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OSEI Corporation Overview Summary Of All 54 Toxicity Tests with OSE II

The OSEI Corporation has had 54 Toxicity tests performed on our product Oil Spill Eater II (OSE II). These tests have been carried out in seven (7) different countries.

The US EPA has tested OSE II themselves, in fact they have performed 11 Toxicity test on OSE II. The UK Government has tested OSE II on 2 Toxicity tests, and Environment Canada has tested on OSE II on 10 Fresh water Toxicity tests, these are completely independent tests, that OSE II had no part in.

OSE II has been tested on 41 Salt water/ocean water toxicity tests with multiple types of species, each country has its own species to be tested. OSE II has also been tested on 13 freshwater toxicity tests on multiple species as well.

OSE II has also been tested with ECO and Indocrine disruption tests which are even more sensitive than LC50 or LD50 tests. OSE II was tested by Environment Canada on single celled Phosphoreum bacterium, which are very sensitive species due to their limited size and

the fact they live in intertidal zones, are a good indicator of the virtual nontoxicity of OSE II, even in intertidal zones/beaches/shorelines. The UK also has a rocky shoreline test using Patella vulgate are shoreline species. The UK has two tests, one for open water approval of a product and the second one for the approval of a product for shoreline use. OSE II was shown to be non-toxic on both their tests, therefore OSE II is approved for open water and shoreline use in the UK.

The US EPA set the standard for what is virtually non-Toxic at 100> mg/l, OSE II's LC50 or LD50 Toxicity test are from 1900mg/l plus to 10,000 mg/l. This proves OSE II is far and away less toxic than the UE EPA's 100> mg/l, which means OSE II is safe for marine species.

Steven Pedigo

CEO OSEI Corporation

Salt / Ocean Water Toxicity Tests

OIL SPILL RESPONSE BIOREMEDIATION AGENTS
EVALUATION METHODS VALIDATION TESTING
DISCUSSION OF RESULTS

The following data are provided for the oil spill response bioremediation agent producer as a means to begin to assess how this bioremediation agent may behave in response to an oil spill in the environment.

The Tier II 96-hour toxicity test data was conducted with Mysidopsis bahia test species. Mortality was the single measure response, therefore, survival data were used to calculate the 96-hour LC50. LC50 is the lowest concentration effecting 50% mortality of the test organism during a 96 hour exposure period. Sub-lethal and lethal responses were noted at concentrations between 1,000-10,000 mg/L (> 1,900 mg/L) following acute exposure of M.bahia to your bioremediation product.

Oil Spill Eater II was shown to cause a statistically significant reduction (p = 0.05) in the survival of Mysidopsis when animals were exposed during a chronic estimator test for a 7 day period. In general, 7 day exposure (2,500 mg/L) correlated well with values calculated following the 96 hour exposure (> 1,900 mg/L).NETAC101

TIER II TOXICITY DATA
TABLE 1

ACUTE TOXICITY VALUES FOR 96 HOUR LC_{50} – MYSIDOPSIS BAHIA

LC_{50} = Lethal concentration of product that will cause the death of 50% of the test species population within a defined exposure time.
a = LC50 presented as a range of test concentrations since data were from 96-hour acute range-finding test.
b = LC50 presented as a single, numerical value since data were from a definitive 96-hour acute toxicity test.
ND = Not Determined

TABLE 2

CHRONIC TOXICITY VALUES FOR 7 DAY LC_{50} – ***MYSIDOPSIS BAHIA***

NOEC = No Observable Effect Concentration
LOEC = Lowest Observable Effect Concentration
CI = Confidence Interval
NE = No Effect
Fecundity = Egg Production

As we indicated prior and to better understand the data presented above we are including a copy of the Evaluation Methods Manual. The Statistical Method Summary is found in Section 4, Method #8, page 40, of the manual and is intended to help a scientist understand the basis of the experimental objectives developed for this test.

Max. Test
Concentration
(mg/L)
Confidence
Interval

(95%)
96 hour LC50
(mg/L)
Product
1,000-10,000[a]
>1,900[b]
Oil Spill
Eater II
10,000
ND
7 Day LC50
(mg/L)
(95% CI)
Endpoints
(mg/L)
Effects
Measurement
Product

NOEC LOEC

5,700
NE
1,900
1,900
1,900
633
Survival
Growth
Fecundity
2,500(mg/L)
(2,225-3,313)

Oil Spill
Eater II NETAC102
Static Acute Toxicity of
Oil Spill Eater II, Batch 329,

To the Mysid, Mysidopsis bahia
Study Completed
March 9, 1990
Performing Laboratory
EnviroSystems Division

Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842

I. SUMMARY

The acute toxicity of Oil Spill Eater II, batch 329 to the mysid, Mysidopsis bahia, is described in this report. The test was conducted for Incorporated for 96 hours during March 5-9, 1990 at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 19.5◦C. The dilution water was filtered natural seawater collected from the Atlantic Ocean at Hampton, New Hampshire. Aeration was not required to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of Oil Spill Eater II were: 0 mg/L (control), 1 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L. Nominal concentrations were used for all calculations.

Mysids used in the test were less than 5 days old at the start of the test. They were produced at Resource Analysts, Inc. and acclimated under test conditions for their entire life. All mysids were in good condition at the beginning of the study.

Exposure of mysids to the test substance resulted in a 96 hour LC50 of 2,100 mg/L Oil Spill Eater II, with a 95 percent confidence level of 100 – 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

IV. METHODS AND MATERIALS

TEST SUBSTANCE:

Oil Spill Eater II (EnviroSystems Sample Number 2351E) was delivered to EnviroSystems on March 5, 1990. It was contained in a 500 ml plastic bottle that was labeled with the following information: Oil Spill Eater II, Batch 329. The sample was supplied by Incorporated. Prior to use the test material was stored at room temperature. Nominal concentrations were added to test media on a weight/vol basis and are reported as mg/L.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was seawater collected from the Atlantic Ocean at EnviroSystems in Hampton, New Hampshire. Water was adjusted to a salinity of 11-17 ppt (parts per thousand) and stored in 500-gallon polyethylene tanks, where it was aerated.

TEST ORGANISM:

Juvenile mysids employed as test organisms were from a single source and were identified using an approximate taxonomic key. They were produced and acclimated at the Resource Analysts, Inc. facility for their entire life. During acclimation mysids were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. Mysids were fed newly hatched Artemia salina nauplii (EnviroSystems lot number BS01) once or twice daily before the test.

TOXICITY TESTING:

The definitive toxicity test was performed during March 5-9, 1990. It was based on procedures of the U.S. Environmental Protection Agency (1986, 1987). The test was conducted at a target temperature of 20 ± 2◦C with five concentrations of test substance and a dilution water control. A stock solution was prepared by combining 20.0 g of test substance with 2,000 ml of dilution water. The stock solution was added directly to dilution water contained in the test vessels without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L.

Twenty mysids were randomly distributed among a single replicate of each treatment. The test was performed in 2 liter glass dishes (approximately 25 cm in diameter and 8 cm deep) that contained 1.0 liter of test solution (water depth was approximately 4 cm). Test vessels were randomly arranged in an incubator during the 96 hour test. A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 40 eEs-$_1$m-$_2$. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Mysids were fed newly hatched Artemia salina nauplii once per day during the test.

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-3), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Labcomp SCT meter, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate the 96 hour median lethal concentration (LC50). The no observed effect level is the highest tested concentration at which 90% or more of the exposed organisms were unaffected.

V. RESULTS

No insoluble material was observed in any test vessel during the test. Biological and water quality data generated by the acute toxicity test are presented in Table 1 and Appendix A, respectively. One hundred percent survival occurred in the control exposure.

The dose – response curve for organisms exposed to the test substance for 96 hours is presented in Figure 1. Exposure of mysids to the Oil Spill Eater II, batch 329, resulted in a 96 hour LC50 of 2,100 mg/L, with a 95 percent confidence interval of 100 – 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

Table 1. Survival data from toxicity test

Nominal Concentration (mg/L)	Number Alive					Number Affected				
	0hr	24hr	48hr	72hr	96hr	0hr	24hr	48hr	72hr	96hr
0 (control)	1	10	10	10	10	10	0	0	0	0
1	1	10	10	9	9	9	0	0	0	0
10	1	10	10	9	9	9	0	0	0	0
100	1	10	10	10	9	9	0	0	0	0

1,000 1 10 9 9 8 8 0 0 0 0 0
10,000 1 10 0 0 0 0 0 - - - -

Resource ana

TOXICITY TEST
FOR ARTEMIA SALINA

To gain acceptance on the U.S. EPA's National Contingency Plan List, we were requested to perform an additional Toxicity Test on Artemia Salina using EPA's Standard Dispersant Toxicity Test.

OSE II Concentrate was presented to the laboratory, but the laboratory refers to the product as a Dispersant instead of OSE II throughout the write-up, since it was a Dispersant Toxicity Test. The Test proved that OSE II Concentrate is once again virtually non-toxic. This particular test proved OSE II helps to detoxify oil. The fuel oil had a higher toxicity rate than did the fuel and OSE II, which shows OSE II to immediately starts reducing the toxicity of hydrocarbons once OSE II is applied. The fuel oils toxicity was 12.4 ppm, and the fuel oil and with OSE II applied showed a drop in the fuel oils toxicity to 29.4, over a 100 percent reduction of the toxicity of the fuel oil. This shows real value in utilizing OSE II since the toxicity of the spilled contaminant would be reduced immediately lesoning the impact of a spill to the associated environment and marine species.

OSE II gained acceptance to the EPA's National Contingency Plan once this test was presented to the EPA.

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

Standard Dispersant Toxicity Test with
the OSE II, Batch #9820 and
Artemia salina

Authors
Timothy J. Ward & Robert L. Boeri
Performing Laboratory EnviroSystems
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Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842
October, 1990

II. TABLE OF CONTENTS

III. Index of Tables

IV. INTRODUCTION

The objective of the study was to determine the acute toxicity of the dispersant – Batch # 9820, No. 2 fuel oil, and a 1:10 mixture of dispersant and oil to *Artemia salina*, a marine invertebrate. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the tests.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

The dispersant – Batch # 9820 (EnviroSystems Sample Number 2591E) was delivered to EnviroSystems on August 17, 1990. It was contained in two 1,000 ml plastic bottles that were labeled with the following information: “Batch # 9820”. The No. 2 fuel oil (EnviroSystems Sample Number 2599E) was delivered to EnviroSystems on August 28, 1990. It was contained in a 1,000 ml plastic bottle that was labeled with the following information: “# 2 fuel oil”.

DILUTION WATER:

Water used for hatching and acclimation of test organisms and for all toxicity testing was formulated at EnviroSystems in Hampton, New Hampshire. Water was diluted to a salinity of 20 parts per thousand and stored in polyethylene tanks where it was aerated.

TEST ORGANISM:
Juvenile *Artemia salina* employed as test organisms were from a single source and were identified using an appropriate taxonomic key. *Artemia salina* used in the test were produced from an in-house culture and were 24 hours old at the start of the test. Prior to testing, *Artemia salina* were maintained in 100% dilution water under static conditions. During acclimation *Artemia salina* were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. They were not fed before or during the tests.
TOXICITY TESTING:
Screening tests with the test substances were conducted during October 1 to 3, 1990. The definitive toxicity tests were performed with the dispersant, No. 2 fuel oil, a 1:10 mixture of dispersant and oil, and the standard toxicant, dodecyl sodium sulfate during October 3 to 5, 1990, according to procedures of the U.S. EPA (1984). The tests were conducted at a target temperature of 20 ± 1◦C with five concentrations of each test substance and a dilution water control.

The dispersant and oil stock solutions were prepared by combining 550 ml of sea water and 0.55 ml of test substance in a glass blender jar and mixing the solution at 10,000 rpm for 5 seconds. The combined dispersant and oil stock solution was prepared by mixing 550 ml of sea water at 10,000 rpm and adding 0.5 ml of oil and 0.05 ml of dispersant. This combined mixture was then mixed for 5 seconds. Nominal concentrations of each test material were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L, and 100 mg/L. Media in each test vessel was added at the beginning of the test and not renewed.
Twenty *Artemia salina* were randomly distributed to each of 5 replicates of each treatment. The tests were performed in 250 ml glass Carolina culture dishes that contained 100 ml of test solution (water depth was approximately 2.5 cm). Test vessels were randomly arranged in an incubator during the 48 hour test. A 24 hour light and 0 hour dark photoperiod was maintained below the dishes. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. *Artemia salina* were not fed during the tests.
The number of surviving organisms was determined visually and recorded initially and after 24 and 48 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-18), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Refractometer, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded at the beginning and end of each test in one test chamber of each concentration.
STATISTICAL METHODS:
Results of the toxicity test were interpreted by standard statistical techniques (Stephen, 1983). The binomial method was used to calculate the median lethal concentration (LC50) values.

VI. RESULTS

All test vessels containing dispersant appeared clear throughout the test and all test vessels containing oil or oil and dispersant had an oil slick on the surface of the test media throughout the

test. Biological and water quality data generated by the acute toxicity tests are presented in Table 1 and Appendix A, respectively. Ninety-nine percent survival occurred in the control exposure. The 48 hour LC50 for *Artemia salina* exposed to the reference toxicant dodecyl sodium sulfate is 38.7 mg/L.

The 24 and 48 hour LD50s from the three toxicity tests are presented in Table 2. The 48 hour LC50s for *Artemia salina* exposed to the test substances are: dispersant/OSE II - >100 mg/L, No. fuel oil – 12.6 mg/L (95% confidence interval = 10.0 – 25.0 mg/L), and a 1:10 mixture of dispersant/OSE II and

No. 2 fuel oil – 29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).

Table 1. Survival data from toxicity tests

Number Alive

Nominal Concentration (mg/L)	Dispersant/OSE II rep.	0hr	24hr	48hr	No. 2 fuel oil 0hr	24hr	48hr	Oil + Dispersant/OSE II 0hr	24hr	48hr
0 (control)	1	20	20	20	20	20	20	20	20	20
	2	20	20	19	20	20	19	20	20	20
	3	20	20	20	20	20	20	20	20	20
	4	20	20	20	20	20	20	20	20	20
	5	20	20	20	20	20	20	20	20	20
10	1	20	19	17	20	20	17	20	20	19
	2	20	20	17	20	20	19	20	20	18
	3	20	20	20	20	20	12	20	18	18
	4	20	20	19	20	20	9	20	20	17
	5	20	19	18	20	18	10	20	20	16
25	1	20	20	16	20	18	0	20	19	19
	2	20	19	17	20	19	3	20	18	15
	3	20	20	18	20	19	2	20	20	16
	4	20	19	12	20	20	2	20	20	17
	5	20	19	15	20	20	0	20	19	14
40	1	20	19	16	20	20	0	20	19	0
	2	20	20	14	20	19	0	20	20	0
	3	20	20	19	20	20	0	20	20	0
	4	20	20	15	20	18	0	20	14	0
	5	20	20	17	20	17	0	20	18	2
60	1	20	19	18	20	18	0	20	18	0
	2	20	19	16	20	19	0	20	19	0
	3	20	19	19	20	16	0	20	19	0
	4	20	20	17	20	19	0	20	16	0
	5	20	20	16	20	14	1	20	16	1
100	1	20	20	18	20	13	0	20	20	0
	2	20	20	18	20	8	0	20	20	0
	3	20	19	13	20	9	0	20	20	0
	4	20	20	19	20	10	0	20	20	0
	5	20	20	16	20	8	0	20	20	0

VII. REFERENCES

Stephen, C.E. 1983. Computer program for calculation of LC50 values. Personal communication.

