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
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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

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October, 1990

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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).