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November 3, 2006

Document Control Office (DCO) (7407M)
Office of Pollution Prevention and Toxics (OPPT)
Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460-0001
Attn: 8(d) Health and Safety Reporting Rule (Notification/Reporting)

MR 299949

CONTAIN NO CBI

Re: TSCA 8(d) Submission of Health and Safety Data on CAS# 61788-76-9 Alkanes, chloro (OS#26198H, OS#87319, and OS#26198B)

Dear Sir or Madam:

The Lubrizol Corporation (Lubrizol) is submitting health and safety data on CAS# 61788-76-9 Alkanes, chloro (OS#26198H, OS#87319, and OS#26198B) as follows:

- Static acute toxicity of lubricant additive OS#26198H to the trout, *Oncorhynchus mykiss*
- Acute toxicity of lubricant additive OS#87319 to the Mysid, *Mysidopsis bahia*
- A 96-hour static renewal acute toxicity test with the sheepshead minnow (*Cyprinodon variegates*)
- OS#26198B Oral LD₅₀, skin irritation, eye irritation

A robust summary of the data in IUCLID format also is attached.

Lubrizol does not manufacture or import this chemical. However, because it is possible that we may have imported this in the past, we are submitting this information to EPA under TSCA 8(d).

This submission does not contain any confidential business information.

If you have any questions, please contact Dr. Robert K. Hinderer at 216-447-5181 or robert.hinderer@noveon.com.

Sincerely,



Al Brightwell
Vice President Environmental, Health, Safety & Product Regulatory Compliance

cc: Robert K. Hinderer, Ph.D.

Attachments (5)



Study Title

**STATIC ACUTE TOXICITY
OF LUBRICANT ADDITIVE OS#26198H
TO THE TROUT, *Oncorhynchus mykiss*
Oil-Water Dispersion Study**

Sponsor

**Lubrizol Corporation
29400 Lakeland Boulevard
Wickliffe, Ohio 44092**

Testing Facility

**EnviroSystems, Incorporated
P.O. Box 778
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Hampton, New Hampshire 03842**

Author

Linnea A. Hawthaway

**Study Completed
June 12, 1997**

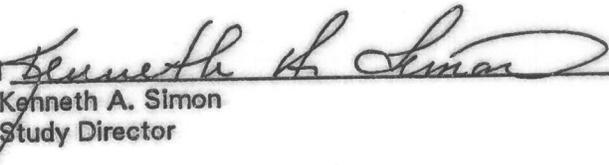
File Reference: LUB5401-96-06

**Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.**

1.0 GOOD LABORATORY PRACTICE STATEMENT

This study was conducted in the spirit of Good Laboratory Practices as defined in 40 CFR part 792. A single protocol deviation was noted during the assay. The dilution water used in the test had a hardness of 204 mg/L CaCO₃ which was outside of the protocol range of 165 - 185 mg/L CaCO₃. It is the opinion of the study director that the deviation did not have an impact on the outcome of the assay. Neither the Study Director nor the sponsor are aware of any other circumstances that would affect the integrity of this study.

EnviroSystems, Incorporated


Kenneth A. Simon
Study Director

6/12/97
Date

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

2.0 TABLE OF CONTENTS

SECTION:	PAGE
1. Good Laboratory Practice Statement	2
2. Table of Contents	3
3. Index of Tables and Figures	4
4. Introduction and Summary	5
5. Methods and Materials	5
6. Results	7
7. References	8
APPENDIX:	
Appendix A. Study Protocol	13
Appendix A. Raw Data	21
Appendix B. Support Data	40

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

3.0 INDEX OF TABLES AND FIGURES

	PAGE
Table 1. Median Lethal Concentration (LC-50), Median Effect Concentration (EC-50), and No Observed Effect Concentration (NOEC) From Acute Range Finding Toxicity Test With The Trout, <i>Oncorhynchus mykiss</i> , Exposed To Experimental Product OS#26198H.	9
Table 2. Survival and Sublethal Effect Data From Acute Range Finding Toxicity Test With The Trout, <i>Oncorhynchus mykiss</i> , Exposed To Experimental Product OS#26198H.	9
Table 3. Summary of Temperature, Dissolved Oxygen, pH, and Specific Conductance Values From Acute Range Finding Toxicity Test With The Trout, <i>Oncorhynchus mykiss</i> , Exposed To Experimental Product OS#26198H.	10
Table 4. Summary of Hardness, Alkalinity, Total Organic Carbon, and Total Solids Data From Acute Range Finding Toxicity Test With The Trout, <i>Oncorhynchus mykiss</i> , Exposed To Experimental Product OS#26198H.	11
Figure 1. Summary of Survival Data After 96-Hours Exposure. Acute Range Finding Toxicity Test With The Trout, <i>Oncorhynchus mykiss</i> , Exposed To Experimental Product OS#26198H.	12

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
 EnviroSystems, Inc. Study Number 5401.

**STATIC ACUTE TOXICITY
OF LUBRICANT ADDITIVE OS#26198H
TO THE TROUT, *Oncorhynchus mykiss*
Oil-Water Dispersion Study**

4.0 INTRODUCTION AND SUMMARY

The acute toxicity of OS#26198H to the trout, *Oncorhynchus mykiss*, is described in this final report. This 96 hour range assay was conducted for Lubrizol Corporation from November 27 through December 1, 1996 at EnviroSystems, Incorporated (ESI) in Hampton, New Hampshire. The assay was conducted by Joel Bedard, Nicole Rocheleau and Linnea Hawthaway according to EnviroSystems, Incorporated Protocol Number LUT-960030.

The test was performed under static conditions using an oil-water dispersion method. Five (5) predetermined concentrations of test material and a dilution water control were evaluated at a temperature of $15 \pm 1^\circ\text{C}$. The dilution water was reconstituted fresh water prepared according to protocol established by the EPA (1993). The water was prepared as hard water having a hardness of 165-185 mg/L CaCO_3 . Target concentrations of the test substance were 0.0 mg/L (control), 0.33 mg/L, 3.30 mg/L, 33.0 mg/L, 333.0 mg/L, and 3330.0 mg/L. Actual concentrations were 0.0 mg/L (control), 0.37 mg/L, 3.37 mg/L, 32.97 mg/L, 330.02 mg/L, and 3299.99 mg/L. Actual concentrations were used for all calculations.

Exposure of test organisms to the test substance resulted in a 96 hour LC-50 of > 3299.99 mg/L. The NOEC of test substance OS#26198H was 3299.99 mg/L.

5.0 METHODS AND MATERIALS

5.1 Test Substance

OS#26198H (ESI Sample Number 5401) was delivered to ESI on June 21, 1996. It was contained in a 250 mL Nalgene® bottle labelled with the following information: "Experimental Product, OS#26198H, Order: 160544-013, Jun. 17, 1996." The test substance (light colored liquid) was shipped from Lubrizol Corporation, 29400 Lakeland Boulevard, Wickliffe, Ohio. The test material was stored in a dark locker at room temperature.

5.2 Dilution Water

Water, used for acclimation of test organisms and all toxicity testing, was reconstituted hard water prepared at ESI according to EPA specifications (EPA 1993). The water was prepared in a 200 gallon batch, stored in a 300 gallon polyethylene tank, and aerated. Dilution water was added to the aquaria 24 hours prior to the start of the assay to allow for temperature acclimation.

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

5.3 Test Organisms

Oncorhynchus mykiss used as test organisms were from a single source and were identified using an appropriate taxonomic key (Eddy 1969). The fish were obtained from cultures at Aquatic Research Organisms (ARO) of Hampton, New Hampshire. The trout were not treated for disease and were apparently free of sickness, injuries, and abnormalities when obtained. The trout were acclimated to the reconstituted hard water for a minimum of 14 days prior to testing. During this period the fish were maintained in a static renewal culture system. Water in the system was the same type of water (hard reconstituted) as used in the assay. The fish were fed commercial flake food once per day up to the start of the test. Fish used in the test ranged in length from 25.1 to 28.2 mm with a mean length of 23.8 mm. Wet weight ranged from 0.0630 to 0.1361 grams with a mean weight of 0.1007 grams. The loading rate was determined to be 0.07 mg/L.

5.4 Toxicity Testing

The test was conducted as a screening assay. Each treatment utilized a single replicate with 10 organisms. The assay was conducted from November 27 through December 1, 1996. Target test concentrations were 0.0 mg/L, 0.33 mg/L, 3.30 mg/L, 33.0 mg/L, 330.0 mg/L, and 3300.0 mg/L. Actual nominal test concentrations were 0.0 mg/L, 0.37 mg/L, 3.37 mg/L, 32.97 mg/L, 330.02 mg/L, and 3299.99 mg/L.

Ten test organisms were arbitrarily and equally distributed among each of the five test treatments plus diluent control. The test was performed in 5 gallon aquaria, each containing 15 L of test solution. Each test vessel was equipped with a glass cylinder, 8 cm in diameter, with openings just below the water surface and approximately 2 cm above the bottom. A 3-blade plastic propeller was suspended inside the cylinders and rotated at approximately 1000 rpm to mix the test substance and dilution water. Test material was weighed out on polyethylene weigh boats. The product and weigh boats were then placed inside the glass cylinders. The photoperiod was automatically controlled and set to 16 hours light and 8 hours dark. Cool-white fluorescent lights provided a light intensity of 60 foot-candles. There was no renewal of the test solution during the exposure period.

The number of surviving organisms and the occurrence of sublethal effects (immobilization) were determined visually and recorded initially and after 24, 48, 72, and 96 hours exposure. Dissolved oxygen (YSI Model 57 dissolved oxygen meter; instrument number ESI #19), pH (Accumet model 50 pH meter; instrument number ESI #7), specific conductance (YSI Model 35 specific conductance meter; instrument number ESI #1), and temperature (ASTM mercury thermometer; thermometer number 2829) were measured and recorded in each test chamber at 24 hour intervals. Temperature was also monitored on an hourly basis in the control treatment using an electronic data logger (Grant Squirrel Logger; instrument number ESI #15). Hardness, alkalinity, and solids (APHA 1992) were measured in the dilution water at the start of the assay. Hardness and alkalinity were also measured in all treatments at the start of the assay. Total organic carbon was measured in each treatment after 24 hours exposure and at the test initiation wherever there was total mortality within the first few hours (APHA 1992).

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

5.5 Statistical Analysis

Results of the toxicity test were interpreted, when appropriate, using standard statistical techniques. LC-50 and EC-50 values were computed using a program developed by EPA (Stephen 1983) which computes LC-50 and EC-50 values and their confidence limits using the probit, binomial, and Spearman-Kärber methods. The No Observed Effect Concentration (NOEC) is the highest concentration of test substance that allows at least 90% survival of exposed organisms and does not cause sublethal effects.

6.0 RESULTS

Table 1 provides a summary of LC-50, EC-50, and NOEC values developed from the assay. Survival and sublethal effects data are presented in Table 2. Table 3 provides a summary of general water quality data: temperature, dissolved oxygen, pH, and specific conductance collected during the assay. Table 4 provides data on hardness, alkalinity, solids, and total organic carbon levels. Figure 1 provides a graphic summary of survival after 96 hours exposure to the test substance.

Review of solubility observations showed varying levels of product remaining on the water surface and in the water column. Throughout the assay no product was observed in the 0.37 and 3.37 mg/L concentrations. During the test a slight film was observed on the water surface of the 32.97 mg/L concentration. A slight film was observed on the water surface as well as some white globules on the bottom of the tank with the 330.02 mg/L concentration. Throughout the test an oily film was observed on the water surface as well as white to clear globules covering the bottom of the tank in the 3299.99 mg/L treatment.

Survival in the diluent control was 100% after 96 hours exposure, with no signs of sublethal effects indicating stress. Based on this finding, the test organisms were considered healthy and had no adverse impact on the outcome of the test. In addition, the dilution water was determined to have no adverse impact on survival.

Data obtained from the hourly temperature measurements showed that the mean temperature was 15.2°C. Minimum and maximum values were 14.8°C and 15.6°C, respectively. The standard deviation was 0.23°C. Daily mercury thermometer readings ranged from 13.8 to 15.6°C in the control and from 13.6 to 15.8°C in the test treatments. Dissolved oxygen levels in the control treatment ranged from 96% saturation to 100% saturation. Dissolved oxygen levels in treatments receiving OS#26198H ranged from 95% saturation to 100% saturation. Measurement of pH showed values ranging from 7.84 to 8.20 SU in the control and 7.92 to 8.24 SU in the treatments receiving test product, with no observable pattern in the pH values as product concentration increased or over exposure time. Specific conductance ranged from 495 to 540 μ mhos/cm in the control. Specific conductance in the treatments ranged from 492 to 538 μ mhos/cm. There was an increase in specific conductance values over exposure time. Analysis of water samples for total organic carbon (TOC) levels showed the dilution water contained 0.50 mg/L organic carbon. TOC levels in the test treatments range from <0.25 mg/L organic carbon in 0.37 mg/L test treatment to 6.66 mg/L organic carbon in the 3299.99 mg/L test treatment.