U.S. EPA. 1984. Revised Standard Dispersant Toxicity Test. Federal Register, Volume 49, Number 139, Wednesday, July 18, 1984, pages 29204 to 29207.

Appendix A. WATER QUALITY DATA FROM TOXICITY TESTS

I. Summary

The acute toxicity of the dispersant – Batch #9820, No. 2 fuel oil, and a 1:10 mixture of dispersant/OSE II and No. 2 fuel oil to *Artemia salina*, is described in this report. The test was conducted for OSEI 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 ± 1◦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/OSE II >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/OSE II and No. 2 fuel oil-29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).

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EPA in Cooperation with NETAC a Group out of
Pittsburgh University performed Efficacy and ToxicityTesting
on OSE II for the EPA NCP Protocol Development.
The Summary follows

The OSEI Corporation supplied OSE II to Hap Prichard of the US EPA in 1992. The EPA performed two separate tests a 48 hour exposure test and a 96 hour exposure test, on two different species Mysidopsis Bahia, and Menidia beryllina. The Mysidopsis Bahia tests also contained a static renewal LC50 for 48 hours and 96 hours with OSE II, and a 7 day toxicity test as well.

The test information is contained in the five pages following this summary, as well as the freedom of information request that was honored over five (5) years after it was requested for these tests shows the OSEI Corporation received this information from the US EPA. The test information with the redacted black outs, is as the OSEI Corporation received them, from the US EPA.

Toxicity tests are performed to show the potential effects of a product to marine species. The larger or higher the number the less toxic the product is. LC 50, the LC means lethal concentration, or the concentration of a product to produce death of the test species.

The US EPA 's first toxicity test of OSE II was on Mysidopsis Bahia for 48 hours of exposure, and for 96 hours of exposure. The 48 hour exposure toxicity test showed OSE II 's toxicity value to be between 5,661 to 7,927 for an average of 6,698. The 96 hour exposure toxicity test showed OSE II 's toxicity value to be between 3,125 to 6,250 for an LC 50 of 5,970. These two test shows the US EPA has proven OSE II to be virtually non toxic.

The US EPA static renewal LC 50 with OSE II and the Mysidopsis Bahia was >5,700 for the 48 hour exposure, and >5,700 for the 96hr as well. The EPA established values for OSE II with this species for both exposure times proves OSE II is virtually non toxic.

The US EPA went on to perform a seven (7) day toxicity test with OSE II and the Mysidopsis Bahia. The LC 50 was 2,225 to 3,133, for an LC 50 value of 2,500 which for a seven (7) day toxicity test is phenomenally non toxic.

The US EPA performed toxicity tests on a second species for the EPA/NETAC testing Menidia beryllina. The first test on this species was for an exposure time of 48 hours, and the LC 50 value was 6,250 to 12,500 for an LC 50 value of 8,839. The second test with the Menidia beryllina was for the exposure time of 96 hours, and the value was

between 6,250 and 12,500 as well for an LC 50 of 8,839. These two test show the US EPA proving OSE II is virtually non toxic on a second species

These toxicity tests associated with the US EPA/NETAC testing as well as the numerous other toxicity tests that have been performed with OSE II by the US EPA and other governments, and for other governments by the OSEI Corporation overwhelmingly prove OSE II is safe for any marine environments species. These toxicity tests show that when OSE II is utilized for a spill there is real value obtained by using OSE II since it converts a spill to CO 2 and water while limiting and or reducing the toxicity of the spill to the environment.

Steven Pedigo
OSEI Corporation

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

June 25, 2003

OFFICE OF
RESEARCH AND DEVELOPMENT

Mr. George Lively
Oil Spill Eater International Corp.
13127 Chandler Drive
Dallas, Texas 75243

RECEIVED
BY ORD | DATE 6-30-03

re: Freedom of Information Act Request HQ-RIN-01971-02

Dear Mr. Lively:

In response to your request for records under the Freedom of Information Act, we were asked to search for and provide data generated using Product C at the Gulf Ecology Division (GED) during the development of oil spill bioremediation protocols. The research involved several laboratories, both within the Office of Research and Development and outside of the Agency.

We are providing these data as an enclosure to this letter, at no cost to you. We also offer a quick explanation of these data in the hopes that it will facilitate your understanding and use .

It is important to note that we used a variety of commercial bioremediation products (CBAs) to develop and evaluate test systems and protocols for the purpose of assessing the efficacy and environmental safety (toxicity) of current and future oil spill bioremediation agents; thus, any data generated with a particular (CBA) was not primarily for the intent of evaluating the product but rather for the purpose of evaluating the test systems under development. These CBAs were provided to us, blind coded, by NETAC–at no time during the collection of these data did we know the actual name of the vendor or product, and thus none of the data will have a vendor's name or product identification associated with it.

In our data, we sometimes refer to Product C as Product 1 - 3 or as CBA C; we have also referred to it by another letter (see manuscript information, below). Data generated at GED was developed through collaborative studies (two cooperative agreements) with the University of West Florida. Throughout the course of evaluating the tests systems, data from more than one CBA might be discussed in notebooks on the same day. Where we have included copies of this data, we have crossed through information that does not respond to FOIA Request HQ RIN-01971-02.

In order to put the data provided in its proper perspective, a copy of a publication and parts of a manuscript are provided to serve as entry points to understanding the data, logs, and materials in this package.

Protocol development utilized a tiered approach of increasingly complex test systems for product evaluation, which is described in more detail in the EPA publication EPA/600/X-93/001 (mentioned below). There were three primary aspects of this research which were conducted at GED that generated data with CBA C:

TOXICOLOGY

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MENIDIA BERYLLINA 96-H STATIC TEST WITH PRODUCT C (CBA C)

Table 3. 48, 96 h, and 7-d LC50 values (95% conf. lim.)[a] for CBAs in static and static-renewal tests using *M. beryllina* and *M. bahia*.

	static LC50		static-renewal LC50		
CBA	48-h	96-h	48-h	96-h	7-d
Mysidopsis bahia					
B	6,698 (5,661-7,927)	5,970 (3,125-6,250)	>5,700	>5,700	2,500 (2,225-3,133)
Menidia beryllina					
B	8,839 (6,250-12,500)	8,839 (6,250-12,500)	---[b]		

[a]Nominal concentrations (mg/L).
[b]Short-term chronic test not conducted.

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United Kingdom Toxicity Test with OSE II Summary

The United Kingdom Marine Management Organization, who's Marine Pollution Response Team, established the requirements to gain approval in the UK. This group required an efficacy test similar to the US EPA's efficacy test requirements to gain approval to the US NCP List. This group in the UK also required an Agitation Test, a Sea Test, and a Rocky Shore Test. The Agitation and Sea Test were to determine if OSE II was non toxic enough to be used on off shore, OSE II was tested with the species the brown shrimp (Crangon crangon), where OSE II proved it was non-toxic, and OSE II was approved for open waters in the UK.

A second test was performed on rocky shores to see if OSE II was nontoxic/safe enough to be used on UK shorelines. OSE II was utilized with Patella vulgata, and was shown to be nontoxic and safe therefore OSE II can be used on shorelines in the UK as well.

"3.2 There are 2 toxicity tests. The first test, the sea test, is carried out using the brown shrimp (Crangon crangon). This test compares the relative toxicity of an oil-product mix to that of the oil alone.

The second test is called the rocky shore test and is carried out using the common limpet (Patella vulgata). This test compares the toxicity of the product alone to that of the standard test oil."

OSE II is approved for use in the UK for open water and shorelines, once again proving to how safe and non-toxic OSE II is to marine species.

Steven Pedigo

CEO OSEI Corporation

Marine
Management
Organisation

Marine Pollution Response Team, Marine Management Organisation,
Lancaster House, Hampshire Court, Newcastle-upon-Tyne, NE4 7YH
Tel: 0191 376 2511
Fax: 0191 376 2682
Email: dispersants@marinemanagement.org.uk

Approval for the use of oil spill treatment products in the sea under the provisions of the Marine and Coastal Access Act 2009

Approval reference number ODA 241/2015

Name and address of approval holder
Mr SRP Pedigo
Oil Spill Eater International Corporation (OSEI Corporation)
P.O. BOX 515429
Dallas, Texas 75251 USA

Postcode

The Secretary of State for Environment, Food and Rural Affairs (referred to as "the licensing authority") in exercise of the power conferred by Section 15 of the Marine Licensing (Exempted Activities) Order 2011 approves the use of Oil Spill Eater II as a bioremediation product within United Kingdom controlled waters (other than waters adjacent to Scotland and Northern Ireland).

This approval shall remain in force for a period of 5 years from the date given below subject to the following conditions.

1. The product shall not be used except as stated at the time of application for approval, or in accordance with any subsequent instructions issued by the manufacturer or approval holder and approved by the licensing authority.
2. Only the product label provided by the approval holder and accepted by the licensing authority shall be used on supplies of the product marketed in the United Kingdom.
3. The approval holder shall not change the composition of the product, or the source of its raw material from that given in the application for approval without the prior notification to and the agreement of the licensing authority. If any change in any respect is made without the agreement of the licensing authority the product must be withdrawn from use. In such cases the agreement of the licensing authority must be obtained before the product is put back into use.
4. Any changes to the name and address details must also be brought to the attention of the Marine Management Organisation.

Signature K Morton Date 23/01/2015

Katherine Morton

Marine Pollution Response Team
Marine Management Organisation
for and on behalf of the licensing authority

Reference: Fresh Kuwait Crude 04/08/11 : 5.4ml per tank

Tank no.	no. dead	no. alive	no. in tank	%Mortality
5	4	16	20	20.0
7	7	13	20	35.0
10	4	16	20	20.0
Total	15	45	60	25.00

Chi-squared 1.600
d.f. 2
p-value for chi-squared test 0.449

Testing at 5% significance level,
Reference tanks are HOMOGENEOUS

! 3 tanks used

Test Treatment: Oil Spill Eater (557) : 5.4ml per tank
Bioremediation , 50 parts water to 1 part OSE

Tank no.	no. dead	no. alive	no. in tank	%Mortality
1	3	17	20	15.0
4	2	18	20	10.0
8	6	14	20	30.0
9	5	15	20	25.0
Total	16	64	80	20.00

Chi-squared 3.125
d.f. 3
p-value for chi-squared test 0.373

Testing at 5% significance level,
Treatment tanks are HOMOGENEOUS

! 4 tanks used

COMPARISON OF MORTALITY RATES

Reference %mortality 25.00
Treatment %mortality 20.00

D, Treatment %mortality - Reference %mortality **-5.00**

Standard error of **D** 7.16

95% Confidence interval for **D** -19.0 to 9.0

H0: treatment mort. = reference mort. , H1: treatment mort. > reference mort.

Test statistic -0.70 p-value = 0.758

Treatment mortality < reference mortality

Tanks where motors stopped have been removed from analysis

Notes: Tanks where motors stopped have been removed from analysis

Reference: Fresh Kuwait Crude : 18ml per 18L SW

Tank no.	no. dead	no. alive	no. in tank	%Mortality
2	2	18	20	10.0
5	6	14	20	30.0
7	5	15	20	25.0
9	0	20	20	0.0
13	5	15	20	25.0
Total	18	82	100	18.00

Chi-squared 8.537
d.f. 4
p-value for chi-squared test 0.074

Testing at 5% significance level,
Reference tanks are HOMOGENEOUS

Test Treatment: Oil Spill Eater (557) : 18ml per 18ml oil
Type 2 , 10% in sea water

Tank no.	no. dead	no. alive	no. in tank	%Mortality
4	4	16	20	20.0
6	1	19	20	5.0
10	0	20	20	0.0
12	2	18	20	10.0
14	0	20	20	0.0
Total	7	93	100	7.00

Chi-squared 8.602
d.f. 4
p-value for chi-squared test 0.072

Testing at 5% significance level,
Treatment tanks are HOMOGENEOUS

COMPARISON OF MORTALITY RATES

Reference %mortality 18.00
Treatment %mortality 7.00

D, Treatment %mortality - Reference %mortality **-11.00**

Standard error of **D** 4.61

95% Confidence interval for **D** -20.0 to -2.0

H0: treatment mort. = reference mort. , H1: treatment mort. > reference mort.

Test statistic -2.39 p-value = 0.991

Treatment mortality < reference mortality

Notes: Pass

Reference: Fresh Kuwait Crude 04/08/11 : 16ml per plate

Tank no.	no. dead	no. alive	no. in tank	%Mortality
1	8	12	20	40.0
4	16	4	20	80.0
5	17	3	20	85.0
9	18	2	20	90.0
12	18	2	20	90.0
Total	77	23	100	77.00

Chi-squared	20.102
d.f.	4
p-value for chi-squared test	0.000

Testing at 5% significance level,
Reference tanks are NOT HOMOGENEOUS

Test Treatment: Oil spill eater (557) : 16ml per plate
Bioremediation , 10% in seawater

Tank no.	no. dead	no. alive	no. in tank	%Mortality
6	5	15	20	25.0
8	4	16	20	20.0
11	3	17	20	15.0
13	1	19	20	5.0
15	1	19	20	5.0
Total	14	86	100	14.00

Chi-squared	5.316
d.f.	4
p-value for chi-squared test	0.256

Testing at 5% significance level,
Treatment tanks are HOMOGENEOUS

COMPARISON OF MORTALITY RATES

Reference %mortality 77.00
Treatment %mortality 14.00

D, Treatment %mortality - Reference %mortality **-63.00**

Standard error of **D** 5.45

95% Confidence interval for **D** -73.7 to -52.3

H0: treatment mort. = reference mort. , H1: treatment mort. > reference mort.

