OIL SPILL EATER INTERNATIONAL, CORP.

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MARINE TOXICITY TEST SUMMARY

OSEI Corporation, in its attempt to prove ***"Oil Spill Eater II"*** is virtually non-toxic, had the following tests performed:

The **MYSIDOPSIS BAHIA (or Mysid)** is one of the more sensitive marine organisms found in the oceans. LC50's (Lethal Concentration) is the level in which there is mortality with 50% of the species being tested. The lethal concentration calculated for OSEII on the Mysid was calculated once 10% of the test species showed equilibrium problems or mortality. At 96 hours, only 10% of the test species showed equilibrium problems or mortality at a calculated level of 2100 mg/L or 2,100 parts per million. This shows OSEII to have a low toxicity level, and had a true LC50 been performed the toxicity level would have been even lower.

The **MUMMICHOG (Fundulus Heteroclitus)** a somewhat larger organism (1 to 1.5 inches long) was tested to see how toxic OSEII was to it. 5,258 mg/L was established. 5,285 parts per million shows a very little toxicity for the Mummichog when exposed to Oil Spill Eater II.

MEDIAN LETHAL CONCENTRATIONS (LG50's) were calculated on Artemia Salina. The tests were run for 48 hours. OSEII alone tested greater than 100 mg/L so the true LG50 was not determined, but OSEII toxicity was greater than the EPA's cut-off for approving a product for the National Contingency Plan. There were other interesting facts involved with this toxicity test. The test calculation was based on using our product at a stronger concentration than our instructions allow. So at our instructed use rate, the toxicity level would have been even lower, even though the test was based on 100 mg/L or greater value. No. 2 fuel oil was tested alone and showed a level of 12.6 mg/L at 48 hours and No. 2 fuel oil and OSEII together at 48 hours showed a level of 29.4 helping prove our point that once OSEII is applied, it immediately starts detoxifying hydrocarbons so bacteria can devour the hydrocarbons. (It is more beneficial to the environment to apply OSEII immediately, than to wait around for evaporation or to try to pick up the hydrocarbons mechanically.)

OSEI Corporation feels the toxicity tests run in conjunction with OSEII help prove OSEII is virtually non-toxic. The EPA established that 35 mg/L LC50 was acceptable for a particular product to be used on the Exxon Valdez spill. If you compare OSEII to this established toxicity of 35 mg/L, then OSEII is far less toxic than that.

OSEI Corporation had two (2) fresh water toxicity tests run also. Environmental Canada, the U.S. EPA's equivalent in Canada, performed a toxicity test on rainbow trout. Rainbow trout are very sensitive fresh water species. The LC50 was greater than 10,000 mg/L. This shows OSEII to have virtually no toxicity in fresh water as well as salt water.

The other 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.

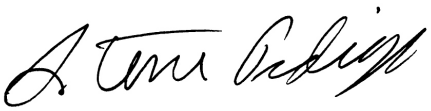
Two gallons of gasoline was poured onto a low area in a commercial business parking lot, and OSEII was applied, allowed to set 3 minutes, and then washed to another low area for collection.

Approximately 1 ½ gallons of runoff was collected and taken to the lab where a 48 hour fathead minnow survival test was initiated. The resulting LC50 test was 9,300 mg/L which shows that gasoline which has had OSEII applied to it is rendered virtually non-toxic.

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 lot allowing the site to stay within its NPDES permitted discharge levels.

Sincerely,



Steven Pedigo
Chairman

SP/eem

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SUMMARY

EPA/NETAC TOXICITY TEST

MYSIDOPSIS BAHIA

The Environmental Protection Agency in Gulf Breeze, Florida tested OIL SPILL EATER II Concentrate, for toxicity using a sensitive species named "Mysidopsis Bahia". This test was in conjunction with Efficacy Tests performed by the EPA and NETAC.

The LC50 for the acute (96 hr.) test was greater than 1,900 and up to 10,000 mg/L which shows OSE II to be virtually non-toxic.

The EPA allowed the use of Inipol during the Valdez Spill and Inipol's LC50 was 135 mg/L which would seem to OSEI, Corp to be somewhat toxic considering Environmental Canada's cut off is 1,000 mg/L.