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

At the start of the assay, hardness in the control was 204 mg/L CaCO₃. Hardness in the test treatments ranged from 184 mg/L CaCO₃ in the 330.02 mg/L test treatment to 196 mg/L CaCO₃ in the 32.97 mg/L test treatment. Initial alkalinity in the control was 123 mg/L (as CaCO₃), and range from 98 mg/L (as CaCO₃) in the 0.37 mg/L test treatment to 102 mg/L (as CaCO₃) in the 330.02 mg/L test treatment. Total solids and total suspended solids were measured in the control water at the beginning of the assay resulting in 392 mg/L and <10 mg/L, respectively.

Review of data collected after 24, 48, 72 and 96 hours exposure showed 100% survival in all the test concentrations. Based on these data, the 24, 48, 72 and 96 hour LC-50 and EC-50 values were determined to be >3299.99 mg/L with an associated NOEC of 3299.99 mg/L. No sublethal effects were observed during the 96 hour test period.

7.0 REFERENCES

- APHA. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th edition. Washington D.C.
- Eddy, S. 1969. *The Freshwater Fishes*. Second Edition. Wm.C. Brown Company Publishers. Dubuque Iowa.
- Stephen, C.E. 1983. Computer Program for Calculation of LC-50 Values. U.S. EPA. Duluth, MN. Personal Communication.
- OECD. 1984. OECD Guidelines for Testing of Chemicals. Method 203, Fish Acute Toxicity Test.
- U.S. EPA. 1986. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Federal Register, Monday, January 6, 1986.
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- U.S. EPA. 1989. 40 CFR Part 792. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Final Rule. Federal Register, Thursday, August 17, 1989.
- U.S. EPA. 1993. *Methods for Measuring the Acute Toxicity of Effluent to Freshwater and Marine Organisms*. Fourth Edition. EPA-600/4-90/027.

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*. EnviroSystems, Inc. Study Number 5401.

Table 1. Median Lethal Concentration (LC-50), Median Effect Concentration (EC-50), and No Observed Effect Concentration (NOEC) From Acute Range Finding Toxicity Test With The Trout, *Oncorhynchus mykiss*, Exposed To Experimental Product OS#26198H.

Exposure Period	LC-50	EC-50	NOEC
24, 48, 72 & 96 Hours	> 3299.99 mg/L	> 3299.99 mg/L	3299.99 mg/L

Table 2. Survival and Sublethal Effect Data From Acute Range Finding Toxicity Test With The Trout, *Oncorhynchus mykiss*, Exposed To Experimental Product OS#26198H.

Affected Target Concentration of Test Substance	Rep	Number of Survivors								Number
		0 Hr	24 Hr	48 Hr	72 Hr	96 Hr	24 Hr	48 Hr	72 Hr	
0.00 mg/L	A	10	10	10	10	10	0	0	0	0
0.33 mg/L	A	10	10	10	10	10	0	0	0	0
3.30 mg/L	A	10	10	10	10	10	0	0	0	0
33.00 mg/L	A	10	10	10	10	10	0	0	0	0
330.00 mg/L	A	10	10	10	10	10	0	0	0	0
3300.00 mg/L	A	10	10	10	10	10	0	0	0	0

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

Table 3. Summary of Temperature, Dissolved Oxygen, pH, and Specific Conductance Values From Acute Range Finding Toxicity Test With The Trout, *Oncorhynchus mykiss*, Exposed To Experimental Product OS#26198H.

Target Concentration of Test Substance	TEMPERATURE (°C)						DISSOLVED OXYGEN (% Saturation)				
	Rep	0 Hr	24 Hr	48 Hr	72 Hr	96 Hr	0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
	0.00 mg/L	A	13.8	15.0	15.6	15.4	15.3	97	97	100	96
0.33 mg/L	A	13.8	15.0	15.5	15.2	15.0	97	97	99	96	95
3.30 mg/L	A	13.8	15.2	15.8	15.5	15.4	97	99	100	96	95
33.00 mg/L	A	13.9	14.8	15.4	15.3	15.1	99	99	99	98	98
330.00 mg/L	A	14.0	15.0	15.5	15.4	15.2	99	99	100	96	97
3300.00 mg/L	A	13.6	15.0	15.4	15.5	15.2	97	98	100	95	95

Target Concentration of Test Substance	pH (SU)						SPECIFIC CONDUCTANCE (µmhos/cm)				
	Rep	0 Hr	24 Hr	48 Hr	72 Hr	96 Hr	0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
	0.00 mg/L	A	8.13	7.84	7.92	8.09	8.20	495	511	521	514
0.33 mg/L	A	8.13	7.92	8.04	8.15	8.16	497	513	519	512	533
3.30 mg/L	A	8.15	7.98	8.08	8.19	8.21	495	513	519	515	538
33.00 mg/L	A	8.19	8.02	8.14	8.24	8.19	494	511	517	513	535
330.00 mg/L	A	8.22	8.03	8.16	8.23	8.19	498	514	515	511	534
3300.00 mg/L	A	8.24	8.03	8.13	8.18	8.18	492	510	514	506	525

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

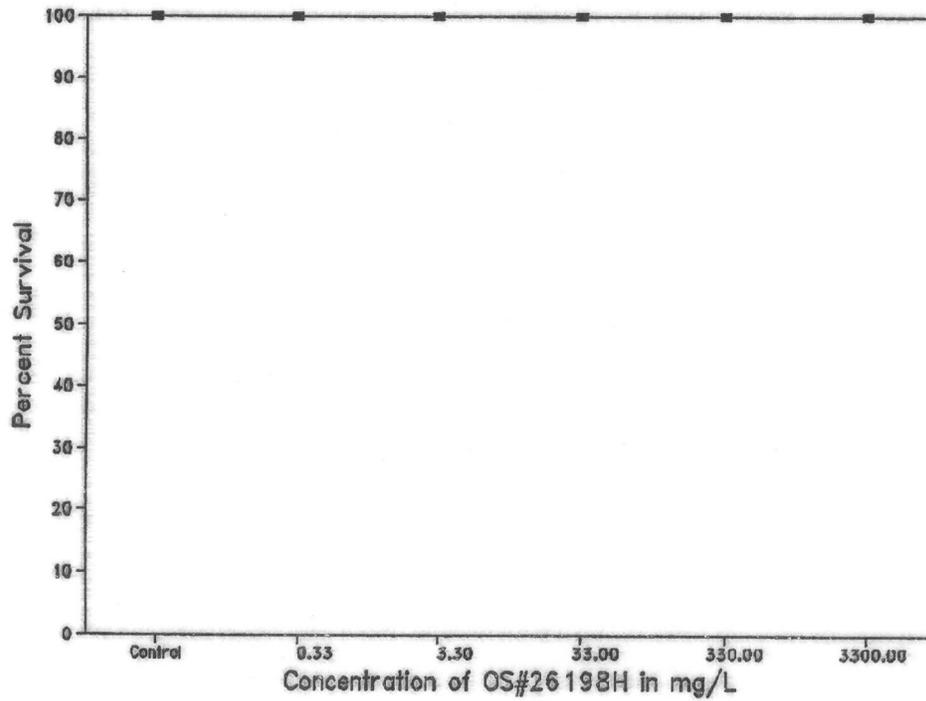
Table 4. Summary of Hardness, Alkalinity, Total Organic Carbon, and Total Solids Data From Acute Range Finding Toxicity Test With The Trout, *Oncorhynchus mykiss*, Exposed To Experimental Product OS#26198H.

Nominal Concentration of Test Substance	TOC (mg/L)	HARDNESS (mg/L CaCO ₃)	ALKALINITY (mg/L CaCO ₃)	TOTAL SOLIDS (mg/L)
	24 Hr	0 Hr	0 Hr	0 Hr
0.00 mg/L	0.50	204	123	392
0.33 mg/L	<0.25	192	98	NA
3.30 mg/L	1.40	190	100	NA
33.00 mg/L	0.96	196	100	NA
330.00 mg/L	3.73	184	102	NA
3300.00 mg/L	6.66	188	100	NA

COMMENTS:
NA - Not applicable.

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

Figure 1. Summary of Survival Data After 96-Hours Exposure. Acute Range Finding Toxicity Test With The Trout, *Oncorhynchus mykiss*, Exposed To Experimental Product OS#26198H.



Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

APPENDIX A
STUDY PROTOCOL

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

EnviroSystems Protocol Number: LUT-960030

ENVIROSYSTEMS, INCORPORATED**AQUATIC TOXICOLOGY STUDY PROTOCOL****Acute Toxicity of Lubricant Additive OS#26198H
To The Rainbow Trout, *Oncorhynchus mykiss*****Sponsor****Lubrizol Corporation
29410 Lakeland Boulevard
Wickliffe, Ohio 44092**Sponsor Approval of Protocol: *Debra L. Cooper*Date: *8/22/96***Testing Facility****EnviroSystems, Incorporated
1 Lafayette Road
Hampton, NH 03842**Estimated Test Initiation Date: *August 1996*Estimated Test Completion Date: *November 1996*Study Director Approval of Protocol: *Frank A. Ross* Date *8/18/96*

1.0 TITLE

Acute Toxicity of the Lubricant Additive OS#26198H to rainbow trout, *Oncorhynchus mykiss*

2.0 PURPOSE

To determine the 24, 48, 72, and 96 hour median lethal concentration, LC50, of the Lubricant Additive OS#26198H to trout exposed under static conditions.

3.0 TEST MATERIAL

3.1 The test substance and related stability and purity data will be supplied by the sponsor. Test material will be stored in the original shipping container at a temperature specified by the client. Handling of the test material will be in accordance with information contained in the sponsor-supplied Material Safety Data Sheet and Standard Operating Procedure 01-02-1106. All unused test substance will be returned to the sponsor.

3.2 Calculations are based on nominal concentrations of the test substance. Test material stock solutions are prepared in deionized or dilution water without the use of a solvent.

4.0 TEST SPECIES

4.1 Healthy trout (2.5 - 3.5 cm in length) from a single source will be used to initiate the test. They will be obtained from in house cultures. The wet weight and length of control organisms will be determined at the end of the test.

4.2 Identification of the test animals shall be verified using appropriate taxonomic keys.

5.0 PRETEST OBSERVATIONS AND PROCEDURES

5.1 Pretest observation data concerning the source, handling procedures, receipt date, disease treatment (if any), health, feeding, and mortality of test animals will be recorded and reported.

5.2 Trout will be maintained under static, replacement, or flow-through conditions prior to test initiation, as appropriate, in the same water and at the same photoperiod and temperature that will be used for testing for a 14 day period prior to starting the assay. The animals will be fed during the acclimation period.

6.0 EXPOSURE CONDITIONS

6.1 Test animals will be added to test media within 30 minutes of test substance preparation.

6.2 Dilution water will be natural groundwater or hard reconstituted laboratory water. The diluent will have a hardness of 165-185 mg/L, alkalinity of 110-120 mg/L, (both measured as CaCO_3), TOC of <2 mg/L, be free of measurable concentrations of pesticides, and meet the requirements specified in U.S. EPA (1986, 1987). Water will be passed through a <20 micron filter and chemically characterized (at least yearly) prior to use.

6.3 Water temperature will be $15 \pm 1^\circ\text{C}$.

6.4 Dissolved oxygen concentration will be maintained between 60 and 105% saturation. If aeration is required during the test it will be supplied to all test chambers. Sponsor approval will be obtained prior to aeration.

6.5 Photoperiod will be automatically controlled and adjusted to 16 hours light and 8 hours dark. A 15-30 minute transition period will be provided between light and dark periods. Light intensity will be approximately 60 foot-candles (ambient lab light).

6.6 The test vessels will be 20 L, or larger, glass beakers containing at least 15 L of solution. Loading rate will be ≤ 5 grams per liter.