Test statistic -11.55 p-value = 1.000

Treatment mortality < reference mortality

TEST INVALID: Reference tanks are not homogeneous

Notes: Reference tanks are not homogeneous, however the test treatment tanks are all lower mortality than the reference tanks. This will not be repeated, as it is a clear pass.

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Australia Government Required Toxicity Test

With OSE II Summary

The Astralian Government requires 48-hr larval development test using the milky oyster *Saccostrea echinate, a* 48-hr larval development test using the mussel *Mytilus galloprovincialis,* There were four (4) toxicity tests carried on the two separate species. The Oyster had, 48-hr IC10, 48-hr EC50, NOEC, and LOEC. The Mussel had 72-hr EC10, 72-hr EC50, NOEC, LOEC carried out with OSE II.

The Oyster results are, 48-hr IC10 = 11.0 (10.0-11.9)mg/L 48-hr EC50 = 16.5 (16.0-17.1)mg/L NOEC = 10.0mg/L This test was extended to 72 hours, producing even more impressive results.
LOEC = 20.0mg/L, and the Mussels results are 72-hr EC10 = >20.0mg/L 72-hr EC50 = >20.0mg/L NOEC = 20.0mg/L LOEC = >20.0mg/L. There were a total of 8 tests performed.

The next set of tests, the 48-hr acute survival test using the copepod Parvocalanus crassirostris ESA SOP 124 (2012), the 96-hr acute toxicity test using the amphipod Melita plumulosa. The results are for the copepod 48-hr IC10 = >20.0mg/L 48-hr EC50 = >20.0mg/L NOEC = 20.0mg/L LOEC = >20.0mg/L and the results for the Amphipod are 96-hr EC10 = >20.0mg/L 96-hr EC50 = >20.0mg/L NOEC = 20.0mg/L
LOEC = >20.0mg/L. There were a total of 8 more Toxicity tests performed in this section.

The next set of tests, the 96-hr fish imbalance toxicity test using barramundi Lates calcarifer and the 96-hr fish imbalance toxicity test using Australian Bass Macquaria. The results for the Barramundi are 96-hr EC10 = >20.0mg/L 96-hr EC50 = >20.0mg/L NOEC = 20.0mg/L LOEC = >20.0mg/L and the results for Australian Bass Macquaria are 96-hr IC10 = 15.7mg/L*,96-hr EC50 = >20.0mg/L, NOEC = 20.0mg/L, LOEC = >20.0mg/L There were 8 more toxicity tests in this section.

There were a total of 24 toxicity tests carried out which covers larval stages to full grown stages as well. These tests all showed that OSE II met the Australian governments requirements and proved that OSE II was non toxic.

OSE II was approved for use in Australia.

Steven Pedigo

CEO OSEI Corporation

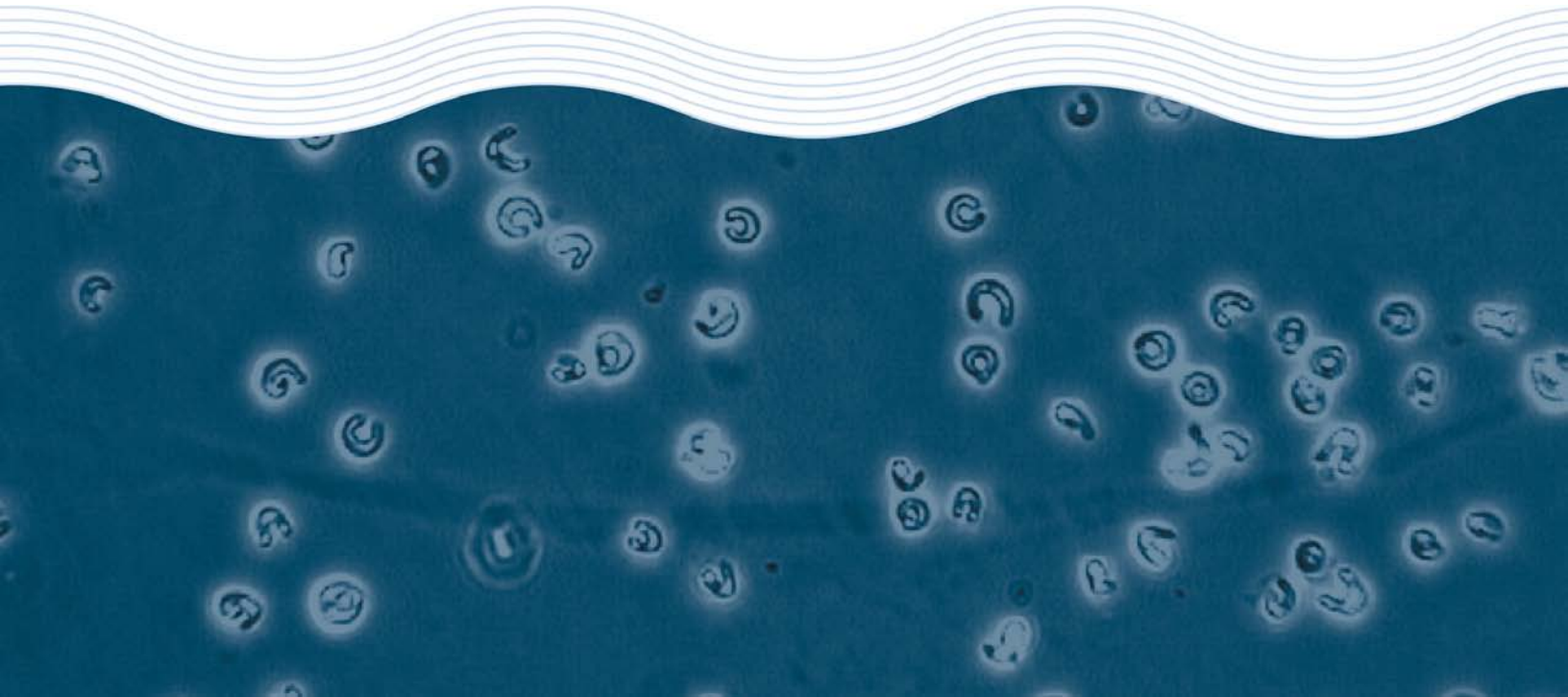
ecotox
SERVICES AUSTRALASIA

Toxicity Assessment of Oil Spill Eater II

CMTA

Test Report

August 2013



Toxicity Assessment of Oil Spill Eater II

CMTA

Test Report

August 2013

ECOTOX Services Australasia Pty Ltd ABN>45 094 714 904
unit 27/2 chaplin drive lane cove nsw 2066 T>61 2 9420 9481 F>61 2 9420 9484 W>www.ecotox.com.au

Toxicity Test Report: TR1083/1

This document is issued in accordance with NATA's accreditation requirements

Client:	CMTA 158 Garretts Rd Longford VIC 3851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1083 Not supplied 19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	48-hr larval development test using the milky oyster *Saccostrea echinata*
Test Protocol:	ESA SOP 106 (ESA 2011), based on APHA (1998) and Krassoi (1995)
Test Temperature:	The test was performed at 29±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	Field collected from Mackay, QLD.
Test Initiated:	20 August 2013 at 1800h

Sample 6232: *Oil Spill Eater II* **Concentration (mg/L)**	**% Normal larvae (Mean ± SD)**	*Vacant*	*Vacant*
FSW Control	72.0 ± 2.2		
1.3	73.3 ± 4.6		
2.5	73.8 ± 2.1		
5.0	74.0 ± 3.7		
10.0	72.0 ± 4.3		
20.0	23.3 ± 16.7 *		
48-hr IC10 = 11.0 (10.0-11.9)mg/L **48-hr EC50 = 16.5 (16.0-17.1)mg/L** **NOEC = 10.0mg/L** **LOEC = 20.0mg/L**			

*Significantly lower percentage of normal larvae compared with the FSW Control (Steel's Many-One Rank Test, 1-tailed, P=0.05)

Toxicity Test Report: TR1083/1

QA/QC Parameter	Criterion	This Test	Criterion met?
FSW Control mean % normal	≥70%	72.0%	Yes
Reference Toxicant within cusum chart limits	13.1-18.8µg Cu/L	15.2µg Cu/L	Yes

Test Report Authorised by:  Dr Rick Krassoi, Director on 3 September 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

APHA (1998) Standard Methods for the Examination of Water and Wastewater. 20th Ed. American Public Health Association, American Water Works Association and the Water Environment Federation, Washington, DC.

ESA (2011) SOP 106 – *Bivalve Larval Development Test*. Issue No. 10. Ecotox Services Australasia, Sydney, NSW.

Krassoi, R (1995) Salinity adjustment of effluents for use with marine bioassays: effects on the larvae of the doughboy scallop Chlamys asperrimus and the Sydney rock oyster *Saccostrea commercialis*. *Australasian Journal of Ecotoxicology*, 1: 143-148.

Toxicity Test Report: TR1083/2

This document is issued in accordance with NATA's accreditation requirements

Client:	CMTA	**ESA Job #:**	PR1083
	158 Garretts Rd	**Date Sampled:**	Not supplied
	Longford VIC 3851	**Date Received:**	19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	48-hr larval development test using the mussel *Mytilus galloprovincialis*
Test Protocol:	ESA SOP 106 (ESA 2011), based on APHA (1998) and USEPA (1996)
Test Temperature:	The test was performed at 20±1°C.
Deviations from Protocol:	The test was extended to 72 hours.
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	Farm-reared, Mercury Passage, TAS
Test Initiated:	26 August 2013 at 1545h

Sample 6232: *Oil Spill Eater II*	*Vacant*	*Vacant*
Concentration (mg/L) / **% Normal larvae (Mean ± SD)**		
FSW Control: 75.8 ± 4.4		
1.3: 72.5 ± 1.3		
2.5: 77.8 ± 7.0		
5.0: 75.3 ± 5.8		
10.0: 77.8 ± 5.0		
20.0: 75.3 ± 5.3		
72-hr EC10 = >20.0mg/L		
72-hr EC50 = >20.0mg/L		
NOEC = 20.0mg/L		
LOEC = >20.0mg/L		

Toxicity Test Report: TR1083/2

QA/QC Parameter	Criterion	This Test	Criterion met?
FSW Control mean % normal	≥70%	75.8%	Yes
Reference Toxicant within cusum chart limits	7.3-17.2µg Cu/L	7.5µg Cu/L	Yes

Test Report Authorised by:  Dr Rick Krassoi, Director on 3 September 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

APHA (1998) *Standard Methods for the Examination of Water and Wastewate*r. 20th Ed. American Public Health Association, American Water Works Association and the Water Environment Federation, Washington, DC, USA.

ESA (2011) *Bivalve Larval Development Test*. Issue No. 10. Ecotox Services Australasia, Sydney, NSW

USEPA (1996) *Bivalve acute toxicity test (embryo larval) OPPTS 850.1055. Ecological Effects Test Guidelines.* United States Environmental Protection Agency. Prevention, Pesticides and Toxic Substances. EPA/712/C-96/137.

Toxicity Test Report: TR1083/3

Client:	CMTA	**ESA Job #:**	PR1083
	158 Garretts Rd	**Date Sampled:**	Not supplied
	Longford VIC 3851	**Date Received:**	19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	48-hr acute survival test using the copepod *Parvocalanus crassirostris*
Test Protocol:	ESA SOP 124 (2012)
Test Temperature:	The test was performed at 27±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	In house culture
Age of Test Organisms:	<7 days old
Test Initiated:	14 November 2013 at 1300h

Sample 6232: *Oil Spill Eater II*	*Vacant*	*Vacant*
Concentration (mg/L) / **% Survival (Mean ± SD)**		
FSW Control: 95.0 ± 10.0		
1.3: 95.0 ± 10.0		
2.5: 100 ± 0.0		
5.0: 90.0 ± 11.6		
10.0: 95.0 ± 10.0		
20.0: 90.0 ± 11.6		
48-hr IC10 = >20.0mg/L **48-hr EC50 = >20.0mg/L** **NOEC = 20.0mg/L** **LOEC = >20.0mg/L**		

QA/QC Parameter	**Criterion**	**This Test**	**Criterion met?**
Control mean % survival	≥80.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	4.4-30.5µg Cu/L	10.0µg Cu/L	Yes

Toxicity Test Report: TR1083/3

Test Report Authorised by:  Dr Rick Krassoi, Director on 25 November 2013

Results are based on the samples in the condition as received by ESA. This document shall not be reproduced except in full.

Citations:

ESA (2012) *SOP 124 – Acute toxicity test using the copepod Gladioferens imparipes*. Issue No. 1. Ecotox Services Australasia, Sydney, New South Wales.

Toxicity Test Report: TR1083/4

This document is issued in accordance with NATA's accreditation requirements

Client:	CMTA 158 Garretts Rd Longford VIC 3851	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1083 Not supplied 19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	Sample Name:	Sample Description:
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	96-hr acute toxicity test using the amphipod *Melita plumulosa*
Test Protocol:	ESA SOP 108 (ESA 2011), based on USEPA (2002) and Department of Transport and Communications (1990)
Test Temperature:	The test was performed at 20±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	In-house culture, originally sourced from Hawkesbury River, NSW
Test Initiated:	14 November 2013 at 1230h

Sample 6232: *Oil Spill Eater II* **Concentration (mg/L)**	**% Unaffected (Mean ± SD)**	*Vacant*	*Vacant*
FSW Control	95.0 ± 10.0		
1.3	95.0 ± 10.0		
2.5	100 ± 0.0		
5.0	90.0 ± 11.6		
10.0	100 ± 0.0		
20.0	100 ± 0.0		

96-hr EC10 = >20.0mg/L
96-hr EC50 = >20.0mg/L
NOEC = 20.0mg/L
LOEC = >20.0mg/L

Toxicity Test Report: TR1083/4

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥90.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	69.6-456.4µg Cu/L	140.8µg Cu/L	Yes

Test Report Authorised by:  Dr Rick Krassoi, Director on 25 November 2013

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

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Citations:

Department of Transport and Communications (1990) Guidelines for Acceptance of Oil Spill Dispersants in Australian Waters. Pollution Prevention Section, Department of Transport and Communications, Canberra ACT.

ESA (2011) SOP 108 – *Amphipod Acute Toxicity Test*. Issue No 8. Ecotox Services Australasia, Sydney, NSW.

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth Edition. United States Environmental Protection Agency, Office of Research and Development, Washington DC, EPA/600/4-90/027F.