A second LC50 was performed at 7 days to see if there was any problem with chronic toxicity. The LC50 was 2,500 mg/L, which once again shows OSE II to be virtually non-toxic even when the species was exposed in a closed environment for 7 days. It would be extremely difficult for a species to be exposed to OSE II for 7 days in an open system due to currents, wind and tidal actions.

This 3rd party, U.S. EPA Toxicity Test absolutely proves OSE II is virtually non-toxic.

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

SRP/AJL

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).

TIER II TOXICITY DATA

TABLE 1

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

Product	Max. Test Concentration (mg/L)	96 hour LC50 (mg/L)	Confidence Interval (95%)
Oil Spill Eater II	10,000	1,000-10,000[a] >1,900[b]	ND

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***

Product	Endpoints (mg/L)		Effects Measurement	7 Day LC50 (mg/L) (95% CI)
	NOEC	LOEC		
Oil Spill Eater II	1,900 1,900 633	5,700 NE 1,900	Survival Growth Fecundity	2,500(mg/L) (2,225-3,313)

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.

NETAC

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	0
1	1	10	10	9	9	9	0	0	0	0	0
10	1	10	10	9	9	9	0	0	0	0	0
100	1	10	10	10	9	9	0	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	-	-	-	-

Acute, Definitive Toxicity Tests of the Material
OSE II
to the Mummichog (Fundulus heteroclitus)

Toxicity Test Report

Submitted by:

Biomonitoring Services Laboratory
6600 East Bay Boulevard
Gulf Breeze, Florida 32561
(904) 932-2717

Project Number: 52-01-AA467-AA469
Report Number: 52-AA467-469-1-BSL-8-90

August 1990

Toxicity Test Summary Sheet

Client Contact: Carol Wilson

Report Date & Number: August 1990, 52-AA467-469-1-BSL-8-90

Project Number: 52-01-AA467-AA469

Study Director: Dan Johnson/Jamie McKee

Test Material: OSE II alone, #2 Fuel alone, OSE II #2 Fuel Oil

Description: Brownish, odorless liquid

Dates Materials Received: June 1990

Dates of Definitive Test: 28 June through 2 July 1990

Test Conditions: Static, aerated, 96-hour duration

Test Procedure: U.S. Environmental Protection Agency. 1989. National Oil and Hazardous Substance Pollution Contingency Plan; Final Rule. Federal Register, 40CFR Part 300, July 18, pp 29192-29207.

Test Animals: Mummichog (Fundulus heteroclitus); Lot number: FH-90-2

Source: Commercial aquaculture supply company
Size: 1 – 1.5 inches

Dilution/Control Water: Artificial seawater at 20 parts per thousand salinity.

Test Concentrations: 500, 1,000, 2,000, 4,000 and 8,000 parts per million OSE II.

Effect Criterion: Mortality

96-Hour LC50 for OSE II: 5,258.09 parts per million with a 95% confidence interval of 4,000.0 – 8,000.0 per million.

96-Hour LC50 for #2 Fuel Oil: 320.03 parts per million with a 95% confidence interval of 129.19 – 644.39 parts per million.

96-Hour LC50 for OSE II #2 Fuel Oil: 125.00 parts per million with a 95% confidence interval of 0.0 – infinity per million.

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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 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 the oil in some organisms. The fuel had a higher toxicity rate than did the fuel and OSE II.

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.

SRP/AJL

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

Authors

Timothy J. Ward
Robert L. Boeri

Performing Laboratory

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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 - >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 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

Nominal Concentration (mg/L)	rep.	Number Alive								
		Dispersant			No. 2 fuel oil			Oil + Dispersant		
		0hr	24hr	48hr	0hr	24hr	48hr	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

Table 2. Medial lethal concentrations (LC50s) from toxicity tests

Test substance	Time	LC50	95 percent confidence limits	Calculation method
Dispersant	24 hour	> 100 mg/L	--	--
	48 hour	> 100 mg/L	--	--
No. 2 fuel oil	24 hour	> 100 mg/L	--	--
	48 hour	12.6 mg/L	10 – 25 mg/L	Binomial
Dispersant and	24 hour	> 100 mg/L	--	--
No. 2 fuel oil	48 hour	29.4 mg/L	25 – 40 mg/L	Binomial

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 and No. 2 fuel oil to *Artemia salina*, is described in this report. The test was conducted for corp for 48 hours during October 3 to 5, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire.

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

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