6.7 Test media will be supplied to the exposure vessels only once at the beginning of the test.

6.8 The test substance will be evaluated as an oil water dispersion. The oil-water dispersion will be prepared by adding a measured volume of product to a known volume of water. The product will be added to the test chamber within the confines of a glass mixing chamber. The chamber will be constructed with holes approximately 1 cm from the bottom of the chamber and 1 cm below the surface of the water. Holes will be covered with Nytex screen to prevent fish from entering the mixing chamber. A stirring motor, with polyethylene covered shaft and mixing blade, will be centered over the mixing chamber. The product will be allowed to mix for one (1) hour prior to adding the fish. Mixing will be done at approximately 1000 revolutions per minute.

7.0 STUDY CONDUCT

7.1 The animals will be exposed for 96 hours to a series of five (5) concentrations of test substance plus a control (dilution water without test substance). Test concentrations will be 0.33, 3.3, 33, 330, and 3,300 mg/L.

7.2 Ten (10) animals will be exposed to each treatment. The animals will be exposed in groups of 10 animals per vessel, each containing at least 15 L of solution. The animals will be equally and randomly assigned to the test vessels (a random numbers table will be used to choose the test vessel for each specimen), and they will not be fed during the assay.

7.3 Dissolved oxygen concentration, pH, temperature, and conductivity will be measured and recorded at the beginning of the test and daily thereafter in all test vessels, as long as living animals are present in those vessels. Temperature of one test vessel, or surrogate test vessel, will be recorded at least every 1 hour during the test.

7.4 Treatments will not be aerated. If dissolved oxygen falls below 60% of saturation, the sponsor will be notified and a determination made with respect to aeration. If aeration is used in the assay, all concentrations will receive the same level of treatment.

7.5 Total Organic Carbon (TOC) will be measured in each test treatment after 24 hours.

7.6 Hardness, and alkalinity, will be measured at the start of the assay in the dilution water and all treatments. Particulate matter will be measured at the start of the assay in the dilution water.

7.7 Mortality, sublethal effects (immobilization), and any observation of insoluble material will be recorded after 0, 24, 48, 72, and 96 hours. Dead animals will be removed when first observed.

7.8 Data Analysis and Statistical Methods

The 24, 48, 72, and 96-hour median lethal concentration (LC50) values and their 95% confidence intervals will be calculated by the non-linear interpolation, moving average, Spearman-Kärber and/or probit methods. If toxicity occurs, the slope of the dose-response curve will be provided. The NOEC will be determined as the highest concentration where 90% survival is observed.

8.0 QUALITY ASSURANCE AND QUALITY CONTROL

8.1 The test will be considered invalid if any of the following conditions occur:

8.1.1 Control survival at the end of the test is less than 90%, or if behavioral abnormalities are observed in the controls.

8.1.2 Temperature in any test vessel is outside of the range of 14-16°C.

8.2 The test will be conducted in the spirit of the TSCA Good Laboratory Practice Standards.

8.3 Selected, representative critical phases of the test conducted at EnviroSystems Inc. will be audited by the Quality Assurance Unit.

8.4 The Study Director will be responsible for reviewing all data and for recording any changes to procedures outlined in this protocol in a protocol amendment. Protocol amendments will be approved by the sponsor.

8.5 Original data will be archived at EnviroSystems, Incorporated for 10 years or it will be transferred to the sponsor for archiving.

9.0 REPORT

9.1 A final report will be prepared after review of an audited draft report by the sponsor. The final report will be signed by the Study Director and staff that conducted the study and prepared the report, and by the Quality Assurance Unit Representative.

9.2 The final report will consist of at least the following information:

9.2.1 Title page, including the study title, data requirement, author, study initiation and completion dates, testing facility, and sponsor.

9.2.2 Sponsor-supplied confidentiality claims.

9.2.3 Good Laboratory Practice Statement.

9.2.4 Quality Assurance Statement.

EnviroSystems Protocol Number: LUT-960030

- 9.2.5 Table of Contents.
- 9.2.6 Summary of test procedures and results.
- 9.2.7 Introduction.
- 9.2.8 Methods and Materials, including a description of test methods, organisms, dilution water, and statistical techniques.
- 9.2.9 Results, including all data from screening tests, and LC50 values.
- 9.2.10 References.
- 9.2.11 Appendices, including all water quality.

10.0 REFERENCES

OECD. 1984. OECD Guidelines for Testing of Chemicals. Method 203, Fish Acute Toxicity Test.

U.S. EPA. 1986. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Federal Register, Monday, January 6, 1986. Section 797.1300.

U.S. EPA. 1987. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Amendments. Federal Register, Wednesday, May 20, 1987. Section 797.1300.

U.S. EPA. 1989. 40 CFR Part 792. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Final Rule. Federal Register, Thursday, August 17, 1989.

SUMMARY OF TEST CONDITIONS

Acceptable Species:	<i>Oncorhynchus mykiss</i>
Reference:	TSCA 797.1400; OECD 203
Test Type:	Static
Test Duration:	96 Hours
Flow Rate:	NA
Dilution Water:	Natural Groundwater or Hard Reconstituted Lab Water
Age/Size of Test Organisms:	Juveniles; 2.5 - 3.5 cm in length
Acclimation Period:	14 days
Temperature:	15 ± 1°C
Photoperiod:	16 Hours Light/8 Hours Dark with 15-30 minute transition period
Light Intensity:	Ambient Laboratory Lighting
Number of Concentrations:	5; 0.33, 3.3, 33, 330, and 3,300 mg/L
Dilution Factor:	NA
Number of Replicates:	1
Number of Organisms/Replicate:	10
Test Vessel Size:	20 L
Test Vessel Volume:	15 L
Solvent:	NA
Loading Rate:	< 5 g/L
Feeding:	None During Exposure
Dissolved Oxygen Levels:	60-105% saturation
Effects Measured:	Death; note sublethal effects
Test Concentration Analysis:	Nominal Concentration
Water Quality Measurements:	DO, pH, Temperature and Conductivity at 0, 24, 48, 72 and 96 Hours. Temperature at least every hour in 1 exposure vessel. Hardness, alkalinity, and particulate matter in dilution water at start of assay. TOC in all concentrations after 24 hours exposure.
Acceptability Criteria:	≤ 10% Control Mortality; Acceptable Temperature Range; No control behavioral abnormalities

000021

APPENDIX B
RAW DATA AND STATISTICAL SUPPORT

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

PSCTOX000104942

LABORATORY SAMPLE RECEIVING LOG

1. ESI Sample Number 5401
2. Sponsor Name and Address Lubrizol Corp.
29400 Lakeland Blvd.
Wickliffe, Ohio
3. Sponsor Sample Identification Experimental Product as# 26198H
Order: 160544-013
4. Sample Container Label Data Jun 17, 196
5. Data Received 6/21/96 6. Data Photographed _____
7. Method of Shipment Shipped from Lubrizol
Via UPS
8. Description of Shipping Containers(s) 1, cardboard box
(Number, Type, Size)
9. Description of Sample Container(s) 1, 250ml bottle - Nalgene
(Number, Type, Size)
10. Sample Storage Location and Required Storage Conditions Locker PRL-15
cool dry, dark
11. Signature L. Hawthorn Date 6/26/96
12. Notes _____

13. Date and Location of Archive Sample Removal and Storage Location _____

000023

ENVIROSYSTEMS, INCORPORATED
PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 5401 SAMPLE NUMBER: CS# 26984

TEST SUBSTANCE: _____ TEST TYPE: SCREEN X DEFINITIVE _____

TEST CONDITION SUMMARY:

DATE: 8/22/96 RECORDED BY: zjt

TEST ORGANISM: Rainbow Trout LOT NUMBER: _____

TEST DURATION: 96 hours FLOW RATE: _____

DILUTION WATER: Hard Recon (HARDNESS/SALINITY: 165-185 mg/l %a)

AGE / SIZE OF TEST ORGANISM: 2.5 - 3.5 cm. in length

ACCLIMATION PERIOD IN DAYS: 14

TREATMENT DURING ACCLIMATION PERIOD: None TREATMENTS: _____

TEST TEMPERATURE: 15 ± 1 °C INCUBATOR: _____

PHOTOPERIOD: Time On 16 Hrs Time Off 8 Hrs INTENSITY: 60 foot candles

15 MINUTE TRANSITION PERIOD: YES NO _____

NUMBER OF TREATMENTS: 5; 0.33, 3.3, 33 330 and 3,300 mg/L

DILUTION FACTOR: _____

NUMBER OF REPLICATES: 1 ORGANISMS PER REPLICATE: 10

FEEDING (food): _____ FREQUENCY: _____ AMOUNT: _____

TEST CHAMBER SIZE (L): 20 SOLUTION VOLUME (L): 15

SOLUTION DEPTH (CM): _____ LOADING RATE: ≤ 5 g/L

DISSOLVED OXYGEN LEVELS (Minimum): 60 % OF SATURATION

SOLVENT: _____ SOLVENT CONCENTRATION: _____

EFFECTS MEASURED: mortality, sublethal + insolubility

WATER QUALITY MEASUREMENTS: pH, temp, cond., DO daily; Temp - once/hour

TOC in all at 24hrs. 3 Alk + 2 in all at 6hrs. + 13, 25 in control

ANALYSIS OF TEST SUBSTANCE CONCENTRATIONS: _____

NOTES: Control: LHW at end;

C00026

ENVIROSYSTEMS, INCORPORATED
 PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 535401 SAMPLE NUMBER: OS# 26198H

TEST SUBSTANCE: _____ TEST TYPE: SCREEN DEFINITIVE

STOCK SOLUTION PREPARATION:

PERCENT ACTIVE INGREDIENT (% AI): _____

NOMINAL CONCENTRATIONS CORRECTED FOR % AI: YES _____ NO _____

BALANCE USED FOR STOCK SOLUTION PREPARATION: _____

PREPARATION NOTES:

<u>Nominal Conc.</u>	<u>Nominal Conc in g/15L</u>	<u>Actual Amt Measured</u>
<u>0.33 mg/L</u>	<u>0.0050 g/15L</u>	<u>0.0056</u>
<u>3.30</u>	<u>0.0495</u>	<u>0.0505</u>
<u>33.0</u>	<u>0.4950</u>	<u>0.4945</u>
<u>330.0</u>	<u>4.9500</u>	<u>4.9503</u>
<u>3300.0</u>	<u>49.5000</u>	<u>49.4998</u>

product added/spinning started: 1055 11/27 JH

fish added: 1230 11/27 JH

<u>Nominal Conc.</u>	<u>Actual Conc.</u>
<u>0.33 mg/L</u>	<u>0.37 mg/L</u>
<u>3.30</u>	<u>3.37</u>
<u>33.0</u>	<u>32.97</u>
<u>330.0</u>	<u>330.02</u>
<u>3300.0</u>	<u>3299.99</u>

000027

ENVIROSYSTEMS, INCORPORATED
PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 540SAMPLE NUMBER: OS #26198HTEST SUBSTANCE: _____ TEST TYPE: SCREEN DEFINITIVE _____

RANDOMIZATION SCHEME: _____

STUDY INITIATION DATE: _____

TEST SPECIES: O. mykiss

TEST VESSEL	NOMINAL CONC.	REP	VESSEL LOCATION
CONTROL	0	A	5
		B	
	0.33 mg/L	A	2
		B	
	3.30	A	4
		B	
	33.0	A	6
		B	
	330.0	A	3
		B	
	3300.0	A	1
		B	
		A	
		B	
RECORDED BY:			LH
DATE:			11/26/96

0.784970 0.197223 0.669453 0.871852 0.672169
0.313911 0.102469 0.067245 0.799519 0.876726
0.901895 0.800698 0.062117 0.465844 0.354891
0.271829 0.461265 0.788686 0.669886 0.799510
0.491743 0.558419 0.088450 0.886916 0.741501
0.466454 0.553328 0.953367 0.061648 0.456856
0.861646 0.330697 0.167285 0.517110 0.267356
0.783739 0.863297 0.780405 0.808705 0.451552
0.670803 0.614568 0.001619 0.097255 0.700095
0.527941 0.756203 0.559617 0.600379 0.534041
0.595743 0.872649 0.489477 0.990834 0.082183
0.130888 0.312814 0.315561 0.762740 0.250538
0.641994 0.914364 0.012080 0.083691 0.188935
0.730075 0.254253 0.605543 0.980285 0.037112
0.758710 0.735142 0.174878 0.158822 0.698693
0.340064 0.983117 0.500280 0.246909 0.007666
0.287885 0.343290 0.872910 0.803261 0.661440
0.875857 0.786878 0.048864 0.572414 0.655408
0.505341 0.626631 0.621285 0.270264 0.265256
0.875782 0.697574 0.587277 0.387795 0.213801
0.651021 0.138749 0.697687 0.631504 0.153148
0.614199 0.907137 0.159377 0.506604 0.548567
0.010164 0.371765 0.966087 0.651433 0.443358
0.080911 0.939203 0.916600 0.227254 0.266442
0.830796 0.533517 0.457971 0.378293 0.929573
0.671668 0.182788 0.761964 0.694308 0.939714
0.793229 0.893479 0.375267 0.415718 0.475900
0.160105 0.973349 0.082689 0.761777 0.518734
0.270072 0.376354 0.868185 0.937212 0.061525

Arbitrarily enter the random number grid. Using this as a starting point move either up or down the column the first two digit number that is encountered that falls within the range of test vessels being located is allocated to "Control Replicate A". The next two digit number is allocated to "Control Replicate B", and so on until all vessels are located.