Toxicity Test Report: TR1083/5

Client:	CMTA	**ESA Job #:**	PR1083
	158 Garretts Rd	**Date Sampled:**	Not supplied
	Longford VIC 3851	**Date Received:**	19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	96-hr fish imbalance toxicity test using barramundi *Lates calcarifer*
Test Protocol:	ESA SOP 117 (ESA 2012), based on USEPA (2002)
Test Temperature:	The test was performed at 25±2°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	Hatchery reared, SA
Test Initiated:	14 November 2013 at 1500h

Sample 6232: *Oil Spill Eater II*	*Vacant*	*Vacant*
Concentration (mg/L) / **% Unaffected (Mean ± SD)**		
FSW Control: 95.0 ± 10.0		
1.3: 100 ± 0.0		
2.5: 85.0 ± 19.2		
5.0: 100 ± 0.0		
10.0: 90.0 ± 11.6		
20.0: 95.0 ± 10.0		
96-hr EC10 = >20.0mg/L **96-hr EC50 = >20.0mg/L** **NOEC = 20.0mg/L** **LOEC = >20.0mg/L**		

Toxicity Test Report: TR1083/5

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥80.0%	95.0%	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 25 November 2013

Results are based on the samples in the condition as received by ESA. This document shall not be reproduced except in full.

Citations:

ESA (2012) SOP 117 –*Freshwater and Marine Fish Imbalance Test*. Issue No 9. Ecotox Services Australasia, Sydney, NSW

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth edition EPA-821-R-02-012. United States Environmental Protection Agency, Office of Research and Development, Washington FC, USA

Toxicity Test Report: TR1083/6

Client:	CMTA	**ESA Job #:**	PR1083
	158 Garretts Rd	**Date Sampled:**	Not supplied
	Longford VIC 3851	**Date Received:**	19 August 2013
Attention:	Joel Farhadian	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1083_q01

Lab ID No.:	**Sample Name:**	**Sample Description:**
6232	Oil Spill Eater II	Chemical received at room temperature in apparent good condition

Test Performed:	96-hr fish imbalance toxicity test using Australian Bass *Macquaria Novemaculeata*
Test Protocol:	ESA SOP 117 (ESA 2012), based on USEPA (2002)
Test Temperature:	The test was performed at 20±2°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The highest test concentration of 20mg/L was prepared by adding a weighed aliquot of sample 6232 'Oil Spill Eater II' into filtered seawater (FSW). The remaining test concentrations were achieved by serially diluting the highest test concentration with FSW. A FSW control was tested concurrently with the prepared sample.
Source of Test Organisms:	Hatchery reared, SA
Test Initiated:	8 November 2013 at 1200h

Sample 6232: *Oil Spill Eater II*		*Vacant*	*Vacant*
Concentration (mg/L)	**% Unaffected (Mean ± SD)**		
FSW Control	95.0 ± 10.0		
1.3	93.3 ± 11.6		
2.5	100 ± 0.0		
5.0	100 ± 0.0		
10.0	95.0 ± 10.0		
20.0	80.0 ± 20.0		
96-hr IC10 = 15.7mg/L*			
96-hr EC50 = >20.0mg/L			
NOEC = 20.0mg/L			
LOEC = >20.0mg/L			

*95%confidence limits are not reliable

Toxicity Test Report: TR1083/6

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % unaffected	≥80.0%	95.0%	Yes
Reference Toxicant within cusum chart limits	58.3-3473.8µg Cu/L	347.6µg Cu/L	Yes

Test Report Authorised by: Dr Rick Krassoi, Director on 25 November 2013

Results are based on the samples in the condition as received by ESA. This document shall not be reproduced except in full.

Citations:

ESA (2012) SOP 117 –*Freshwater and Marine Fish Imbalance Test*. Issue No 9. Ecotox Services Australasia, Sydney, NSW

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth edition EPA-821-R-02-012. United States Environmental Protection Agency, Office of Research and Development, Washington FC, USA

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Statistical Printouts for the Milky Oyster Larval Development Tests

Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Conc-mg/L	1	2	3	4
FSW Control	0.7200	0.7400	0.6900	0.7300
1.3	0.7200	0.7900	0.6800	0.7400
2.5	0.7600	0.7200	0.7500	0.7200
5	0.7600	0.7000	0.7800	0.7200
10	0.7800	0.7200	0.6800	0.7000
20	0.4600	0.1900	0.2200	0.0600

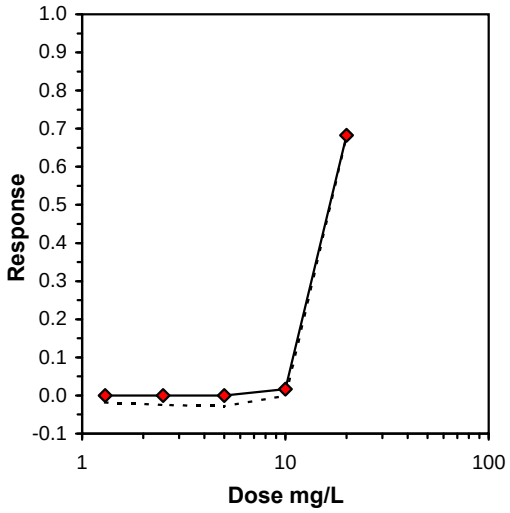
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
FSW Control	0.7200	1.0000	1.0134	0.9803	1.0357	2.359	4			0.7325	1.0000
1.3	0.7325	1.0174	1.0283	0.9695	1.0948	5.070	4	19.00	10.00	0.7325	1.0000
2.5	0.7375	1.0243	1.0331	1.0132	1.0588	2.272	4	21.00	10.00	0.7325	1.0000
5	0.7400	1.0278	1.0364	0.9912	1.0826	4.025	4	20.50	10.00	0.7325	1.0000
10	0.7200	1.0000	1.0141	0.9695	1.0826	4.832	4	16.50	10.00	0.7200	0.9829
*20	0.2325	0.3229	0.4830	0.2475	0.7454	42.321	4	10.00	10.00	0.2325	0.3174

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.791823	0.916	0.475743	7.130866
Bartlett's Test indicates unequal variances (p = 1.05E-03)	20.41248	15.08627		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	10	20	14.14214	
Treatments vs FSW Control				

Log-Logit Interpolation (200 Resamples)

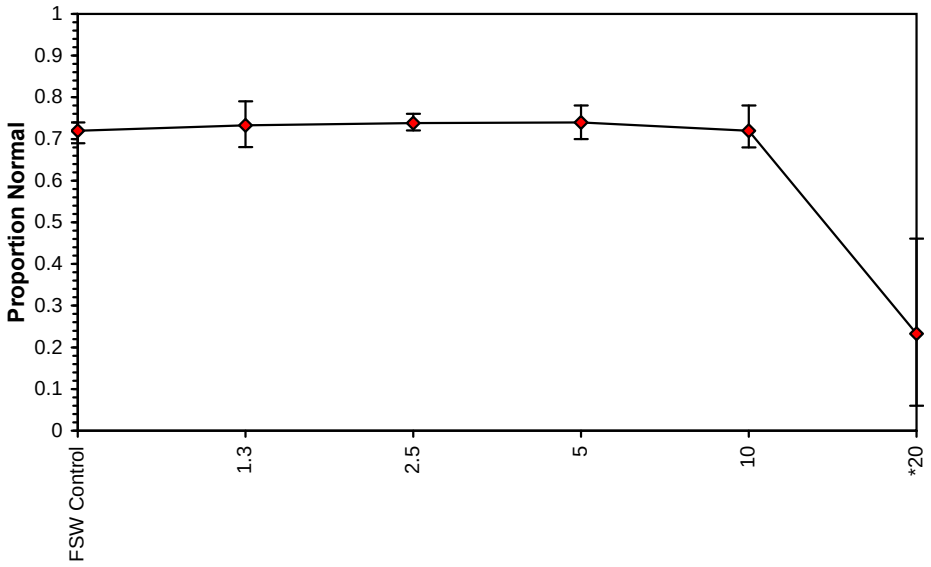
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	10.395	0.623	6.739	10.906	-2.2999
IC10	10.988	0.303	10.037	11.886	0.3357
IC15	11.579	0.389	10.443	12.867	0.7568
IC20	12.176	0.507	10.793	14.080	0.9655
IC25	12.784	0.647	11.168	15.266	1.0823
IC40	14.752				
IC50	16.275				



Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Dose-Response Plot



Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	72.00	69.00	74.00	2.16	2.04	4
1.3		73.25	68.00	79.00	4.57	2.92	4
2.5		73.75	72.00	76.00	2.06	1.95	4
5		74.00	70.00	78.00	3.65	2.58	4
10		72.00	68.00	78.00	4.32	2.89	4
20		23.25	6.00	46.00	16.68	17.57	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.10	8.10	8.10	0.00	0.00	1
2.5		8.10	8.10	8.10	0.00	0.00	1
5		8.10	8.10	8.10	0.00	0.00	1
10		8.10	8.10	8.10	0.00	0.00	1
20		8.10	8.10	8.10	0.00	0.00	1
FSW Control	Salinity ppt	34.80	34.80	34.80	0.00	0.00	1
1.3		34.30	34.30	34.30	0.00	0.00	1
2.5		34.40	34.40	34.40	0.00	0.00	1
5		34.50	34.50	34.50	0.00	0.00	1
10		34.50	34.50	34.50	0.00	0.00	1
20		34.50	34.50	34.50	0.00	0.00	1
FSW Control	DO %	99.30	99.30	99.30	0.00	0.00	1
1.3		98.70	98.70	98.70	0.00	0.00	1
2.5		97.50	97.50	97.50	0.00	0.00	1
5		97.20	97.20	97.20	0.00	0.00	1
10		96.80	96.80	96.80	0.00	0.00	1
20		97.20	97.20	97.20	0.00	0.00	1

Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Conc-mg/L	1	2	3	4
FSW Control	0.7200	0.7400	0.6900	0.7300
1.3	0.7200	0.7900	0.6800	0.7400
2.5	0.7600	0.7200	0.7500	0.7200
5	0.7600	0.7000	0.7800	0.7200
10	0.7800	0.7200	0.6800	0.7000
20	0.4600	0.1900	0.2200	0.0600

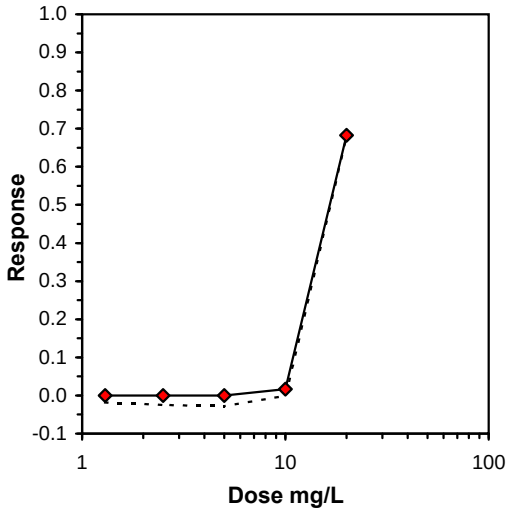
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
FSW Control	0.7200	1.0000	1.0134	0.9803	1.0357	2.359	4			112	400
1.3	0.7325	1.0174	1.0283	0.9695	1.0948	5.070	4	19.00	10.00	107	400
2.5	0.7375	1.0243	1.0331	1.0132	1.0588	2.272	4	21.00	10.00	105	400
5	0.7400	1.0278	1.0364	0.9912	1.0826	4.025	4	20.50	10.00	104	400
10	0.7200	1.0000	1.0141	0.9695	1.0826	4.832	4	16.50	10.00	112	400
*20	0.2325	0.3229	0.4830	0.2475	0.7454	42.321	4	10.00	10.00	307	400

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.791823	0.916	0.475743	7.130866
Bartlett's Test indicates unequal variances (p = 1.05E-03)	20.41248	15.08627		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	10	20	14.14214	
Treatments vs FSW Control				

Trimmed Spearman-Karber

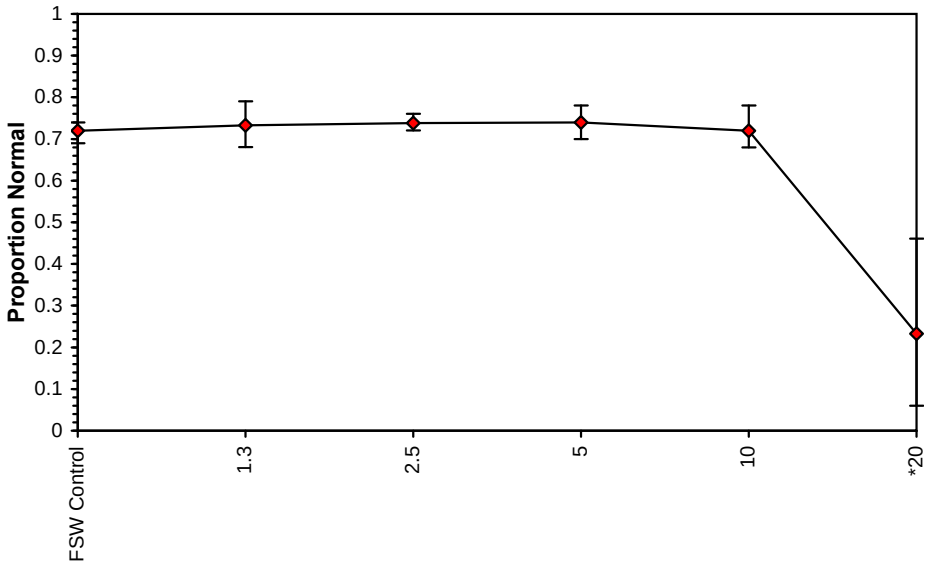
Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%			
Auto-31.7%	16.536	15.962	17.132



Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Dose-Response Plot



Bivalve Larval Development Test-Proportion Normal

Start Date:	20/08/2013 18:00	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	22/08/2013 18:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	72.00	69.00	74.00	2.16	2.04	4
1.3		73.25	68.00	79.00	4.57	2.92	4
2.5		73.75	72.00	76.00	2.06	1.95	4
5		74.00	70.00	78.00	3.65	2.58	4
10		72.00	68.00	78.00	4.32	2.89	4
20		23.25	6.00	46.00	16.68	17.57	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.10	8.10	8.10	0.00	0.00	1
2.5		8.10	8.10	8.10	0.00	0.00	1
5		8.10	8.10	8.10	0.00	0.00	1
10		8.10	8.10	8.10	0.00	0.00	1
20		8.10	8.10	8.10	0.00	0.00	1
FSW Control	Salinity ppt	34.80	34.80	34.80	0.00	0.00	1
1.3		34.30	34.30	34.30	0.00	0.00	1
2.5		34.40	34.40	34.40	0.00	0.00	1
5		34.50	34.50	34.50	0.00	0.00	1
10		34.50	34.50	34.50	0.00	0.00	1
20		34.50	34.50	34.50	0.00	0.00	1
FSW Control	DO %	99.30	99.30	99.30	0.00	0.00	1
1.3		98.70	98.70	98.70	0.00	0.00	1
2.5		97.50	97.50	97.50	0.00	0.00	1
5		97.20	97.20	97.20	0.00	0.00	1
10		96.80	96.80	96.80	0.00	0.00	1
20		97.20	97.20	97.20	0.00	0.00	1