PSCTOX000104948

ENVIROSYSTEMS, INCORPORATED
 PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 5401SAMPLE NUMBER: OS# 2619811TEST SUBSTANCE: _____ TEST TYPE: SCREEN DEFINITIVE _____

SOLUBILITY DATA:

STUDY INITIATION DATE: 11/27/96

TEST VESSEL ID	NOMINAL CONC. -	REP	TEST MATERIAL PRESENCE				
			0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
Control	0	A	none	none	none	NONE	NONE
		B					
	0.33 mg/L	A	none	none	none	NONE	NONE
		B					
	3.30	A	none	none	none	NONE	NONE
		B					
	33.0	A	very little	very little	very little	VERY LITTLE	VERY LITTLE
		B					
	330.0	A	little	little	little	LITTLE	LITTLE
		B					
	3300.0	A	some	some	SOME	SOME	SOME
		B					
		A					
		B					
RECORDED BY:			NRL	NRL	NRL	jm	jm
DATE:			11.27.96	11.28.96	11.29.96	11/30/96	12/1/96

If no insoluble material is observed enter the word "NONE" in the appropriate box. If insoluble material is observed enter the word "SOME" in the appropriate box and describe the observation below.

NOTES: very little: very slight film on surface
little: slight film on surface, white globules on bottom of tank
some: oily surface, film, clear to whitish globules covering bottom of tank

000029

ENVIROSYSTEMS, INCORPORATED
 PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: ²⁴ OS# 5401 SAMPLE NUMBER: OS# 261984

TEST SUBSTANCE: _____ TEST TYPE: SCREEN DEFINITIVE

SURVIVAL DATA:

STUDY INITIATION DATE: 11/27/96 TEST SPECIES: O. mykiss

TEST VESSEL ID	NOMINAL CONC.	REP	NUMBER ALIVE				
			0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
CONTROL	0	A	10	10	10	10	10
		B					
	0.33 mg/L	A	10	10	10	10	10
		B					
	3.30	A	10	10	10	10	10
		B					
	33.0	A	10	10	10	10	10
		B					
	330.0	A	10	10	10	10	10
		B					
	3300.0	A	10	10	10	10	10
		B					
		A					
		B					
RECORDED BY:			NK	NK	NK	ju	ju
DATE:			11.27.96	11.28.96	11.29.96	11/30/96	12/1/96

NOTES: _____

000030

ENVIROSYSTEMS, INCORPORATED
 PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 540

SAMPLE NUMBER: as⁺ 26198H

TEST SUBSTANCE: _____ TEST TYPE: SCREEN DEFINITIVE _____

SUBLETHAL EFFECTS DATA:

STUDY INITIATION DATE: 11/27/96

TEST SPECIES: O. mykiss

TEST VESSEL ID	NOMINAL CONC.	REP	NUMBER AFFECTED				
			0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
CONTROL	0	A	0	0	0	∅	∅
		B					
	0.33 ms/L	A	0	0	0	∅	∅
		B					
	3.30	A	0	0	0	∅	∅
		B					
	33.0	A	0	0	0	∅	∅
		B					
	330.0	A	0	0	0	∅	∅
		B					
	3300	A	0	0	0	∅	∅
		B					
		A					
		B					
RECORDED BY:			NVL	NVL	NVL	ju	ju
DATE:			11/27/96	11/28/96	11/29/96	11/30/96	12/1/96

NOTES: _____

Data file - LU9701

C00031

Logger details:

Logger number : 10076
Logger type : 8-bit

Run details:

Site ID :
Run number : 1
Channels used : 1
Recording interval : 00:60:00.
Recording period :
Start : 27-Nov-96 11:00:00.
Finish : 02-Dec-96 10:00:00.
Readings per channel: 120

Channel 1 Statistics

Sample Period

Start 27-Nov-96 11:00:00.

Finish 01-Dec-96 10:00:00.

Range : 0.000 to 30.000 degC
Minimum Value : 14.760 degC
Maximum Value : 15.600 degC
Mean : 15.243 degC
Standard Deviation : 0.226
Variance : 0.051

at 27-Nov-96 19:00:00.
at 30-Nov-96 23:00:00.

All readings

000032

Rdg no	Date and Time	Ch1	degC
	27-Nov-96 11:00:00.		15.240
	27-Nov-96 12:00:00.		15.120
3	27-Nov-96 13:00:00.		15.120
4	27-Nov-96 14:00:00.		14.880
5	27-Nov-96 15:00:00.		14.880
6	27-Nov-96 16:00:00.		14.880
7	27-Nov-96 17:00:00.		14.880
8	27-Nov-96 18:00:00.		14.880
9	27-Nov-96 19:00:00.		14.760
10	27-Nov-96 20:00:00.		14.880
11	27-Nov-96 21:00:00.		14.880
12	27-Nov-96 22:00:00.		14.880
13	27-Nov-96 23:00:00.		14.880
14	27-Nov-96 24:00:00.		14.880
15	28-Nov-96 01:00:00.		14.880
16	28-Nov-96 02:00:00.		14.880
17	28-Nov-96 03:00:00.		14.880
18	28-Nov-96 04:00:00.		14.880
19	28-Nov-96 05:00:00.		14.880
20	28-Nov-96 06:00:00.		14.880
21	28-Nov-96 07:00:00.		14.880
22	28-Nov-96 08:00:00.		14.880
23	28-Nov-96 09:00:00.		14.880
24	28-Nov-96 10:00:00.		14.880
25	28-Nov-96 11:00:00.		15.000
26	28-Nov-96 12:00:00.		15.000
27	28-Nov-96 13:00:00.		15.120
28	28-Nov-96 14:00:00.		15.120
	28-Nov-96 15:00:00.		15.120
	28-Nov-96 16:00:00.		15.120
31	28-Nov-96 17:00:00.		15.120
32	28-Nov-96 18:00:00.		15.120
33	28-Nov-96 19:00:00.		15.240
34	28-Nov-96 20:00:00.		15.240
35	28-Nov-96 21:00:00.		15.240
36	28-Nov-96 22:00:00.		15.240
37	28-Nov-96 23:00:00.		15.240
38	28-Nov-96 24:00:00.		15.240
39	29-Nov-96 01:00:00.		15.240
40	29-Nov-96 02:00:00.		15.240
41	29-Nov-96 03:00:00.		15.360
42	29-Nov-96 04:00:00.		15.360
43	29-Nov-96 05:00:00.		15.360
44	29-Nov-96 06:00:00.		15.360
45	29-Nov-96 07:00:00.		15.360
46	29-Nov-96 08:00:00.		15.360
47	29-Nov-96 09:00:00.		15.360
48	29-Nov-96 10:00:00.		15.360
49	29-Nov-96 11:00:00.		15.360
50	29-Nov-96 12:00:00.		15.360
51	29-Nov-96 13:00:00.		15.360
52	29-Nov-96 14:00:00.		15.360
53	29-Nov-96 15:00:00.		15.360
54	29-Nov-96 16:00:00.		15.360
55	29-Nov-96 17:00:00.		15.360
	29-Nov-96 18:00:00.		15.360
	29-Nov-96 19:00:00.		15.360
58	29-Nov-96 20:00:00.		15.360
59	29-Nov-96 21:00:00.		15.360
60	29-Nov-96 22:00:00.		15.360
61	29-Nov-96 23:00:00.		15.360

62	29-Nov-96 24:00:00.	15.360
63	30-Nov-96 01:00:00.	15.360
64	30-Nov-96 02:00:00.	15.360
65	30-Nov-96 03:00:00.	15.360
66	30-Nov-96 04:00:00.	15.360
67	30-Nov-96 05:00:00.	15.360
68	30-Nov-96 06:00:00.	15.360
69	30-Nov-96 07:00:00.	15.360
70	30-Nov-96 08:00:00.	15.360
71	30-Nov-96 09:00:00.	15.360
72	30-Nov-96 10:00:00.	15.360
73	30-Nov-96 11:00:00.	15.360
74	30-Nov-96 12:00:00.	15.360
75	30-Nov-96 13:00:00.	15.360
76	30-Nov-96 14:00:00.	15.360
77	30-Nov-96 15:00:00.	15.360
78	30-Nov-96 16:00:00.	15.360
79	30-Nov-96 17:00:00.	15.480
80	30-Nov-96 18:00:00.	15.480
81	30-Nov-96 19:00:00.	15.480
82	30-Nov-96 20:00:00.	15.480
83	30-Nov-96 21:00:00.	15.480
84	30-Nov-96 22:00:00.	15.480
85	30-Nov-96 23:00:00.	15.600
86	30-Nov-96 24:00:00.	15.480
87	01-Dec-96 01:00:00.	15.480
88	01-Dec-96 02:00:00.	15.600
89	01-Dec-96 03:00:00.	15.480
90	01-Dec-96 04:00:00.	15.480
91	01-Dec-96 05:00:00.	15.480
92	01-Dec-96 06:00:00.	15.480
93	01-Dec-96 07:00:00.	15.480
94	01-Dec-96 08:00:00.	15.480
95	01-Dec-96 09:00:00.	15.600
96	01-Dec-96 10:00:00.	15.600

Ch.1 ---

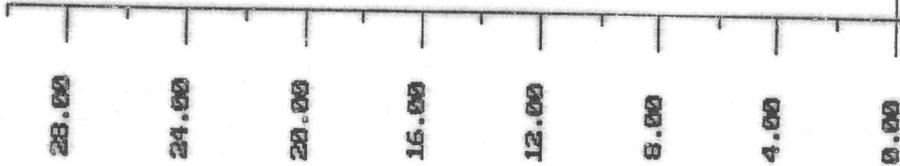
Analogue Graph Summary

Real time mode

Channel 1

Start time : 27-Nov-96 11:00:00.
End time : 01-Dec-96 10:00:00.
Time divisions : 12 hours
Divisions on y axis : 2:00 degC
No accuracy lost drawing curve

degC



27:00:00
27-Nov-96

24:00:00
27-Nov-96

24:00:00
28-Nov-96

24:00:00
29-Nov-96

000033

000034

ENVIROSYSTEMS, INCORPORATED
PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 5401SAMPLE NUMBER: OS # 26198HTEST SUBSTANCE: _____ TEST TYPE: SCREEN DEFINITIVE _____

TEMPERATURE MEASUREMENTS:

STUDY INITIATION DATE: 11/27/96TEST SPECIES: C. mullissTHERMOMETER NUMBER: 2829

TEST VESSEL ID	NOMINAL CONC.	REP	TEMP °C				
			0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
CONTROL	0	A	13.8	15.0	15.6	15.4	15.3
		B					
	0.33 mg/L	A	13.8	15.0	15.5	15.2	15.0
		B					
	3.30	A	13.8	15.2	15.8	15.5	15.4
		B					
	33.0	A	13.9	14.8	15.4	15.3	15.1
		B					
	330.0	A	14.0	15.0	15.5	15.4	15.2
		B					
	3300.0	A	13.6	15.0	15.4	15.5	15.2
		B					
		A					
		B					
RECORDED BY:			<u>NR</u>	<u>NR</u>	<u>NR</u>	<u>NR</u>	<u>NR</u>
DATE:			<u>11.27.96</u>	<u>11.28.96</u>	<u>11.29.96</u>	<u>11/30/96</u>	<u>12/1/96</u>

NOTES: _____

PSCTOX000104955

000035

ENVIROSYSTEMS, INCORPORATED
 PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 5401 SAMPLE NUMBER: OS# 26198H
 TEST SUBSTANCE: _____ TEST TYPE: SCREEN DEFINITIVE _____

DISSOLVED OXYGEN CONCENTRATION MEASUREMENTS:

STUDY INITIATION DATE: 11/27/96 TEST SPECIES: O. mykiss

INSTRUMENT NUMBER: TSI #19

TEST VESSEL ID	NOMINAL CONC.	REP	D.O. IN MG/L				
			0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
CONTROL	0	A	10.0	9.8	9.9	9.6	9.6
		B					
	0.33 mg/L	A	10.0	9.8	9.9	9.6	9.6
		B					
	3.30	A	10.0	9.9	9.9	9.6	9.5
		B					
	33.0	A	10.2	10.0	9.9	9.8	9.8
		B					
	330.0	A	10.2	10.0	10.0	9.6	9.7
		B					
	3300.0	A	10.1	9.9	10.0	9.5	9.5
		B					
		A					
		B					
RECORDED BY:			NRL	NRL	NRL	JW	JW
DATE:			11/27/96	11/28/96	11/29/96	11/30/96	12/1/96

NOTES: _____

000036

ENVIROSYSTEMS, INCORPORATED
 PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 5401 SAMPLE NUMBER: 05th 26 1984

TEST SUBSTANCE: _____ TEST TYPE: SCREEN DEFINITIVE _____

DISSOLVED OXYGEN PERCENT SATURATION MEASUREMENTS:

STUDY INITIATION DATE: 11/27/96 TEST SPECIES: O. mykiss

TEST VESSEL ID	NOMINAL CONC.	REP	D.O. % SATURATION				
			0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
CONTROL	0	A	97	97	100	96	96
		B					
	0.33 mg/L	A	97	97	99	96	95
		B					
	3.30	A	97	99	100	96	95
		B					
	33	A	99	99	99	98	98
		B					
	330	A	99	99	100	96	97
		B					
	3300	A	97	98	100	95	95
		B					
		A					
		B					
RECORDED BY:		LH	LH	LH	LH	LH	
DATE:		11/27	11/28	11/29	11/30	12/1	

NOTES: calculated 1/8/97 JH

ENVIROSYSTEMS, INCORPORATED
 PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 5401 SAMPLE NUMBER: OS# 261984

TEST SUBSTANCE: _____ TEST TYPE: SCREEN DEFINITIVE _____

pH MEASUREMENTS:

STUDY INITIATION DATE: 11/27/96

TEST SPECIES: O. mykiss

INSTRUMENT NUMBER: Probe #7

TEST VESSEL ID	NOMINAL CONC.	REP	pH VALUES IN SU				
			0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
CONTROL	0	A	8.13	7.84	7.92	8.09	8.20
		B					
	0.33 mg/L	A	8.13	7.92	8.04	8.15	8.16
		B					
	330	A	8.15	7.98	8.08	8.19	8.21
		B					
	33.0	A	8.19	8.02	8.14	8.24	8.19
		B					
	330.0	A	8.22	8.03	8.16	8.23	8.19
		B					
	3300.0	A	8.24	8.03	8.13	8.18	8.18
		B					
		A					
		B					
RECORDED BY:			<u>NRL</u>	<u>NRL</u>	<u>NRL</u>	<u>ju</u>	<u>ju</u>
DATE:			<u>11.27.96</u>	<u>11.28.96</u>	<u>11.29.96</u>	<u>11/30/96</u>	<u>12/1/96</u>

NOTES: _____

030028

**ENVIROSYSTEMS, INCORPORATED
PRODUCT REGISTRATION LABORATORY - RAW DATA FORM**

STUDY NUMBER: 5401 SAMPLE NUMBER: 05[#] 26198H
 TEST SUBSTANCE: _____ TEST TYPE: SCREEN X DEFINITIVE _____

CONDUCTIVITY MEASUREMENTS:

STUDY INITIATION DATE: 11/27/96 TEST SPECIES: C. mykiss

INSTRUMENT NUMBER: ESI#1

TEST VESSEL ID	NOMINAL CONC.	REP	CONDUCTIVITY IN μ mhos/cm				
			0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
CONTROL	0	A	495	511	521	514	540
		B					
	0.33 mg/L	A	497	573	579	512	533
		B					
	3.30	A	495	573	579	515	538
		B					
	33.0	A	494	571	577	513	535
		B					
	330.0	A	498	574	575	511	534
		B					
	3300.0	A	492	570	574	506	525
		B					
		A					
		B					
RECORDED	BY:						
DATE:							

NOTES: _____

000089

ENVIROSYSTEMS, INCORPORATED
 PRODUCT REGISTRATION LABORATORY - RAW DATA FORM

STUDY NUMBER: 5401
 TEST SUBSTANCE: _____

SAMPLE NUMBER: OS^H 26198H
 TEST TYPE: SCREEN DEFINITIVE

LENGTHS AND WEIGHTS OF CONTROL ORGANISMS:

REP	WEIGHT g	LENGTH _{mm}	REP	WEIGHT	LENGTH
1	0.0784	25.2	11		
2	0.1021	25.9	12		
3	0.1170	26.8	13		
4	0.0919	25.6	14		
5	0.0630	25.1	15		
6	0.1361	26.2	16		
7	0.1011	26.4	17		
8	0.1145	27.1	18		
9	0.0908	25.5	19		
10	0.1117	27.4	20		
RECORDED BY:		<i>ju</i>			
DATE:		12/1/76			

MEAN WEIGHT: 0.1007 g

MEAN LENGTH: 23.8 mm

VOLUME OF TEST VESSEL: 15

LOADING RATE: 0.07 g/L

PSCTOX000104960

000040

APPENDIX C
SUPPORT DATA

Static Acute Toxicity of Lubricant Additive OS#26198H to the Trout, *Oncorhynchus mykiss*.
EnviroSystems, Inc. Study Number 5401.

PSC TOX000104961

LUBRIZOL Material Safety Data Sheet

000041

Lubrizol Material Safety Data Sheet: OS#26198H

1

CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

OS#26198H

THE LUBRIZOL CORPORATION
29400 LAKELAND BOULEVARD
WICKLIFFE, OHIO 44092
216/943-4200

CAS Registry Number	Confidential.
Synonyms	Confidential.
Generic/Chemical Name	Chlorinated long chain paraffin
Product type	Not applicable.
Preparation/Revision Date	06/10/1996
Transportation Emergency Phone No	(CHEMTREC) 1-800-424-9300.

2

COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous Ingredients - This material has no known hazards under applicable laws.

3

HAZARDS IDENTIFICATION

Principal Hazards - This material has no known hazards.
See Section 11 for complete health hazard information.

4

FIRST AID MEASURES

Oral	DO NOT INDUCE VOMITING. If conscious, give 2 glasses of water. Get immediate medical attention.
Eye	Flush with water at least 15 minutes. Get medical attention if eye irritation develops or persists.
Skin	Wash with soap and water. Get medical attention if irritation develops. Launder contaminated clothing before reuse.
Inhalation	Remove exposed person to fresh air if adverse effects are observed.
Additional	Note to physician: Treat symptomatically.

5

FIRE FIGHTING MEASURES

Flash Point (Typical)	205 Deg C, 401 Deg F (COC)
Upper Flammable Limit	Not Determined.
Lower Flammable Limit	Not Determined.
Extinguishing Media	CO2, dry chemical, or foam. Water can be used to cool and protect exposed material.
Special Firefighting Procedures	Recommend wearing self-contained breathing apparatus. Water may cause splattering.

5

FIRE FIGHTING MEASURES (CONTINUED)**Unusual Fire & Explosion Hazards**

Toxic fumes, gases or vapors may evolve on burning. Closed containers may explode on heating. At high temperatures chlorinated paraffin decomposes to release hydrogen chloride gas.

**Autoignition Temperature
Explosion Data**

Not Determined.

Material does not have explosive properties.

6

ACCIDENTAL RELEASE MEASURES**Spill Procedures**

Personal Protective Equipment must be worn, see Personal Protection Section for PPE recommendations. Ventilate area if spilled in confined space or other poorly ventilated areas. Prevent entry into sewers and waterways. Pick up free liquid for recycle and/or disposal. Residual liquid can be absorbed on inert material. Check under Transportation and Labeling (DOT/CERCLA) and Other Regulatory Information Section (SARA) for hazardous substances to determine regulatory reporting requirements for spills.

7

HANDLING AND STORAGE**Handling Procedures**

Keep containers closed when not in use. Wash thoroughly after handling. Empty container contains product residue which may exhibit hazards of product.

Storage Procedures

Storage temperatures should not exceed 151 deg F (66 deg C).

8

EXPOSURE CONTROLS/PERSONAL PROTECTION**Ventilation Procedures**

Use with adequate ventilation.

Gloves Protection

Plastic gloves.

Eye Protection

Safety Glasses.

Respiratory Protection

Under normal use conditions, respirator is not usually required.

Clothing Recommendation

Long sleeve shirt is recommended.

9

PHYSICAL AND CHEMICAL PROPERTIES**Vapor Pressure**

Not Determined.

pH

Not Determined.

Specific Gravity

1.18 at 25.0 Deg C

Water Solubility

Insoluble.

Percent Volatile

Unknown.

Vapor Density

Not Determined.

Evaporation Rate

Not Determined.

Odor

Mild

Appearance

Clear, light amber liquid

Viscosity

5000 Centistokes at 37.8 Deg C

35.6 Centistokes at 100 Deg C

Odor Threshold

Unknown.

Boiling Point

Not Determined.

Freezing Point

Not Determined.

Molecular Weight

Not Determined.

10

STABILITY AND REACTIVITY**Stability
Incompatibility**

Material is normally stable at moderately elevated temperatures and pressures.

This product will deteriorate with subsequent darkening and eventual decomposition when exposed to elevated temperatures or alkalis. The presence of iron or zinc in any form will catalyze this deterioration.

Polymerization

Will not occur.

Thermal Decomposition

Smoke, carbon monoxide, aldehydes and other products of incomplete combustion. Hydrogen chloride and Chlorinated hydrocarbons will also form. Held at 175 degrees C for 4 hours, this material will give off a maximum of 0.5% by weight of hydrogen chloride.

11

TOXICOLOGICAL INFORMATION**-- ACUTE EXPOSURE --****Oral Toxicity**

Material has a low order of acute toxicity.

Eye Irritation

Not expected to cause eye irritation. Based on actual data.

Skin Irritation

Not expected to be a primary skin irritant. Based on actual data.

Dermal Toxicity

The LD50 in rabbits is > 2000 mg/kg. Based on actual data.

Inhalation Toxicity

Not expected to be an inhalation hazard. Based on similar materials.

Respiratory Irritation

Not expected to cause nose, throat and lung irritation. Based on actual data.

Dermal Sensitization

Not expected to cause skin sensitization. Based on actual data.

Inhalation Sensitization

No data available to indicate product or components may be respiratory sensitizers.

-- CHRONIC EXPOSURE --**Chronic Toxicity**

Repeated and prolonged overexposure to chlorinated paraffins may cause liver toxicity.

Carcinogenicity

The National Toxicology Program (NTP) has reported that chlorinated paraffins of various molecular weights which are different than those used in this product caused cancer at high doses when fed to laboratory animals. The relevance of these results to human workplace exposure has not been established.

Mutagenicity

No data available to indicate product or any components present at greater than 0.1% are mutagenic or genotoxic.

Reproductive Toxicity

No data available to indicate either product or components present at greater than 0.1% that may cause reproductive toxicity.

Teratogenicity

No data available to indicate product or any components contained at greater than 0.1% may cause birth defects.

-- ADDITIONAL INFORMATION --**Other**

No other health hazards known.

Exposure Limits

See Hazardous Ingredients Section for any applicable exposure limits for components.

12

ECOLOGICAL INFORMATION**Freshwater Fish Toxicity**

An environmental effects test program for freshwater fish is in progress.

**Freshwater
Invertebrates Toxicity**

An environmental effects test program for freshwater invertebrates is in progress.

12

ECOLOGICAL INFORMATION (CONTINUED)

Algae Toxicity	Environmental effects data for algae is not available on all materials.
Saltwater Fish Toxicity	Environmental effects data for saltwater fish is not available on all materials.
Saltwater Invertebrates Toxicity	Environmental effects data for saltwater invertebrates is not available on all materials.
Bacteria Toxicity	An environmental effects test program for bacteria is in progress.
Miscellaneous Toxicity	Environmental effects data for flora and fauna is not available on all materials.
Environmental Fate	An environmental effects test program for biodegradation is in progress.

13

DISPOSAL CONSIDERATIONS

Waste Disposal	Material, if discarded, is not expected to be a characteristic hazardous waste under RCRA. Waste management should be in compliance with federal, state, and local laws.
-----------------------	--

14

TRANSPORT INFORMATION

U.S.DOT Bulk Shipping Description	Not applicable.
U.S.DOT Non-Bulk Shipping Description	Not applicable.
IMDG Code Shipping Description	Not applicable.
ICAO Shipping Description	Not applicable.
ADR/RID Hazard Class	Not applicable.