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Statistical Printouts for the Mussel Toxicity Tests

Bivalve Larval Development Test-Proportion Normal

Start Date:	26/08/2013 15:45	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	29/08/2013 15:45	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	MG-Mytilus galloprovincialis
Comments:					

Conc-mg/L	1	2	3	4
FSW Control	0.8200	0.7400	0.7200	0.7500
1.3	0.7300	0.7200	0.7400	0.7100
2.5	0.8500	0.7400	0.8200	0.7000
5	0.8300	0.6900	0.7400	0.7500
10	0.7800	0.7900	0.8300	0.7100
20	0.7300	0.7400	0.8300	0.7100

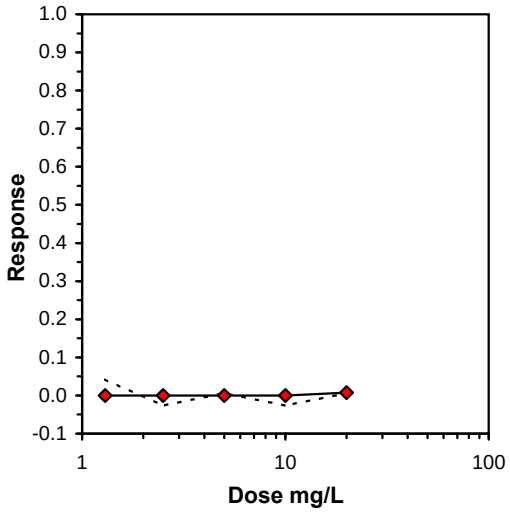
			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
FSW Control	0.7575	1.0000	1.0572	1.0132	1.1326	4.942	4				0.7580	1.0000
1.3	0.7250	0.9571	1.0189	1.0021	1.0357	1.419	4	0.887	2.410	0.1041	0.7580	1.0000
2.5	0.7775	1.0264	1.0832	0.9912	1.1731	7.771	4	-0.601	2.410	0.1041	0.7580	1.0000
5	0.7525	0.9934	1.0523	0.9803	1.1458	6.545	4	0.114	2.410	0.1041	0.7580	1.0000
10	0.7775	1.0264	1.0813	1.0021	1.1458	5.501	4	-0.558	2.410	0.1041	0.7580	1.0000
20	0.7525	0.9934	1.0520	1.0021	1.1458	6.090	4	0.120	2.410	0.1041	0.7525	0.9927

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.942211	0.916	0.356552	-0.59913
Bartlett's Test indicates equal variances (p = 0.30)	6.045919	15.08627		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	20	>20			0.094079	0.124016	0.002221	0.003735	0.704366	5, 18
Treatments vs FSW Control										

Log-Logit Interpolation (200 Resamples)

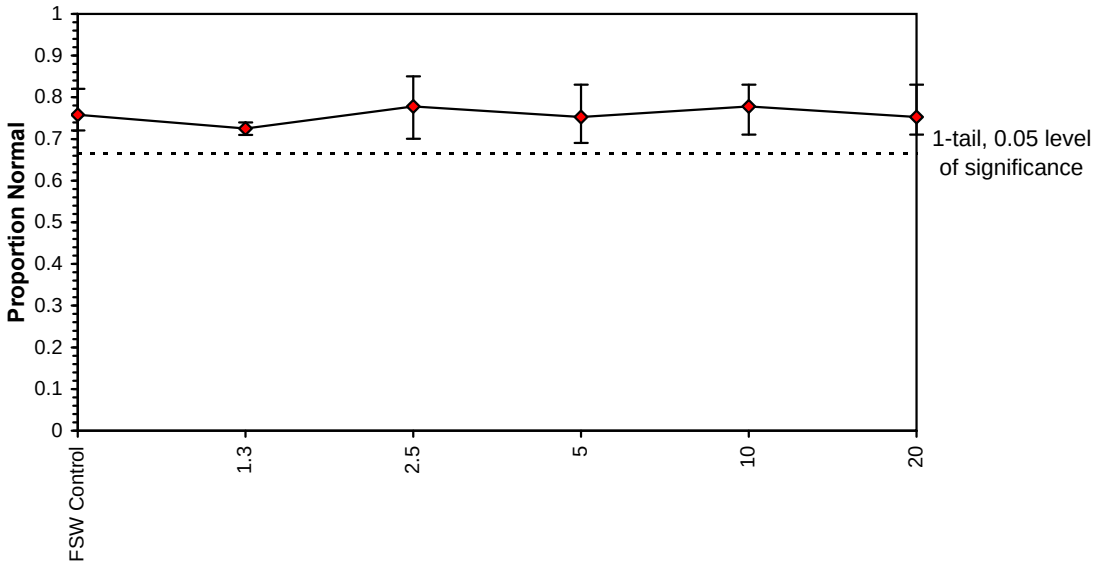
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	>20			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Bivalve Larval Development Test-Proportion Normal

Start Date:	26/08/2013 15:45	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	29/08/2013 15:45	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	MG-Mytilus galloprovincialis
Comments:					

Dose-Response Plot



Bivalve Larval Development Test-Proportion Normal

Start Date:	26/08/2013 15:45	Test ID:	PR1083/01	Sample ID:	Oil Spill Eater II
End Date:	29/08/2013 15:45	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 106	Test Species:	MG-Mytilus galloprovincialis
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	75.75	72.00	82.00	4.35	2.75	4
1.3		72.50	71.00	74.00	1.29	1.57	4
2.5		77.75	70.00	85.00	6.95	3.39	4
5		75.25	69.00	83.00	5.80	3.20	4
10		77.75	71.00	83.00	4.99	2.87	4
20		75.25	71.00	83.00	5.32	3.06	4
FSW Control	pH	8.20	8.20	8.20	0.00	0.00	1
1.3		8.20	8.20	8.20	0.00	0.00	1
2.5		8.20	8.20	8.20	0.00	0.00	1
5		8.20	8.20	8.20	0.00	0.00	1
10		8.20	8.20	8.20	0.00	0.00	1
20		8.20	8.20	8.20	0.00	0.00	1
FSW Control	Salinity ppt	34.20	34.20	34.20	0.00	0.00	1
1.3		34.30	34.30	34.30	0.00	0.00	1
2.5		34.30	34.30	34.30	0.00	0.00	1
5		34.30	34.30	34.30	0.00	0.00	1
10		34.40	34.40	34.40	0.00	0.00	1
20		34.30	34.30	34.30	0.00	0.00	1
FSW Control	DO %	99.00	99.00	99.00	0.00	0.00	1
1.3		99.90	99.90	99.90	0.00	0.00	1
2.5		99.70	99.70	99.70	0.00	0.00	1
5		99.70	99.70	99.70	0.00	0.00	1
10		99.40	99.40	99.40	0.00	0.00	1
20		99.20	99.20	99.20	0.00	0.00	1

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Statistical Printouts for the Juvenile Copepod Tests

Marine Copepod Acute Test-48-hr Survival

Start Date:	14/11/2013 13:00	Test ID:	PR1083/25	Sample ID:	Oil Spill Eater II
End Date:	16/11/2013 12:10	Lab ID:	6232	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 124	Test Species:	PC- Parvocalanus crassirostris
Comments:					

Conc-mg/L	1	2	3	4
FSW Control	1.0000	0.8000	1.0000	1.0000
1.3	1.0000	1.0000	1.0000	0.8000
2.5	1.0000	1.0000	1.0000	1.0000
5	0.8000	0.8000	1.0000	1.0000
10	1.0000	0.8000	1.0000	1.0000
20	0.8000	1.0000	0.8000	1.0000

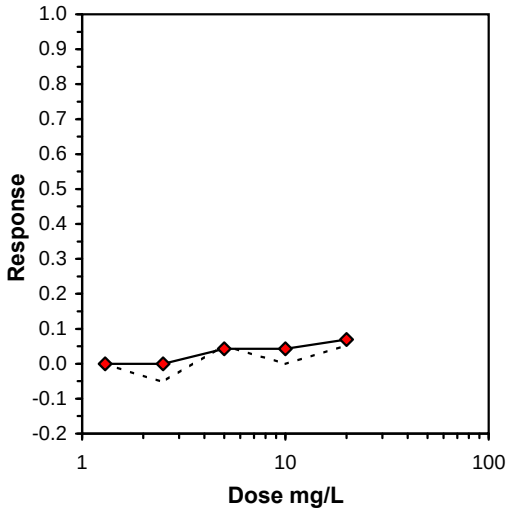
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			0.9667	1.0000
1.3	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9667	1.0000
2.5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9667	1.0000
5	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	0.9250	0.9569
10	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9250	0.9569
20	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	0.9000	0.9310

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.840894	0.916	-0.67177	-0.98034
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	20	>20		
Treatments vs FSW Control				

Log-Logit Interpolation (200 Resamples)

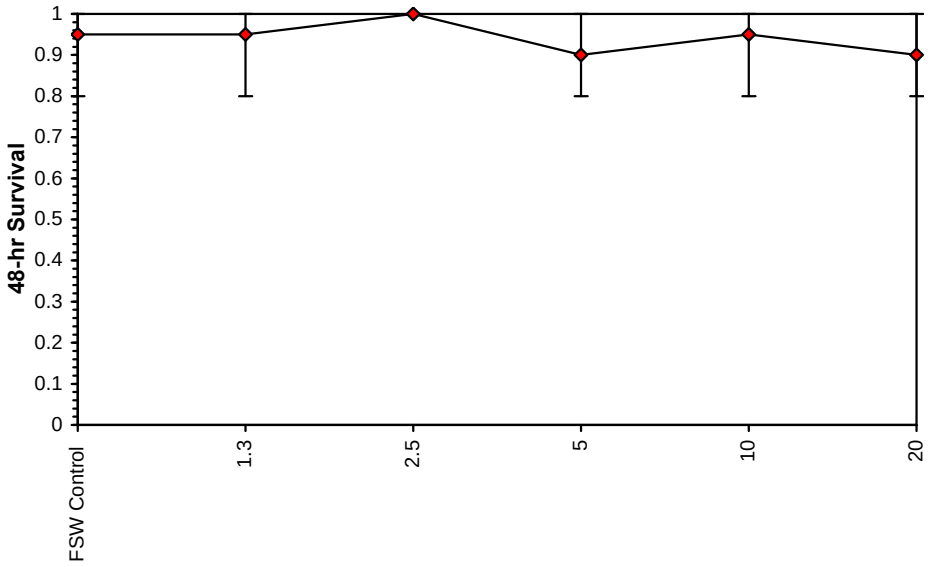
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	12.297			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Marine Copepod Acute Test-48-hr Survival

Start Date:	14/11/2013 13:00	Test ID:	PR1083/25	Sample ID:	Oil Spill Eater II
End Date:	16/11/2013 12:10	Lab ID:	6232	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 124	Test Species:	PC- Parvocalanus crassirostris
Comments:					

Dose-Response Plot



Marine Copepod Acute Test-48-hr Survival

Start Date:	14/11/2013 13:00	Test ID:	PR1083/25	Sample ID:	Oil Spill Eater II
End Date:	16/11/2013 12:10	Lab ID:	6232	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 124	Test Species:	PC- Parvocalanus crassirostris
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Survival	95.00	80.00	100.00	10.00	3.33	4
1.3		95.00	80.00	100.00	10.00	3.33	4
2.5		100.00	100.00	100.00	0.00	0.00	4
5		90.00	80.00	100.00	11.55	3.78	4
10		95.00	80.00	100.00	10.00	3.33	4
20		90.00	80.00	100.00	11.55	3.78	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.30	8.30	8.30	0.00	0.00	1
2.5		8.30	8.30	8.30	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
10		8.30	8.30	8.30	0.00	0.00	1
20		8.40	8.40	8.40	0.00	0.00	1
FSW Control	DO %	110.60	110.60	110.60	0.00	0.00	1
1.3		101.10	101.10	101.10	0.00	0.00	1
2.5		101.40	101.40	101.40	0.00	0.00	1
5		101.50	101.50	101.50	0.00	0.00	1
10		101.10	101.10	101.10	0.00	0.00	1
20		101.30	101.30	101.30	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
1.3		35.50	35.50	35.50	0.00	0.00	1
2.5		35.50	35.50	35.50	0.00	0.00	1
5		35.50	35.50	35.50	0.00	0.00	1
10		35.50	35.50	35.50	0.00	0.00	1
20		35.60	35.60	35.60	0.00	0.00	1

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Statistical Printouts for the Juvenile *Melita plumulosa* Tests

Amphipod Acute Toxicity Test-96 hr survival

Start Date:	14/11/2013 12:30	Test ID:	PR1083/22	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 13:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 108	Test Species:	ML-Melita Plumulosa
Comments:					

Conc-mg/L	1	2	3	4
FSW Control	1.0000	1.0000	0.8000	1.0000
1.3	0.8000	1.0000	1.0000	1.0000
2.5	1.0000	1.0000	1.0000	1.0000
5	1.0000	0.8000	1.0000	0.8000
10	1.0000	1.0000	1.0000	1.0000
20	1.0000	1.0000	1.0000	1.0000

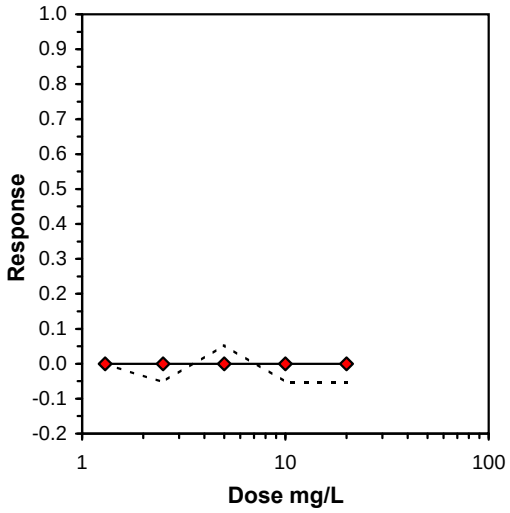
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			0.9667	1.0000
1.3	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9667	1.0000
2.5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9667	1.0000
5	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	0.9667	1.0000
10	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9667	1.0000
20	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9667	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.829814	0.916	-0.99267	0.896104
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	20	>20		
Treatments vs FSW Control				

Log-Logit Interpolation (200 Resamples)