15

REGULATORY INFORMATION

U.S. TSCA Inventory	All components of this material are on the US TSCA Inventory.
Other TSCA Reg.	Section 8d (Alkanes, chlorinated).
SARA Ext. Haz. Subst.	This product does not contain greater than 1.0% of any chemical substance on the SARA Extremely Hazardous Substances list.
SARA Section 313	This product does not contain greater than 1.0% (greater than 0.1% for carcinogenic substance) of any chemical substances listed under SARA Section 313.
CERCLA Hazardous Substances	None known.
Cal. Prop. 65	This product is not known to contain any chemicals known to the state of California to cause cancer or birth defects. However, we do not conduct routine analysis for all listed materials.
U.S. Fuel Registration	Not applicable.
U.S. Dept. of Agriculture	This product has not been filed with the USDA to support H2 approvals.
FDA Approval	Not applicable.
EEC EINES	All components are in compliance with the EC Seventh amendment Directive 92/32/EEC.
Japan MITI	All components are in compliance with the Chemical Substances Control Law of Japan.

15

REGULATORY INFORMATION (CONTINUED)**Australia**

All components are in compliance with chemical notification requirements in Australia.

Canada

All components are in compliance with the Canadian Environmental Protection Act.

**Canadian Fuel Registration
Switzerland**

Not applicable.

All components are in compliance with the Environmentally Hazardous Substances Ordinance in Switzerland.

Korea

All components are in compliance in Korea.

Korea Fuel Registration

Not applicable.

Phillippines

All components are in compliance with the Philippines Toxic Substances and Hazardous and Nuclear Wastes Control Act of 1990 (R.A. 6969).

16

OTHER INFORMATION**NFPA Code**

Health: 3 Fire: 1 Reactivity: 0

HMIS Code

Health: 1 Fire: 1 Reactivity: 0

Precautionary Labels

- This material has no known hazards.

Revision Indicators

- Section 02,HAZARDOUS INGREDIENTS	Changed: 11/03/93
- Section 05,FLASH POINT	Changed: 07/03/95
- Section 07,HANDLING PROCEDURES	Changed: 07/03/95
- Section 08,CLOTHING RECOMMENDATIONS	Changed: 07/03/95
- Section 09,VISCOSITY	Changed: 07/03/95
- Section 10,THERMAL DECOMPOSITION	Changed: 07/26/94

The information presented herein has been compiled from sources c Corporation's knowledge; however, The Lubrizol Corporation makes **MERCHANTABILITY OR FITNESS FOR THE PARTICULAR PURPOSE**, obtained from the use thereof. The Lubrizol Corporation assumes no damage to any property and recipient assumes all such risks.

Study Title

Acute Toxicity of Lubricant Additive OS# 87319
To The Mysid, *Mysidopsis bahia*

Authors

Timothy J. Ward
Robert L. Boeri

Study Initiated

January 10, 1992

Study Completed

March 4, 1992

Sponsor

The Lubrizol Corporation
29400 Lakeland Boulevard
Wickliffe, Ohio 44092

Testing Facility

EnviroSystems Division
Resource Analysts, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

I. GOOD LABORATORY PRACTICE STATEMENT

This study was performed following Good Laboratory Practices as defined in 40 CFR part 792 and the OECD (1981). Neither the Study Director nor the sponsor are aware of any circumstances that would affect the integrity of this study. The screening test was not conducted with duplicate test vessels at each concentration and adult rather than juvenile mysids were used for the test. The photoperiod was 16 hours light/8 hours dark rather than 14 hours light/10 hours dark. Temperature was recorded continuously in a representative beaker rather than a test vessel. No other deviations were made from the protocol.

Timothy J. Ward 3/4/92
Timothy J. Ward
Study Director and Author

Robert L. Boeri 3-4-92
Robert L. Boeri
Coauthor

Jeanne P. Magazu 3-4-92
Jeanne P. Magazu
Aquatic Toxicologist

Peter L. Kowalski 3-4-92
Peter L. Kowalski
Aquatic Toxicologist

Ellen J. Stanford 3-4-92
Ellen J. Stanford
Aquatic Toxicologist

II. QUALITY ASSURANCE STATEMENT

Submitted by: EnviroSystems Division
Resource Analysts, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

Certification: This study meets requirements of 40 CFR part 792 and the OECD (1981). Data presented in this report were derived by methods and with materials identified in the section of the report entitled "Methods and Materials." The test was performed in accordance with EnviroSystems Protocol 91179-LU and the Product Registration Aquatic Toxicology Laboratory Standard Operating Procedures Manual with the exceptions noted on page 2. The toxicity test was performed by Ellen Stanford, Peter Kowalski, Jeanne Magazu, Robert Boeri, and Timothy Ward. The final report and raw data will be archived at EnviroSystems.

All data transcribed from the raw data to the report were checked for accuracy and all data were verified by Quality Assurance Auditors. The following quality assurance audits were performed:

	Audit Date	Reported to Study Director	Reported to Management
Protocol	10/24/91	10/24/91	--
In-Life	1/15/92	1/15/92	3/ 4/92
Draft Report	2/26/92	2/26/92	3/ 4/92
	3/ 4/92	3/ 4/92	3/ 4/92

Arthur P. ... 3-4-92
Quality Assurance Representative

III. TABLE OF CONTENTS

SECTION:	PAGE
I. Good Laboratory Practice Statement	2
II. Quality Assurance Statement	3
III. Table of Contents	4
IV. Index of Tables	5
V. Summary	6
VI. Methods and Materials	7
VII. Results	11
VIII. References	14
APPENDIX:	
Appendix A. Water Quality Data from Toxicity Test	15

IV. INDEX OF TABLES

	PAGE
Table 1. Chemical characterization of a representative sample of natural seawater used as dilution water for toxicity test	8
Table 2. Survival and sublethal effect data from toxicity test	12
Table 3. Median lethal concentrations (LC50s) from toxicity test	13
Table A.1. Salinity, dissolved oxygen concentration, pH, and temperature measured during toxicity test	16

V. SUMMARY

The acute toxicity of OS# 87319 to the mysid, *Mysidopsis bahia* is described in this final report. The test was conducted for Lubrizol Corporation for 96 hours during January 13 to 17, 1992, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Ellen Stanford, Peter Kowalski, Jeanne Magazu, Robert Boeri, and Timothy Ward according to EnviroSystems Protocol Number 91179-LU.

The test was performed under static renewal conditions with five concentrations of test substance and a dilution water control at a temperature of $24 \pm 1^\circ\text{C}$. The dilution water was filtered natural seawater collected from the Atlantic Ocean at Hampton, New Hampshire and adjusted to a salinity of 20 ± 1 ppt (parts per thousand). Nominal concentrations of the test substance were: 0 mg/L (control), 150, 250, 400, 600, and 1,000 mg/L. A mixer (approximately 1,000 rpm) was used to continuously draw floating test substance from the surface down into the dilution water in each test vessel. Nominal concentrations were used for all calculations.

Mysids used in the test were less than 24 hours old at the start of the test. They were produced at EnviroSystems from a culture originally procured from a commercial supplier (Aquatic Research Organisms, Hampton, New Hampshire). After 96 hours of exposure the control organisms had an average wet weight (blotted dry) of 1.5 mg. All mysids were in good condition at the beginning of the study.

Exposure of test organisms to the test substance resulted in a 96 hour LC50 greater than 1,000 mg/L OS# 87319. The 96 hour no observed effect concentration is greater than 1,000 mg/L OS# 87319.

VI. METHODS AND MATERIALS

TEST SUBSTANCE:

OS# 87319 (EnviroSystems Samples Number 5097E) was delivered to EnviroSystems on October 15, 1991. The sample was contained in a 250 ml plastic bottle that was labelled with the following information: "OS# 87319A, Order: 114382-003, 07 Oct. 91". The test substance (a clear yellow liquid) was shipped from Lubrizol Corporation, 29400 Lakeland Boulevard, Wickliffe, Ohio 44092. Prior to use the test material was stored in the dark at room temperature. Reserve samples will be retained at EnviroSystems for a minimum of 10 years, and unused test substance is returned to the sponsor.

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 20 ± 1 ppt and stored in a 500-gallon polyethylene tank where it was aerated. Results of chemical analysis of a representative sample of water are presented in Table 1.

TEST ORGANISM

Juvenile mysids (less than 24 hours old) employed as test organisms were from a single source and were identified using an appropriate taxonomic key. They were produced at EnviroSystems from a culture originally procured from a commercial supplier (the Aquatic Research Organisms Division of Resource Analysts, Inc., Hampton, New Hampshire). Control organisms were weighed at the conclusion of the toxicity test. Mysids were not treated for disease and they were apparently free of sickness, injuries, and abnormalities at the beginning of the test. Mysids were fed newly hatched *Artemia salina* nauplii (EnviroSystems lot number BS02) once or twice daily before and during the test. During the 14 days immediately preceding the start of the definitive toxicity test the temperature of the culture was 24.2 to 25.0°C and the dissolved oxygen concentration was always at least 7.3 mg/L.

TOXICITY TESTING:

A screening test with the test substance was conducted during January 8 to 12, 1992. Nominal concentrations of test substance were 0 mg/L (control), 0.1, 1, 10, 100, and 1,000 mg/L. After 96 hours of exposure there was 100% survival at all concentrations.

Table 1. Chemical characterization of a representative sample of natural seawater used as dilution water for toxicity test.

Parameter	Unit of Measurement	Reporting Limit	Measured Value
pH	pH units	--	8.0
Salinity	parts per thousand	--	20
Total organic carbon	mg/L	1.0	0 hr 96 hr ND ND
Particulate matter	mg/L	10	0 hr 96 hr 34 31
Organochlorine pesticides	ug/L	2	ND
Polychlorinated biphenyls	ug/L	1	ND

- Notes: 1. ND = Not detected above the reporting limit.
 2. pH and salinity were measured in dilution water collected from the control test vessel at the beginning of the test. Total organic carbon and particulate matter (total suspended solids) were measured in dilution water from a control test vessel collected at the beginning and end of the test. Pesticide and PCB data is collected during routine biannual water quality testing.

The definitive toxicity test was performed during January 13 to 17, 1992 according to EnviroSystems Test Protocol 91179-LU (Acute Toxicity of Lubricant Additive OS# 87319 to the Mysid, *Mysidopsis bahia*). The protocol, which was signed by the Study Director on January 10, 1992, is based on procedures of the U.S. Environmental Protection Agency (1985, 1989) and the OECD (1984a, 1984b). The test was conducted at a target temperature of $24 \pm 1^\circ\text{C}$ with five concentrations of test material and a dilution water control. No stock solution was prepared and test substance was added directly to dilution water without the use of a solvent (because test substance adhered well to the weigh boats, the boats were submerged in the test vessels after an attempt had been made to transfer the material from the boats to the dilution water in the test vessels). Nominal concentrations were: 0 mg/L (control), 150, 250, 400, 600, and 1,000 mg/L OS# 87319.

Ten test organisms were distributed to each of two replicates of each treatment. The test was performed in 20 liter glass aquaria that contained 15 liters of media (water depth was approximately 18 cm). Each test vessel was equipped with a cylinder (approximately 8 cm in diameter) with two openings. One opening extended from approximately 1 cm above the water surface to approximately 1 cm below the surface and the second opening was located approximately 1 to 4 cm above the bottom. A three bladed propeller inside this cylinder revolved at a nominal speed of approximately 1,000 rpm to draw water in through the surface openings and discharge it through the bottom openings. The test substance was added into the cylinder after the mixer was switched on. Mysids were contained in a cage that consisted of a glass cylinder closed at the bottom with Nitex screen. The cage was suspended in the test vessels and extended from the surface approximately one third of the way to the bottom.

Media was renewed in each test vessel at approximately 24 hour intervals. Test vessels were randomly arranged in a walk-in incubator during the 96 hour test (a random numbers table was used to select the location of each vessel). A 16 hour light and 8 hour dark photoperiod was automatically maintained during the entire test. Cool-white fluorescent lights provided a light intensity of $30 \text{ uEs}^{-1} \text{ m}^{-2}$.