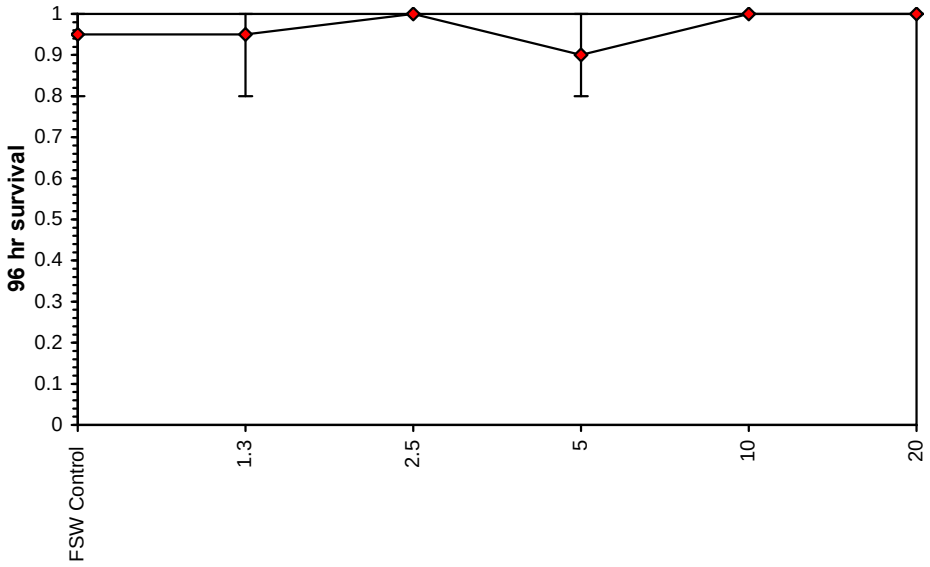
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	>20			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Amphipod Acute Toxicity Test-96 hr survival

Start Date:	14/11/2013 12:30	Test ID:	PR1083/22	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 13:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 108	Test Species:	ML-Melita Plumulosa
Comments:					

Dose-Response Plot



Amphipod Acute Toxicity Test-96 hr survival

Start Date:	14/11/2013 12:30	Test ID:	PR1083/22	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 13:00	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 108	Test Species:	ML-Melita Plumulosa
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Non-immobilised	95.00	80.00	100.00	10.00	3.33	4
1.3		95.00	80.00	100.00	10.00	3.33	4
2.5		100.00	100.00	100.00	0.00	0.00	4
5		90.00	80.00	100.00	11.55	3.78	4
10		100.00	100.00	100.00	0.00	0.00	4
20		100.00	100.00	100.00	0.00	0.00	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.30	8.30	8.30	0.00	0.00	1
2.5		8.30	8.30	8.30	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
10		8.30	8.30	8.30	0.00	0.00	1
20		8.40	8.40	8.40	0.00	0.00	1
FSW Control	DO %	110.60	110.60	110.60	0.00	0.00	1
1.3		101.10	101.10	101.10	0.00	0.00	1
2.5		101.40	101.40	101.40	0.00	0.00	1
5		101.50	101.50	101.50	0.00	0.00	1
10		101.10	101.10	101.10	0.00	0.00	1
20		101.30	101.30	101.30	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
1.3		35.50	35.50	35.50	0.00	0.00	1
2.5		35.50	35.50	35.50	0.00	0.00	1
5		35.50	35.50	35.50	0.00	0.00	1
10		35.50	35.50	35.50	0.00	0.00	1
20		35.60	35.60	35.60	0.00	0.00	1

ecotox
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Statistical Printouts for the Fish Imbalance Tests

Fish Imbalance Test-96 hr Imbalance

Start Date:	14/11/2013 15:00	Test ID:	PR1083/20	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 16:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	LT-Lates calcarifer
Comments:					

Conc-%	1	2	3	4
FSW Control	1.0000	1.0000	0.8000	1.0000
1.3	1.0000	1.0000	1.0000	1.0000
2.5	1.0000	0.8000	0.6000	1.0000
5	1.0000	1.0000	1.0000	1.0000
10	0.8000	1.0000	1.0000	0.8000
20	1.0000	1.0000	0.8000	1.0000

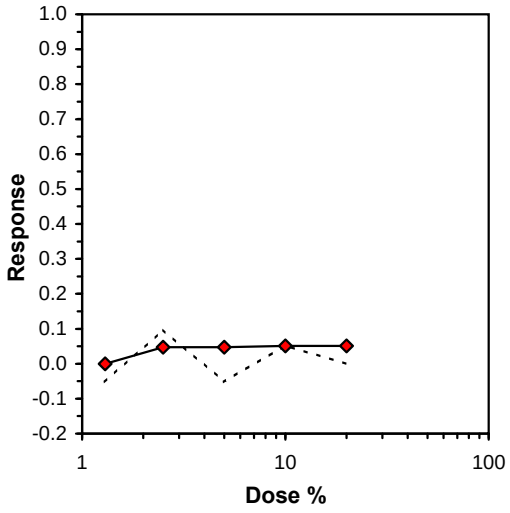
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4			0.9750	1.0000
1.3	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9750	1.0000
2.5	0.8500	0.8947	1.1759	0.8861	1.3652	19.221	4	17.00	10.00	0.9286	0.9524
5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	4	20.00	10.00	0.9286	0.9524
10	0.9000	0.9474	1.2262	1.1071	1.3453	11.212	4	16.00	10.00	0.9250	0.9487
20	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	18.00	10.00	0.9250	0.9487

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.926986	0.916	-0.75635	0.717947
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	20	>20		5
Treatments vs FSW Control				

Log-Logit Interpolation (200 Resamples)

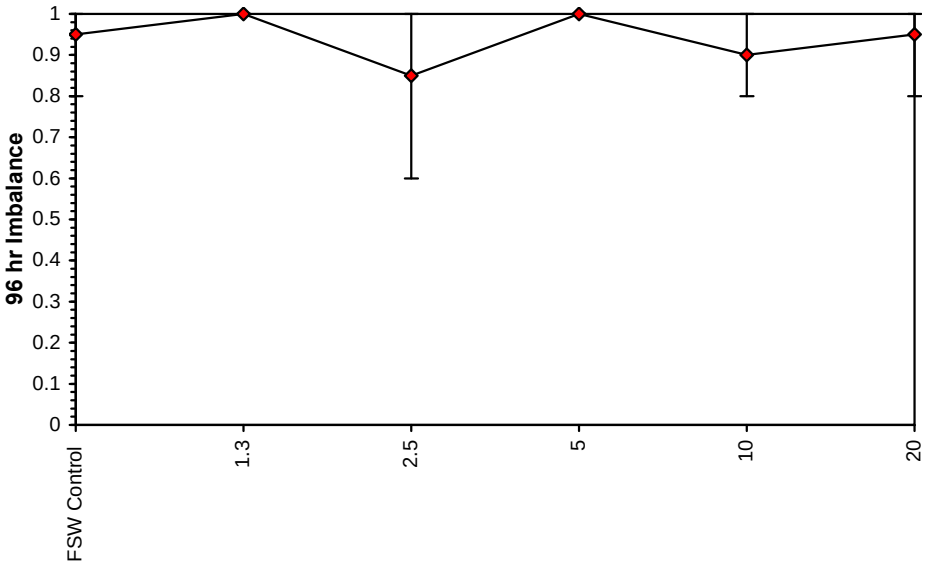
Point	%	SD	95% CL(Exp)	Skew
IC05	7.9248			
IC10	>20			
IC15	>20			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Fish Imbalance Test-96 hr Imbalance

Start Date:	14/11/2013 15:00	Test ID:	PR1083/20	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 16:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	LT-Lates calcarifer
Comments:					

Dose-Response Plot



Fish Imbalance Test-96 hr Imbalance

Start Date:	14/11/2013 15:00	Test ID:	PR1083/20	Sample ID:	Oils Spill Eater II
End Date:	18/11/2013 16:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	LT-Lates calcarifer
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Un-affected	95.00	80.00	100.00	10.00	3.33	4
1.3		100.00	100.00	100.00	0.00	0.00	4
2.5		85.00	60.00	100.00	19.15	5.15	4
5		100.00	100.00	100.00	0.00	0.00	4
10		90.00	80.00	100.00	11.55	3.78	4
20		95.00	80.00	100.00	10.00	3.33	4
FSW Control	pH	8.30	8.30	8.30	0.00	0.00	1
1.3		8.30	8.30	8.30	0.00	0.00	1
2.5		8.30	8.30	8.30	0.00	0.00	1
5		8.30	8.30	8.30	0.00	0.00	1
10		8.30	8.30	8.30	0.00	0.00	1
20		8.40	8.40	8.40	0.00	0.00	1
FSW Control	Salinity ppt	35.50	35.50	35.50	0.00	0.00	1
1.3		35.50	35.50	35.50	0.00	0.00	1
2.5		35.50	35.50	35.50	0.00	0.00	1
5		35.50	35.50	35.50	0.00	0.00	1
10		35.50	35.50	35.50	0.00	0.00	1
20		35.60	35.60	35.60	0.00	0.00	1
FSW Control	DO %	110.60	110.60	110.60	0.00	0.00	1
1.3		101.10	101.10	101.10	0.00	0.00	1
2.5		101.40	101.40	101.40	0.00	0.00	1
5		101.50	101.50	101.50	0.00	0.00	1
10		101.10	101.10	101.10	0.00	0.00	1
20		101.30	101.30	101.30	0.00	0.00	1

Fish Imbalance Test-96 hr Imbalance

Start Date:	8/11/2013 12:00	Test ID:	PR1083/21	Sample ID:	Oils Spill Eater II
End Date:	12/11/2013 10:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	MN-Macquaria novemaculeata
Comments:					

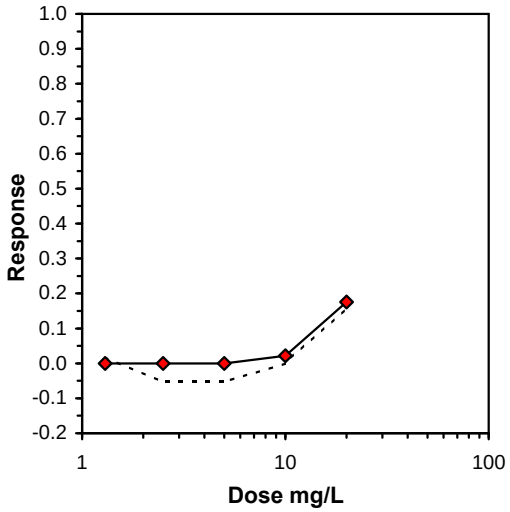
Conc-mg/L	1	2	3	4
FSW Control	1.0000	0.8000	1.0000	1.0000
1.3	0.8000	1.0000	1.0000	
2.5	1.0000	1.0000	1.0000	
5	1.0000	1.0000	1.0000	
10	1.0000	1.0000	0.8000	1.0000
20	0.8000	0.6000	1.0000	

			Transform: Arcsin Square Root					Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
FSW Control	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	0.9708	1.0000
1.3	0.9333	0.9825	1.2659	1.1071	1.3453	10.861	3	0.9708	1.0000
2.5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	3	0.9708	1.0000
5	1.0000	1.0526	1.3453	1.3453	1.3453	0.000	3	0.9708	1.0000
10	0.9500	1.0000	1.2857	1.1071	1.3453	9.261	4	0.9500	0.9785
20	0.8000	0.8421	1.1128	0.8861	1.3453	20.637	3	0.8000	0.8240

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.861842	0.905	-0.54281	0.656
Equality of variance cannot be confirmed				

Log-Logit Interpolation (200 Resamples)

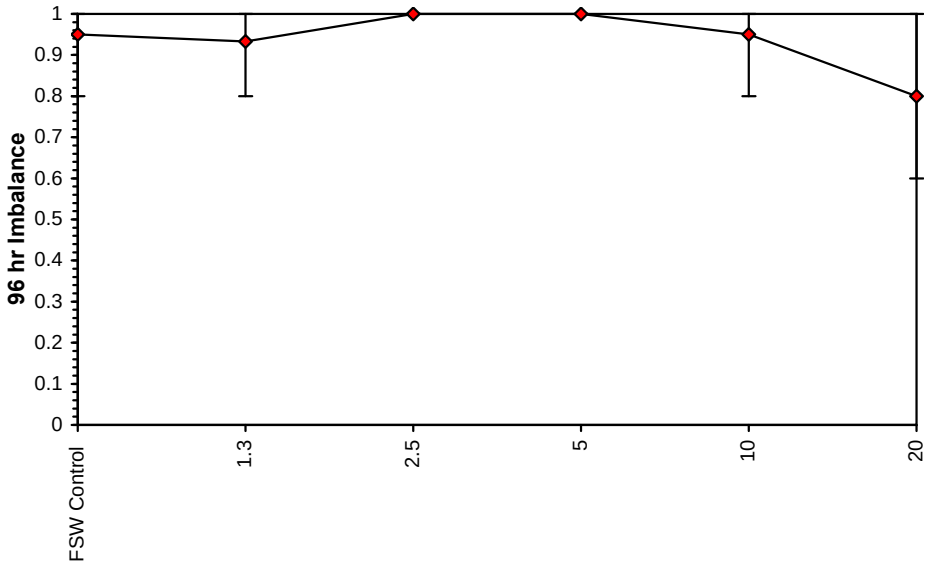
Point	mg/L	SD	95% CL(Exp)	Skew
IC05	12.372			
IC10	15.727			
IC15	18.604			
IC20	>20			
IC25	>20			
IC40	>20			
IC50	>20			



Fish Imbalance Test-96 hr Imbalance

Start Date:	8/11/2013 12:00	Test ID:	PR1083/21	Sample ID:	Oils Spill Eater II
End Date:	12/11/2013 10:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	MN-Macquaria novemaculeata
Comments:					

Dose-Response Plot



Fish Imbalance Test-96 hr Imbalance

Start Date:	8/11/2013 12:00	Test ID:	PR1083/21	Sample ID:	Oils Spill Eater II
End Date:	12/11/2013 10:30	Lab ID:	6232	Sample Type:	CP-Chemical product
Sample Date:		Protocol:	ESA 117	Test Species:	MN-Macquaria novemaculeata
Comments:					

Auxiliary Data Summary

Conc-mg/L	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Un-affected	95.00	80.00	100.00	10.00	3.33	4
1.3		93.33	80.00	100.00	11.55	3.64	3
2.5		100.00	100.00	100.00	0.00	0.00	3
5		100.00	100.00	100.00	0.00	0.00	3
10		95.00	80.00	100.00	10.00	3.33	4
20		80.00	60.00	100.00	20.00	5.59	3
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
1.3		8.20	8.20	8.20	0.00	0.00	1
2.5		8.20	8.20	8.20	0.00	0.00	1
5		8.20	8.20	8.20	0.00	0.00	1
10		8.20	8.20	8.20	0.00	0.00	1
20		8.20	8.20	8.20	0.00	0.00	1
FSW Control	Salinity ppt	35.30	35.30	35.30	0.00	0.00	1
1.3		35.50	35.50	35.50	0.00	0.00	1
2.5		35.40	35.40	35.40	0.00	0.00	1
5		35.40	35.40	35.40	0.00	0.00	1
10		35.30	35.30	35.30	0.00	0.00	1
20		35.20	35.20	35.20	0.00	0.00	1
FSW Control	DO %	98.30	98.30	98.30	0.00	0.00	1
1.3		99.60	99.60	99.60	0.00	0.00	1
2.5		99.50	99.50	99.50	0.00	0.00	1
5		99.80	99.80	99.80	0.00	0.00	1
10		100.70	100.70	100.70	0.00	0.00	1
20		101.70	101.70	101.70	0.00	0.00	1

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Norway Five Toxicity Tests

The Norwegian Institute for Water Research, performed 5 toxicity tests on OSE II, for the Norwegian EPA to understand, the non-toxic characteristics associated with the use of OSE II.

The OSEI Corporation distributor Premium Green Technologies of Norway, had 5 toxicity tests performed in order to show the Norwegian government that OSE II was safe, and non toxic to the particular species they require toxicity testing for Algae Skeletonema Pseudocstatum.

The 72 hour EC50 toxicity test showed n.d. or non detect, the EC50 95/%-CL lower test showed n.d., or non detect, the EC50 95/%-CL upper showed an n.d., or non detect, the LOEC was >100, or greater than 100, and the NOEC was _>100, or greater than or equal to 100.

These test are extremely conclusive in proving OSE II is much less toxic than the required limit of greater than >10, the tests stopped at >100 the upper testing limit for these toxicity tests. These tests are great toxicity tests, once again showing that OSE II is safe and non toxic, and in particular for use, in Norwegian waters.

The toxicity test performed was the Determination of the 72 hour toxicity of OSE II to the marine algae Skeletonema pseudocostatum. The results showed that OSE II has no detection (ND) for the three EC 50 tests showing no toxicity, while the LOEC was greater than 100 ul/l and the NOEC showed great results as well.

The full report follows this page.

Steven Pedigo

CEO/Chairman OSEI Corporation

Norwegian Institute for
Water Research

Gaustadalléen 21
0349 Oslo
Tel: 02348
Fax: 22 18 52 00

TEST REPORT

Determination of the 72 hour toxicity of OSE II to the marine algae *Skeletonema pseudocostatum*

NIVA Report number: 190167

April 2020

PREFACE

SPONSOR

Halvor Gaasrud,
Premium Green Technologies Norway AS,
Strabdveien 50 c, Postboks 415
1325 Lysaker, Norway

TESTING FACILITY

Norwegian Institute for Water Research (NIVA)
Gaustadalléen 21
0349 Oslo
Norway

PERSONNEL INVOLVED IN THE STUDY

Name and Company	Title	Function
Ana Almeida, NIVA, Gaustadalléen 21, 0349 Oslo, Norway	Research scientist	Study Director and Test personnel
Tânia Gomes, NIVA	Research scientist	Test personnel
Adam Lillicrap, NIVA	Research manager	Test Facility Manager

TIME SCHEDULE

Study initiation date: 25th February 2020
Start of test: 25th February 2020
Completion of test: 28th February 2020

REPORT APPROVED BY GLP MANAGER

GLP Manager: _____ Date: 24.04.2020

Adam Lillicrap
Research Manager
Norwegian Institute for Water Research

SUMMARY

The inhibitory effects of OSE II on the growth of the marine microalgae *Skeletonema pseudocostatum*, strain NIVA BAC 1, was investigated. The test was performed according to ISO 10253:2016, Water quality – Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum*. ISO/TC 147/SC 5 Biological methods, ICS:13.060.70, 19p (1).

A series of test solutions were prepared by dissolving different concentrations of the test substance OSE II in ISO media 10253 (1 µL/L, 3.2 µL/L, 10 µL/L, 32 µL/L and 100 µL/L), plus a control.

The test solutions were inoculated with approximately 5×10^3 cells/mL of an exponentially growing culture of *Skeletonema pseudocostatum*. Three replicates of each concentration were incubated in 25 mL glass flasks with 15 mL test volume in an incubator with orbital shaking, set to 20± 2 °C and under continuous light. Six replicate cultures in growth medium were used as controls. Growth was monitored using a coulter counter at 24, 48 and 72 hours.

The test substance showed no effects in *Skeletonema pseudocostatum* growth. The results of the study are summarised as follows:

Endpoint	Test substance OSE II (µL/L)
	72 h
EC_{50}	n.d.
EC_{50} 95%-CL lower	n.d.
EC_{50} 95%-CL upper	n.d.
LOEC	>100
NOEC	≥100

Where:

EC_{50} – effective concentration for 50% reduction
95% – CL 95 – confidence limits
LOEC – lowest observed effect concentration
NOEC – no observed effect concentration
n.d. - not determined

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Trinidad and Tobago Toxicity Test With OSE II Summary

The Trinidad and Tobago Government requires a Toxicity test from their local laboratory CARRIRI with whom the OSEI Corporation contracted the Toxicity test work to be carried out.

The 96-hour Acute (static) Toxicity test was conducted using Metamysidopsis Insularis between 1-5 days old at the test initiation. The test result was nontoxic. The US EPA set the standard for what is virtually non-toxic at 100 mg/l or greater.

This test is another example of how Non-Toxic OSE II and proves OSE II is safe for marine species.

Steven Pedigo

CEO OSEI Corporation

Caribbean Industrial Research Institute
Mailing Address: Tunapuna Post Office, Trinidad and Tobago
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www.cariri.com Email: mail@cariri.com

IAS ACCREDITED Testing Laboratory

REPORT

Attn: Mr. Griffin Pedigo

Project Code: EC03870890/23

Client: OSEI Corporation

Client Address: 1212 Delmonte Circle Plano, Texas 75075, USA

Report Title: Analysis of one (01) Oilfield Chemical Sample

Report No: 01

Project Chief: N/A

Author(s): Gail Ram Ganesh / Monalisa Cooper

Reviewed By: DocuSigned by: 5E0022423F5F470... Kern Mahabir
Technologist / Deputy Laboratory Manager

Date: 2023/09/05

Authorized By: DocuSigned by: 60A3B28236E24C4... Eka Rudder-Fairman, Mphil.
Programme Leader & Laboratory Manager

Date: 2023/09/05

Copy No: 1 of 1

Appendices: ☐

Report Version: **ORIGINAL** ☒ **RE-ISSUE** ☐ **AMENDED** ☐ ☐

Introduction

The sample was submitted by the Client to CARIRI'S Petroleum & Sustainable Energy Services Laboratory located at UWI Campus, St. Augustine for determination of the following MSDS parameters:

- Appearance / Colour
- pH
- Solubility
- Toxicity
- Biodegradability

Toxicity and Biodegradability testing were conducted by other CARIRI laboratories.

Sample Collection

The following sample was submitted by the Client on June 20, 2023:

CARIRI SAMPLE NO.	CLIENT SAMPLE ID.
E0649/23	Oil Spill Eater II, OSE II

Testing and Methodology

Appearance	-	Visual
Solubility	-	Visual
pH	-	Direct Meter Reading
Toxicity	-	CAR.CHEM.TOX.2.1[2,3]
Biodegradability	-	SMEWW 5220-COD[4] / EMICRO.TM.02[5]

Results

Date Analysed: 2023.06.23 – 2023.07.27

Analysis	Specifications	Sample # E0392/23
Appearance (Not IAS accredited)	Amber to Brown Liquid	Amber / Brown Liquid
Solubility in Water (Not IAS accredited)	100	100% soluble in water
pH (Not IAS accredited)	6.36 @ 22.3 °C	8.91
[a]Toxicity, LC_{50} value (mg/L) (Not IAS accredited)	Non-Toxic	9.29
[b]Biodegradability,%	100	100*

* Acceptable limit for Biodegradability is ≥ 70%.

[a] See Appendix A attached.

[b] See Appendix B attached.

References

[1] American Society for Testing and Materials (2018). Annual Book of ASTM Standards. American Society for Testing and Materials, Philadelphia, USA.

[2] U.S. EPA.2002.Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Ed. U.S. Environmental Protection Agency, Office of Research and Development Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. EPA/821/R-02/012

[3] U.S. EPA.1991. Trimmed Spearman-Karber (TSK) program (Version 1.5). Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 45268

[4] Organization for Economic Cooperation and Development (OECD). 1992. Guidelines for testing of chemicals-Biodegradability Test Guidelines: 301 D Closed Bottle Test

[5] Baird, R.B., Eaton A.D., Rice E.W., Bridgewater L.L., Ed. (2017) Standard Methods for the Examination of Water and Wastewater, 23rd ed. Washington, D.C. USA: American Public Health Association / American Water Works Association / Water Environment Federation: USA

END OF REPORT

APPENDIX A

Caribbean Industrial Research Institute
Mailing Address: Tunapuna Post Office, Trinidad and Tobago
Telephone: (868) 299-0210 Telefax: (868) 662-7177
www.cariri.com Email: mail@cariri.com

IAS ACCREDITED Testing Laboratory

REPORT

Attention : Mrs. Eka Rudder-Fairman

Project Code : IC03890901/23

Client : PSES

Client Address : The UWI Campus, St. Augustine

Report Title : Analysis of Chemical

Report No. : 01/01

Project Chief : N/A

Author(s) : Mrs. Nandranie Janglee

Reviewed By : DocuSigned by: L C Nicholas FC046F6C81704B7...
DR. LATISHA NICHOLAS
CHEMIST I & DEPUTY LABORATORY MANAGER
Date: 2023/07/19

Authorized By : DocuSigned by: Gaitri Jeethan BED49217C492436...
MS. GAITRI JEETHAN
CHEMIST II & LABORATORY MANAGER
Date: 2023/07/19

Copy No. : 1 of 1 **Appendices:** ☐

Report Version : **ORIGINAL** ☒ **RE-ISSUE** ☐ **AMENDED** ☐

Introduction

One (1) chemical sample was analysed for the determination of Acute Toxicity to the Mysid shrimp.

Sample Collection

The sample was submitted to the Analytical Chemistry Department on June 22, 2023.

Testing and Methodology

The 96-hour Acute (static) Toxicity test was conducted using *Metamysidopsis Insularis* between 1 to 5 days old at test initiation. Range-finder tests were conducted to determine the maximum and minimum sample concentrations for analysis. The definitive test was conducted with a minimum of five (5) sample dilutions plus a negative control at 25 ± 3 °C. Test solutions were prepared by mixing the product with dilution water (artificial saltwater, 20 ± 2 ppt). The health and sensitivity of the organism was verified using a Reference Toxicant (Potassium Chloride). The test was considered acceptable with a ≥ 90% survival of the test species in the control.

The procedure for the Toxicity test followed CAR.CHEM.TOX.2.1, which is based on "*Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*" (U.S. EPA, 2002)[1]. Toxicity is determined by calculation of an LC_{50} value with a 95% Confidence Interval using Trimmed Spearman-Karber[2] analysis. LC_{50} refers to the concentration of a test sample which results in the mortality of 50% of the test population.

Results

CARIRI Sample No.	Client Sample Description	LC_{50} value, mg/L
A1304/23	E0649/23: Oil Spill Eater II	9.29

Dates Analysed: 2023-06-26 to 2023-06-30

REFERENCES

1. U.S. EPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Ed. U.S. Environmental Protection Agency, Office of Research and Development Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. EPA/821/R-02/012.

2. U.S.EPA. 1991. Trimmed Spearman-Karber (TSK) program (Version 1.5). Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 45268.

END OF REPORT

APPENDIX B

EMICRO PROJECT NO. : IC03894694/23
REPORT NO. : 01
CLIENT ORDER/ REF NO. : EC03870890/23 – PSES – OCP [ex: *Oil Spill Eater II]*
DATE OF REPORT : August 3, 2023

RESULTS

(Apply to samples as received)

Table 1: Microbial Analysis

Laboratory Sample No.	Client Sample Label	SMEWW[1] 5220-COD	EMICRO.TM.02[2]	
		Chemical Oxygen Demand (mg/l)	**Biochemical Oxygen Demand (mg/l)**	***Biodegradability (%)** (Spec ≥70%)
V 1434/23	E 0649/23 – Oil Spill Eater II	30	44	**100.0%**
Dates of Analysis		2023.06.30	2023.06.29 to 2023.07.27	

*% Biodegradability is calculated from the ratio of Biochemical Oxygen Demand to Chemical Oxygen Demand.

REFERENCE

[1.] Baird, R.B., Eaton A.D., Rice E.W., Bridgewater L.L., Ed. (2017) *Standard Methods for the Examination of Water andWastewater, 23rd ed*. Washington, D.C. USA: American Public Health Association/ American Water Works Association/ Water Environment Federation: USA

[2.] Organization for Economic Cooperation and Development (OECD). 1992. *Guidelines for testing of chemicals – Biodegradability: 301D closed bottle test.*

DocuSigned by:
K. Dhanpat
DB5FCCA2AA064AA...

Kavir Dhanpat
Deputy Laboratory Manager
Environmental Microbiology Laboratory

DocuSigned by:
Tricia Singh
0DFD001E2CD5430...

Tricia Singh
Laboratory Manager
Environmental Microbiology Laboratory

SERVICE CONTRACT

Document #: CAR.F.15

Tunapuna Post Office
Trinidad and Tobago
Telephone: (868) 299-0210
Campus Fax: (868) 662-7177
Macoya Fax: 663-9771 ext 3160
Email: mail@cariri.com

Contract No: EC03870890/23
Version No: 1
Department: PSES
Quotation Ref. No.: PSES-Q-057-23
Client Ref. No.:

Attention: **Mr. Michael Joseph/Mr. Griffin Pedigo**
Client: **OSEI CORPORATION**
Address: **1212 DELMONTE CIRCLE PLANO, TEXAS 75075, USA**

Phone:
Fax: -
Email: hardestofmen@gmail.com

Start Date: 2023.06.20 Estimated Completion Date: 2023.08.16

Project Description and Work Required:
Analysis of one (01) Oilfield Chemical -Oil Spill Eater II, OSE II for the following parameters:
1. Appearence - Visual
2. pH - Direct Meter Reading
3. Solubility - Visual
4. Biodegradability Testing - EMICRO. TM.02[2]
5. Toxicity Testing _ CAR. CHEM. TOX.2.1

CARIRI Sample Number: E00649/23

Fee: US $888.89 VAT: $0.00 Total Fee: $888.89 Report Dispatch Method: Email

Terms of Payment: PAYMENT IN ADVANCE (Payment made in USD)

For and on behalf of Client	For and on behalf of CARIRI
---	DocuSigned by: Rudder-Fairman 60A3B38336F24C4...
Name [block letters]	EKA RUDDER-FAIRMAN, PROGRAMME LEADER
---	2023/09/05
Signature	Date
---	---
Position	
---	---
Date	Date

See Terms and Conditions on page 2

SERVICE CONTRACT

EC03870890/23

Terms and Conditions of Contract:

1) Any test or calibration report resulting from this contract relates only to the specific item(s)/sample(s) which has been collected, tested, analysed, or calibrated by CARIRI. It shall be used solely for informing the client of the results of this specific item(s)/sample(s) and not any other. Information contained herein, shall not be used for any other purposes including, but not limited to, Certification, Advertising, and Marketing

2) The report resulting from this contract may not be reproduced other than in full, except with the prior written authorization from the Executive Management of CARIRI

3) The Client shall indemnify CARIRI against all actions proceedings claims or demands in any way connected with this contract brought or threatened against CARIRI by a third party except to the extent that CARIRI is liable to the Client under this contract.