The number of surviving organisms and the occurrence of sublethal effects (immobilization, 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 pH 12 meter; instrument number PRL-4), salinity (refractometer, instrument number PRL-7), and temperature (ASTM mercury thermometer; thermometer number 1686) were measured and recorded daily in each test chamber that contained live animals. The temperature was continuously recorded in a representative beaker placed with the test in the incubator.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques, when warranted. Computer methods (Stephan, 1983) were used to calculate the median lethal concentrations (LC50s). All calculations were performed by the author using nominal concentrations of test substance. The no observed effect concentration is the highest concentration of test substance at and below which at least 90% survival of exposed organisms occurred and sublethal effects were not observed.



VII. RESULTS

All non-control test vessels containing OS# 87319 had insoluble droplets of test material on the bottom of the test vessel throughout the test. After 24 hours of exposure the test vessels also had an oily surface slick that remained throughout the test. Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Table A.1, respectively. Ninety five percent survival occurred in the control exposure. Control organisms had an average wet weight (blotted dry) of 1.5 mg at the end of the test, resulting in a loading rate of 0.001 g/L.

The 24, 48, 72, and 96 hour LC50s for mysids exposed to OS# 87319 are presented in Table 3. The 96 hour LC50 is greater than 1,000 mg/L OS# 87319. The no observed effect concentration is greater than 1,000 mg/L OS# 87319.

Table 2. Survival and sublethal effect data from toxicity test.

Nominal concentration of test substance	Rep	Number of Survivors					Number Affected				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 mg/L (control)	1	10	10	9	9	9	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
150 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
250 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
400 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
600 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
1,000 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	9	9	9	9	0	0	0	0	0



Table 3. Median lethal concentrations (LC50s) from toxicity test.

Exposure period	LC50	95 percent confidence limit	LC50 calculation method
24 hours	>1,000 mg/L	--	--
48 hours	>1,000 mg/L	--	--
72 hours	>1,000 mg/L	--	--
96 hours	>1,000 mg/L	--	--



VIII. REFERENCES

- Stephan, C.E. 1983. Computer Program for Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
- OECD. 1981. OECD Guidelines for Testing of Chemicals. Annex 2. OECD Principals of Good Laboratory Practice. Adopted 1 June 1981.
- OECD. 1984a. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 202, Daphnia sp., Acute Immobilisation Test and Reproduction Test. Adopted 4 April 1984.
- OECD. 1984b. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 203, Fish Acute Toxicity Test. Adopted 4 April 1984.
- U.S. EPA. 1985. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Federal Register, Friday, September 27, 1985.
- U.S. EPA. 1987. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Amendments. Federal Register, Wednesday, May 20, 1987.
- U.S. EPA. 1989. 40 CFR Part 792. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Final Rule. Federal Register, Thursday, August 17, 1989.

Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

EnviroSystems Study Number 91179-LU
Page 15 of 17

Resource Analysis, Inc. 

PSCTOX000034459

Table A.1. Salinity, dissolved oxygen concentration, pH, and temperature measured during toxicity test.

Nominal concentration of test substance	Rep.	Salinity (ppt)						Dissolved oxygen (% saturation)					
		0 hr	24 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	24 hr	48 hr	72 hr	96 hr
0 mg/L (control)	1	20	20	20	20	20	20	90	86	89	88	90	87
	2	20	20	20	20	20	20	90	87	89	87	89	88
150 mg/L	1	20	20	20	20	20	20	90	86	89	88	89	88
	2	20	20	20	20	20	20	90	84	89	87	88	87
250 mg/L	1	20	20	20	20	20	20	90	86	89	90	89	86
	2	20	20	20	20	20	20	90	84	89	87	89	88
400 mg/L	1	20	20	20	20	20	20	90	86	89	86	90	88
	2	20	20	20	20	20	20	90	86	89	86	89	87
600 mg/L	1	20	20	20	20	20	20	90	87	89	88	88	87
	2	20	20	20	20	20	20	90	86	89	89	90	88
1,000 mg/L	1	20	20	20	20	-	-	90	86	89	89	89	86
	2	20	20	20	20	20	20	90	86	89	90	89	87

Notes: 1. All measurements were made before media renewal except the second 24 hour measurement which was made after renewal.
 2. Dissolved oxygen saturation is 8.4 mg/L at 24°C. Percent saturation is calculated as the actual dissolved oxygen concentration in mg/L divided by 8.4 and multiplied by 100.

Table A.1. Continued.

Nominal concentration of test substance	Rep.	Temperature (°C)						pH					
		0 hr	24 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	24 hr	48 hr	72 hr	96 hr
0 mg/L (control)	1	23.3	24.9	24.8	24.6	23.6	23.2	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.4	24.8	24.7	24.4	23.7	23.1	8.0	8.1	8.0	8.5	8.0	7.9
150 mg/L	1	23.4	24.8	24.7	23.7	23.3	23.3	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.3	24.9	24.8	24.5	23.5	23.2	8.0	8.1	8.0	8.5	8.0	7.9
250 mg/L	1	23.3	24.8	24.7	24.0	23.3	23.2	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.3	24.7	24.7	24.4	23.4	23.3	8.0	8.1	8.0	8.5	8.0	7.9
400 mg/L	1	23.4	24.7	24.6	24.8	24.6	23.2	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.4	24.8	24.7	24.8	24.6	23.1	8.0	8.1	8.0	8.5	8.0	7.9
600 mg/L	1	23.3	24.9	24.7	23.5	23.4	23.3	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.5	24.8	24.8	23.6	23.5	23.3	8.0	8.1	8.0	8.5	8.0	7.9
1,000 mg/L	1	23.4	24.9	24.8	23.7	23.2	23.2	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.3	24.7	24.8	24.2	23.2	23.3	8.0	8.1	8.0	8.5	8.0	7.9

Note: All measurements were made before media renewal except the second 24 hour measurement which was made after renewal.



WILDLIFE INTERNATIONAL LTD.

PROJECT NO.: 331A-103

OS 87319:
A 96-HOUR STATIC RENEWAL ACUTE TOXICITY TEST
WITH THE SHEEPSHEAD MINNOW (*Cyprinodon variegatus*)

FINAL REPORT

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 331A-103

TITLE: A 96-Hour Static Renewal Acute Toxicity Test
with the Sheepshead Minnow (*Cyprinodon variegatus*)

TITLE 40 OF THE CODE OF FEDERAL REGULATIONS
Part 797.1440

TITLE 40 OF THE CODE OF FEDERAL REGULATIONS
PART 797.1930

This study was conducted so as to conform with Good Laboratory
Practice Standards as published in the Environmental Protection
Agency, Office of Pesticide Programs, in 40 CFR Part 160, 17 August 1989,
with the following exception:

OECD GUIDELINE 203

AUTHORS:

Daniel Murphy
Catherine M. Holmes
Gregory J. Smith, Ph.D.

STUDY INITIATION DATE: November 12, 1991

STUDY COMPLETION DATE: March 11, 1992

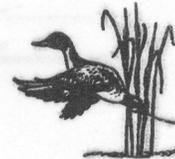
Submitted to

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Wickliffe, Ohio 44092



WILDLIFE INTERNATIONAL LTD.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600



GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: The Lubrizol Corporation

TITLE: OS 87319: A 96-Hour Static Renewal Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 331A-103

STUDY COMPLETION DATE: March 11, 1992

This study was conducted so as to conform with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 792, 17 August 1989, with the following exception:

Water samples were not analyzed to verify test concentrations.

STUDY DIRECTOR:

Catherine M. Holmes
Catherine M. Holmes
Aquatic Toxicology Laboratory Coordinator

DATE: 3/11/92

REPORT APPROVED BY:

Steven P. Lynn
Steven P. Lynn, Ph.D.
Laboratory Management

DATE: 3/11/92

Sponsor

DATE: _____

Applicant/Submitter

DATE: _____

- 3 -

QUALITY ASSURANCE STATEMENT

This study was examined for conformance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 792. The dates of all audits and the date that the results of those audits were reported to the Study Director/Laboratory Management were as follows:

TYPE AUDIT:	DATE OF AUDIT:	DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT:
Aquatic Toxicology Laboratory		
Procedure (Test substance preparation and mixing of test substance with dilution water)	January 21, 1992	January 22, 1992
Data	March 1, 1992	March 1, 1992
Draft	March 1, 1992	March 1, 1992
Final Report	March 11, 1992	March 11, 1992

QUALITY ASSURANCE ARCHIVES: Raw data and an authenticated copy of the final report are filed under Wildlife International Ltd. Project Number 331A-103 in the Wildlife International Ltd. archives.



Susan J. Palmer
Quality Assurance Officer

DATE 3-11-92

TABLE OF CONTENTS

Title/Cover Page.....	Page 1
Good Laboratory Practice Compliance Statement.....	Page 2
Quality Assurance Statement.....	Page 3
Table of Contents.....	Page 4
Summary.....	Page 6
Introduction.....	Page 7
Objective.....	Page 7
Experimental Design.....	Page 7
Materials and Methods.....	Page 8
Results and Discussion.....	Page 12
Conclusion.....	Page 13
References.....	Page 14

TABLES

Table 1. Temperature, Dissolved Oxygen, Salinity, and pH of Water in the Test Chambers.....	Page 15
Table 2. Cumulative Mortality, Percent Mortality, and Treatment-Related Effects.....	Page 17
Table 3. LC50 Values Calculated During the Test.....	Page 18

TABLE OF CONTENTS

SPONSOR:	The Lubrizol Corporation	PAGE 2
CONTACT:	Dr. Robert Meyer	
LOCATION OF STUDY:	Wildlife International Ltd.	
TEST DATE:	1972	
Figure 1	Sheepshead Minnow Test Exposure System.....	Page 19

APPENDICES

Appendix I	Dilution Water Chemistry Parameters for the 4-Week Period Immediately Preceding the Test.....	Page 20
Appendix II	Analyses of Pesticides in Wildlife International Ltd. Saltwater.....	Page 21
Appendix III	Analyses of Metals in Wildlife International Ltd. Saltwater.....	Page 22
Appendix IV	Changes in Protocol.....	Page 23
Appendix V	Personnel Involved in the Study.....	Page 24

TOXIC LEVEL:	1000 mg/l
NO TOXICITY CONCENTRATION:	100 mg/l
NO OBSERVED EFFECT CONCENTRATION:	100 mg/l
TEST ORGANISM:	Sheepshead Minnow (Gambusia affinis holbrooki)
SOURCE OF TEST ORGANISMS:	Wildlife International Ltd., 5000 Centre Drive, Canton, MA 01030
AGE:	Juvenile
WEIGHTS:	Group 1: 0.27 g to 0.40 g (30 Controls)
STANDARD LENGTH:	Group 1: 25 mm to 31 mm (30 Controls)

- 7 -

INTRODUCTION

This study was conducted by Wildlife International Ltd. for The Lubrizol Corporation, at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The test was conducted from January 21, 1992 through January 25, 1992. Raw data and a certified copy of the final report are filed under Project Number 331A-103 in archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of this study was to evaluate the acute toxicity of OS 87319 to the sheepshead minnow (*Cyprinodon variegatus*) during a 96-hour exposure period under daily static renewal test conditions.

EXPERIMENTAL DESIGN

Sheepshead minnows were exposed to a geometric series of five test concentrations and a negative (saltwater) control under static renewal test conditions for 96 hours. Two replicate test chambers were maintained in each treatment and control group, with 10 fish in each test chamber. Nominal test concentrations were selected in consultation with the sponsor, and were based upon results from a range finding test. Nominal test concentrations selected were 130, 216, 360, 600, and 1000 mg/L.

Immediately prior to initiating the test, fish were impartially removed from a holding tank in groups of two and distributed among the test chambers until each contained 10 fish. Observations of mortality and other clinical signs of toxicity were made 3, 6, 24, 48, 72, and 96 hours after test initiation. Cumulative percent mortality values in each treatment group were used to calculate LC50 values and 95% confidence limits at 24, 48, 72, and 96 hours. The no mortality concentration and

- 8 -

no observed effect concentration were determined by visually examining results of mortality and clinical observations.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, OS 87319: A 96-Hour Static Renewal Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*). The protocol was based in part on procedures outlined in TSCA Title 40 of the Code of Federal Regulations, Part 797.1440 (1), TSCA Title 40 of the Code of Federal Regulations, Part 797.1930 (2), ASTM Standard E-729-88, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (3), and OECD, Guideline for Testing of Chemicals, 203 (4).