4) In any event and notwithstanding anything contained in this contract, CARIRl's liability in contract, tort (including negligence or breach of statutory duty) or otherwise arising by reason of or in connection with this contract shall be limited to the sum representing the fee payable by the Client to CARIRI under this contract.

5) CARIRI is accredited by International Accreditation Service Inc. (IAS) to ISO/IEC 17025:2017. Details on the scope of accreditation may be viewed at http://www.iasonline.org using our Accreditation #'s: TL-397 and CL-134

6) The price quoted is for 1 copy of the final report. Additional copies (including fax copies) shall incur additional charges. The contract does not include any activity outside what is disclosed in its scope.

Fresh Water Toxicity Tests

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 (Dahnia 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
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, 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

Environmental Technology Centre
URL: http://www.etc-cte.ec.gc.ca

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.

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
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Email: oseicorp@msn.com
Web: www.osei.us

Texas Plano Fresh Water Minnow Test With Cities Physical Engineer

For Storm Drain Protection

The OSEI Corporation CEO Mr. Steven Pedigo and team, performed a demonstration of how OSE II works and protects the environment, especially any oil or fuel spill where OSE II is applied to these types of materials, would break down their molecular structure, Reducing the toxicity, Eliminating Flammability, reducing Adhesion properties, and cause the oil or fuel to float on waters surface, protecting the water column where 60% of marine species live.

A fresh water test was run on fathead minnows for the physical engineer in Plano, Texas, USA. We were attempting to prove that hydrocarbons which have had OSEII applied to them and then washed in the storm drain would not add any toxicity to the storm drain.

One half gallon of gasoline was poured onto a concrete surface, where ••• 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 ••• gallons were recovered and sent to Bio-Aquatic Laboratory.

Bio-Aquatic Laboratory performed a Static 48 Definitive Toxicity Test using Fathead Minnows (*Pimphales promeas*). 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.

This helped alleviate the physical engineer's concerns for adding anything toxic to the storm drain and ultimately to a creek, river or lake. This test shows that using OSEII would help reduce the toxicity to storm drains from rain water runoff. If OSEII is used periodically to clean the parking lots allowing the sites in Plano to stay within its NPDES permitted discharge levels.

This also shows fire departments as well, who utilize OSE II for the elimination of flammability for fuels and solvents spilled, to also clean up the road area, addressing the environment, and rendering the spilled material non toxic, even when washed into storm drains.

This test also shows that if OSE II is used to clean up a parking lot or emergency spill, and washed into the storm drain there would be no adverse environmental impact.

Steven Pedigo

CEO OSEI Corporation

See Minnow test starting on the next page.

OSEI CORPORATION OSE II/GASOLINE/WATER *Toxicity Test Report*

DECEMBER 7, 1991

BIO-AQUATIC TESTING, INC.

Prepared by: _____ David Smith,
Aquatic Toxicologist125

BIO-AQUATIC TESTING, INC.

1555 Valwood Parkway, Ste. 100 Carrollton, Texas 75006
Tel: (214) 247-5928
Fax: (214) 241-4474 **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

CHEMICAL MEASUREMENTS TEST PROCEDURES

Pimephales promelas

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*).

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.

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.126

LC50 RESULTS

Pimephales promelas

SUMMARY

LC50 value calculated using the Binomial Method: CONC. (mg/L) # EXPOSED # DEAD % DEAD BINOMIAL %

30,000 9,000 3,000 800 200

20

6

1

0

0

100

30

5
0

0.0001
5.7659
0.0020
0.0001
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-Karber Method:
Trim Var. of Ln Est. LC50 95% Conf. Limits
0.00% 0.17396D-01 9,300 mg/L 7,100 to 12,100 mg/L
The 48-hour LC50 for *Pimephales promela*s exposed to a mixture of OSE II, gasoline, and water was 9,300 mg/L.

BIO-AQUATIC TESTING, INC.

48 – HOUR *PIMEPHALES PROMELAS* 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 *Pimephales promelas* DATE COLLECTED 12/05/91 TEST TEMPERATURE ($_{o}$C) 25$_{o}$ ± 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

EFFLUENT MEASUREMENTS

D.O. @ 30,000 mg/L$_{1}$ 8.6/6.6
pH @ 30,000$_{1}$ 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 Cl_2 (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 ' Recorded at the beginning and end of each 24-hour exposure period.

x % Surv.

SURVIVAL SUMMARY

x LIVE PER CONC

100
100
100
95
70

0
%

EFFLUENT CONC Control 200 mg/L 800 mg/L

3,000 mg/L

9,000 mg/L
30,000 mg/L NUMBER LIVE PER REP START 24 HOURS 48 HOURS

abcdabcdabcd

555555555555

555555555555

555555555555

555555555455

555533553155

5 5 5 5 0 0 0 0 0 0 0 0128

P.O. Box 515429
Dallas, Texas 75251
Ph: (972) 669-3390
Email oseicorp@msn.com
Web www.osei.us

Date June 30, 2008

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

Prepared for:

Kwang Keun, Kim
Korea Institute of Construction anticorrosive Technology
95-6 Munjung-dong, Songpa-Ku
Seoul, Korea 138-869
Tel: 02-3401-8388
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Prepared by:

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TABLE OF CONTENTS

ACUTE LC50 PRODUCT REPORT

Client . . . . . OSEI, Corporation
Sample . . . . . .Oil Spill Eater II
Project No. OS457
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 Huther 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 $CaCO_3$. 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^{0}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-Karber 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-Karber 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.

Huther and Associates, Inc.

environmental toxicologists, biologists, consultants

24-HOUR *PIMEPHALES PROMELAS* SURVIVAL

CLIENT: OSE - 2%

PROJECT #: 05457

CONC.	NUMBER ORGANISMS, 0 HRS A	NUMBER ORGANISMS, 0 HRS B	NUMBER ORGANISMS, 24 HRS A	NUMBER ORGANISMS, 24 HRS B
Con	10	10	10	10
250 mg/L	10	10	10	10
500	10	10	10	10
1000	10	10	10	10
2000	10	10	10	10
4000	10	10	10	10
8000	10	10	10	10
16,000	10	10	10	10
DATE/TIME	MM		MM	
TECHNICIAN	6/28/08 1430		6/29/08 1430	

1156 North Bonnie Brae Denton, Texas 76201 Ph: (940) 387-1026 Fax: (940) 387-1036

OIL SPILL EATER

Test @ 2%

CHEMISTRY MEASUREMENTS

Date	Client	Sample #	ST pH 24hr	ST DO 24hr	Hardness	Alkalinity	Cond.*	~~Ammonia~~	Salinity	Analyst
6/28/05										
			ST / 24hr	ST / 24hr				pH @ 96hr	D.O @ 96hr	
	LAB CONTROL		7.84 / 8.60	8.23 / 7.43			390	7.91	7.92	
	250 mg/L		7.86 / 8.59	8.22 / 7.38			389	7.94	8.14	
	500		7.85 / 8.52	8.20 / 7.44			387	7.95	8.27	
	1000		7.85 / 8.48	8.15 / 7.38			387	7.94	8.26	
	2000		7.86 / 8.47	8.10 / 7.53			388	7.93	8.20	
	4000		7.86 / 8.42	8.15 / 7.28			390	7.92	8.21	
	8000		7.85 / 8.31	8.05 / 7.31			393	7.92	8.05	
	16,000		7.81 / 8.14	7.87 / 7.12			399	7.94	8.03	
	LAB WATER CONTROL		7.84		104	64				

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

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
******* 16000.00 BK	20	20

SPEARMAN-KARBER TRIM: .00%

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

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Date June 30, 2008

Toxicity Test Summary for a Ceridaphnia Dubia
Fresh Water Flea

The OSEI Corporation performed a toxicity test for a land, water, and airborn 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:

Oil Spill Eater International, Corporation
13127 Chandler Drive
Dallas, Texas 75243
Tel: 972-669-3390

Prepared by: _____
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huther@flash.net

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ACUTE LC50 PRODUCT REPORT

Client . . . . . OSEI, Corporation
Sample . . . . . 2% Oil Spill Eater II
Project No. OS457
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 Huther 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 $CaCO_3$. 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-Karber 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-Karber Trim:	0.00%
Estimated LC50 (mg/L):	**>16,000.00**
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.

Huther and Associates, Inc.

environmental toxicologists, biologists, consultants

24-HOUR *CERIODAPHNIA DUBIA* SURVIVAL

CLIENT: OSE 2%

PROJECT #: 05457

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			

1445 MacArthur Blvd. Ste. 216 Carrollton, Texas 75007 (972) 242-6844

OIL SPILL EATER

Test @ 2%

CHEMISTRY MEASUREMENTS

Date	Client	Sample #	ST pH 24hr		ST DO 24hr		Hardness	Alkalinity	Cond.	~~Ammonia~~	Salinity	Analyst
6/28/05												
			ST	24hr	ST	24hr				pH @ 96hr	D.O @ 96hr	
	LAB CONTROL		7.84	8.60	8.23	7.43			390	7.91	7.92	
	250 mg/L		7.86	8.59	8.22	7.38			389	7.94	8.14	
	500		7.85	8.52	8.20	7.44			387	7.95	8.27	
	1000		7.85	8.48	8.15	7.38			387	7.94	8.26	
	2000		7.86	8.42	8.10	7.53			388	7.93	8.20	
	4000		7.86	8.42	8.15	7.28			390	7.92	8.21	
	8000		7.85	8.31	8.05	7.31			393	7.92	8.05	
	16,000		7.81	8.14	7.87	7.12			399	7.94	8.03	
	LAB WATER CONTROL		7.84				104	64				

ACUTE REFERENCE TOXICANT TEST RESULTS

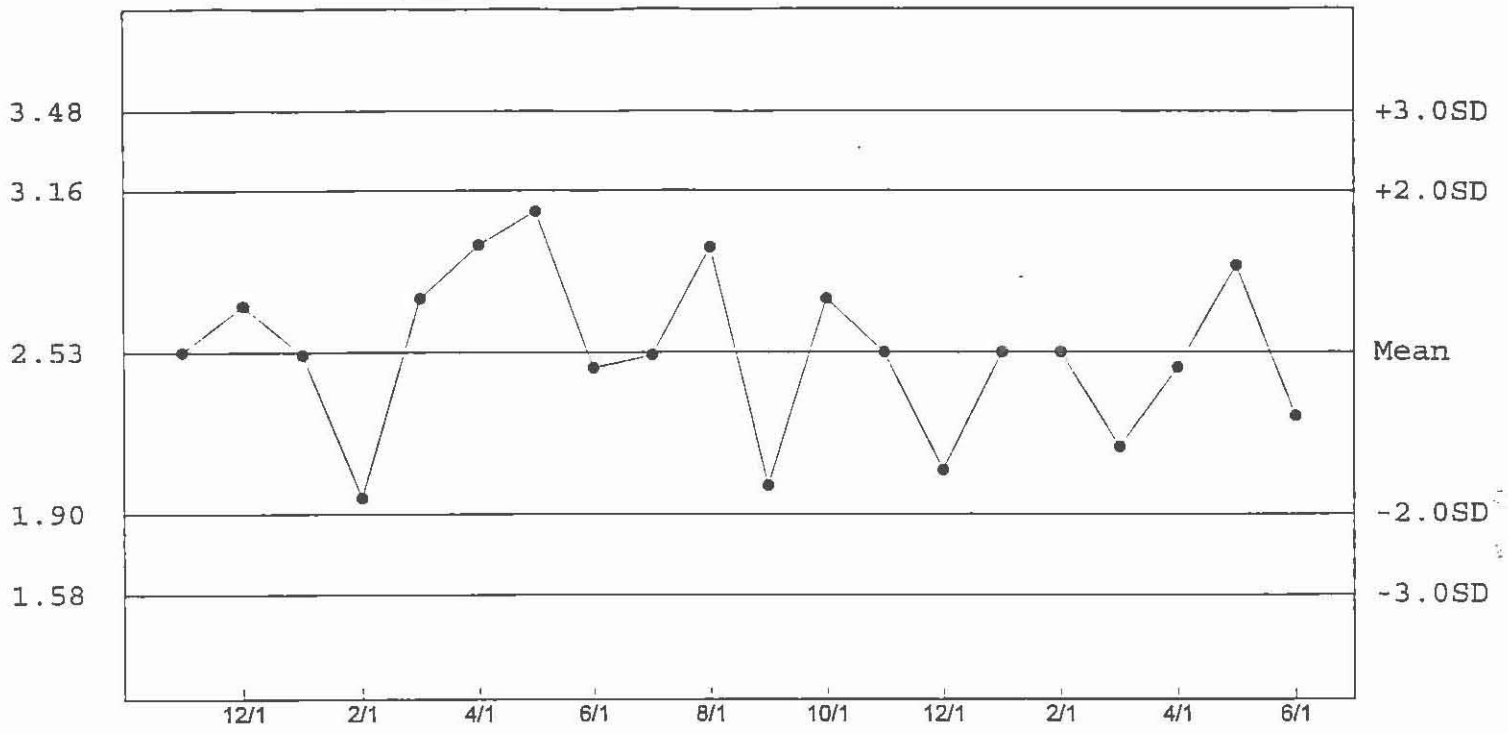
SPECIES:	*Ceriodaphnia dubia*
CHEMICAL:	Sodium Chloride
DURATION:	48-Hours
TEST NUMBER:	6
TEST DATE:	June 2008
STATISTICAL METHOD:	Spearman-Karber

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