Test Substance

The test substance was received in two shipments from The Lubrizol Corporation on October 21, 1991 and on December 17, 1991. The test substance was a yellow liquid and was maintained at ambient room temperature during storage. The shipment, received October 21, 1991, was identified on the label as: EXPERIMENTAL PRODUCT, OS #87319A, ORDER: 114647-004, 17 OCT 91 and was assigned Wildlife International Ltd. Identification Number WIL-2133 upon receipt. The second shipment was identified on the label as: EXPERIMENTAL PRODUCT, OS #87319A, ORDER: 116110-002, 12 DEC 91 and was assigned Wildlife International Ltd. Identification Number WIL-2169 upon receipt. A Material Safety Data Sheet supplied by the Sponsor indicated that the test substance was insoluble in water.

Preparation of Test Concentrations

Portions of the test substance were removed from the test substance container and individually weighed in glass beakers. Test concentrations used during the test were prepared by mixing the appropriate amount of

neat test substance directly into the saltwater contained in each test chamber.

Test Organism

The sheepshead minnow (*Cyprinodon variegatus*) was selected as the test species for this study. The sheepshead minnow was considered representative of an important group of estuarine vertebrates and was selected for use in this test based upon past history of use, and ease of culturing in the laboratory. Fish used in the test were juveniles obtained from cultures maintained by Wildlife International Ltd. at 8651 Brooks Drive, Easton, Maryland 21601.

The juvenile sheepshead minnows were held in cultures for at least 14 days prior to testing. The water used during the 14-day holding period was obtained from the same source and maintained at the same temperature as water used during the test. The test fish were acclimated to test conditions for approximately 74 hours prior to test initiation. While two mortalities occurred during acclimation, all other fish were normal in appearance and behavior throughout the holding or acclimation periods and showed no signs of disease or stress.

All fish used in the test were from the same source and year class. The lengths of 10 control fish were measured at the end of the test, and it was determined that the standard length of the longest fish measured was no more than twice the standard length of the shortest. The average length was 28 mm with a range of 25 mm to 32 mm, while the average weight was 0.67 g with a range of 0.45 g to 1.00 g. Instantaneous loading in the test chambers was calculated to be 0.17 grams of fish per liter of test water.

The juvenile sheepshead minnows were fed from hatch until approximately 89 hours prior to test initiation. During that time the fish

- 10 -

were fed flaked fish food (Zeigler Brothers, Inc., Gardners, PA 17324). Feed was withheld during the test.

Test Apparatus

Test chambers were 60 liter glass aquaria containing 40 L of the test or control saltwater. Within each test chamber a slotted PVC cylinder extended from the surface of the water to the bottom of the aquarium. An electric mixer able to run at approximately 1000 rpm was mounted above each PVC cylinder with a mixing shaft extending into the center of the cylinder. The juvenile fish were protected from the mixer by placing them in a cage within each aquarium. Each cage was approximately 20 cm in diameter and 22.5 cm tall, and constructed with 1100 μm nytex screen. The bottom section was glass with a lip approximately 10 cm high that was designed to hold sufficient water to assist the transfer of the minnows from tank to tank during renewal of the test water. A similar cage was placed around the PVC cylinder. Test chambers were positioned in an environmentally controlled room designed to maintain a temperature of $22 \pm 1^\circ\text{C}$. Test chambers were labeled with the project number, test concentration, and replicate.

Dilution Water

The water used for holding, acclimation, and testing was natural seawater collected at Indian River Inlet, Delaware, and diluted to the appropriate salinity with well water. Salinity and pH measurements taken weekly during the four-week period immediately preceding the test are presented in Appendix I.

The freshly collected seawater was passed through a sand filter to remove particles greater than approximately 25 μm and was pumped into a 37,800-L storage tank. The filtered seawater then was diluted with fresh water from a well located on the Wildlife International Ltd. site and aerated with spray nozzles. Water used to fill the test aquaria was pumped from the storage tank and filtered to remove microorganisms and

- 11 -

particles. Results of analyses performed to measure concentrations of pesticides and metals in saltwater used by Wildlife International Ltd. are presented in Appendix II and III, respectively.

Environmental Conditions

Ambient room light used to illuminate the aquaria during holding, acclimation and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (e.g., Chroma 50). Light intensity was measured at 0 and 96 hours during the experiment and averaged approximately 31 footcandles (range = 24 to 40 footcandles) at the surface of the water in the negative control replicates. The photo-period of 16 hours of light and 8 hours of darkness was controlled with an automatic timer.

Water temperatures during the 14-day holding period ranged from 21.2°C to 21.8°C, and any changes did not exceed 3°C during any 72-hour period. The salinity of the holding water ranged from 24 ‰ to 26 ‰, and the pH ranged from 7.6 to 7.8.

Water temperature was measured continuously throughout the test in one negative control replicate using a Fulscope ER/C Recorder. Temperature also was measured in each test chamber with a hand-held calibrated thermometer at 24-hour intervals during the test. The target test temperature during the study was 22±1°C. Dissolved oxygen and pH were measured in each replicate of each treatment and control group at 24-hour intervals during the test. The salinity of the water was measured in each replicate test chamber at the beginning and end of the test.

Statistical Analyses

The mortality data were evaluated by calculating the LC50 value with the computer program of C. E. Stephan (5). The program was designed to calculate the LC50 and the 95% confidence interval by probit analysis, the moving average method, or binomial probability (6, 7, 8). In this

- 12 -

study the mortality pattern did not facilitate calculation of the LC50 value, and therefore an estimation of the LC50 value was made by a visual inspection of the mortality data.

RESULTS AND DISCUSSION

Observations and Measurements

Measurements of temperature, dissolved oxygen, pH, and salinity are presented in Table 1. Water temperatures taken daily with a hand-held thermometer, ranged from 21.5 to 24.1°C, and occasionally exceeded the established test temperature range (22±1°C). Deviations from the established range did not appear to affect the results of the study. Dissolved oxygen concentrations exceeded 60% of saturation throughout the test.

After the test substance, OS 87319, was added to the vortex a cloudy solution was formed. The degree of cloudiness varied according to concentration and decreased over time. Oil-like droplets were noted on the water surface and on the bottom of the test tanks, and the test solution adhered to glass.

Daily observations of mortality and other signs of toxicity used to evaluate the effects of the test substance on sheepshead minnows are shown in Table 2. LC50 values and 95% confidence limits for the 24, 48, 72, and 96-hour intervals were calculated from the mortality data, and are shown in Table 3.

Fish in the negative control group appeared normal and healthy throughout the test with no signs of disease or stress. Similarly, fish in the 130, 216, 360, 600, and 1000 mg/L treatment groups also appeared normal throughout the test with no clinical signs of toxicity.

- 13 -

CONCLUSION

The 96-hour LC50 value for sheepshead minnows exposed to OS 87319 was greater than 1000 mg/L, the highest concentration tested. Based on a visual interpretation of the data, the no mortality concentration and no observed effect concentration were 1000 mg/L.

- 14 -

REFERENCES

- 1 Title 40 of the Code of Federal Regulations, Part 797, Section 1440, Fish Acute Toxicity Test, July, 1989.
- 2 Title 40 of the Code of Federal Regulations, Part 797, Section 1930, Mysid Shrimp Acute Toxicity Test, July, 1989.
- 3 ASTM Standard E 729-88, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians, American Society for Testing and Materials, 1988.
- 4 OECD, Guideline for Testing of Chemicals. 203: Fish. Acute Toxicity Tests, April 4, 1984.
- 5 Stephan, C.E. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota, 1978. Personal communication.
- 6 Finney, D.J. Statistical Methods in Biological Assay, second edition. Griffin Press, London. 1971.
- 7 Thompson, W.R. Bacteriological Reviews, Vol. II, No. 2. June, 1947. Pp. 115-145.
- 8 Stephan, C.E. "Methods for Calculating an LC50," Aquatic Toxicology and Hazard Evaluations, American Society for Testing and Materials. Publication Number STP 634 (1977), pp 65-84.

Table I
Temperature, Dissolved Oxygen, Salinity, and pH of Water in the Test Chambers⁽¹⁾

Nominal Concentration (mg OS 87319/L)	Replicate	0 HOURS			24 HOURS			48 HOURS						
		TEMP (°C)	DISSOLVED OXYGEN ⁽²⁾ (mg/L) % Saturation	pH	Salinity (‰)	TEMP (°C)	DISSOLVED OXYGEN (mg/L) % Saturation	pH	TEMP (°C)	DISSOLVED OXYGEN (mg/L) % Saturation	pH			
Negative Control	A	21.5 ⁽³⁾	7.4	97 ⁽⁴⁾	8.2	25	22.5	6.8	89	8.1	22.3	7.0	92	8.1
	B	21.7	7.4	97	8.2	25	22.6	6.8	89	8.1	22.3	7.0	92	8.1
130	A	22.4	7.4	97	8.2	25	23.7	6.8	89	8.1	22.0	7.0	92	8.1
	B	22.4	7.2	95	8.2	25	24.0	6.8	89	8.1	22.0	6.8	89	8.1
216	A	22.0	7.4	97	8.2	25	23.3	6.6	87	8.1	22.0	6.8	89	8.1
	B	22.4	7.4	97	8.2	24	24.1	6.6	87	8.1	22.0	6.8	89	8.1
360	A	22.3	7.4	97	8.2	25	23.5	6.8	89	8.1	22.2	6.8	89	8.1
	B	22.3	7.4	97	8.2	25	23.1	6.8	89	8.1	22.2	6.8	89	8.2
600	A	22.5	7.2	95	8.2	25	23.2	6.6	87	8.1	21.9	6.8	89	8.1
	B	22.3	7.4	97	8.2	25	24.1	6.8	89	8.1	21.8	6.8	89	8.1
1000	A	22.5	7.3	96	8.2	25	24.0	6.6	87	8.1	22.3	6.8	89	8.1
	B	22.4	7.4	97	8.2	25	23.7	6.6	87	8.1	22.5	6.8	89	8.1

⁽¹⁾ All measurements taken prior to daily renewal of test solutions.

⁽²⁾ A dissolved oxygen concentration of 4.5 mg/L represents 60% saturation at 22°C and at a salinity of 25 ‰.

⁽³⁾ Temperature measured continuously in Negative Control Replicate A ranged from approximately 20.6°C to 23.3°C.

⁽⁴⁾ A dissolved oxygen concentration of 7.6 mg/L represents 100% saturation at 22°C and at a salinity of 25 ‰.

Table 1 (Continued)
 Temperature, Dissolved Oxygen, Salinity, and pH of Water in the Test Chambers⁽¹⁾

Sponsor: The Lubrizol Corporation		72 HOURS		96 HOURS						
Test Substance: OS 87319	Test Organism: Sheepshead Minnow (<i>Cyprinodon variegatus</i>)	TEMP (°C)	DISSOLVED OXYGEN ⁽²⁾ (mg/L) % Saturation	TEMP (°C)	DISSOLVED OXYGEN (mg/L) % Saturation					
Dilution Water: Seawater	Replicate		pH		Salinity (‰)					
Nominal Concentration (mg OS 87319/L)	A	22.3 ⁽³⁾	7.4	97 ⁽⁴⁾	8.1	22.4	7.8	103	8.0	25
	B	22.4	7.4	97	8.1	22.2	7.8	103	8.0	25
130	A	23.3	7.2	95	8.1	22.3	7.8	103	8.0	25
	B	23.6	7.4	97	8.1	22.2	7.8	103	8.0	25
216	A	23.0	7.4	97	8.1	22.5	7.8	103	8.1	25
	B	23.3	7.4	97	8.1	22.8	7.8	103	8.1	25
360	A	22.9	7.0	92	8.2	22.2	7.8	103	8.1	25
	B	22.9	7.2	95	8.2	22.0	7.8	103	8.0	25
600	A	23.1	7.0	92	8.2	23.0	7.6	100	8.1	25
	B	23.1	7.0	92	8.2	22.7	7.6	100	8.1	25
1000	A	23.6	6.6	87	8.1	22.5	7.6	100	8.1	25
	B	23.5	6.6	87	8.1	22.7	7.6	100	8.1	25

⁽¹⁾ All measurements taken prior to daily renewal of test solutions.

⁽²⁾ A dissolved oxygen concentration of 4.5 mg/L represents 60% saturation at 22°C and at a salinity of 25 ‰.

⁽³⁾ Temperature measured continuously in Negative Control Replicate A ranged from approximately 20.6°C to 23.3°C.

⁽⁴⁾ A dissolved oxygen concentration of 7.6 mg/L represents 100% saturation at 22°C and at a salinity of 25 ‰.

Table 2
Cumulative Mortality, Percent Mortality, and Treatment-Related Effects⁽¹⁾

Nominal Concentration (mg OS 87319/L)	Replicate	Number of Dead / Number Exposed							Cumulative Total	Total Percent Mortality
		3 Hours	6 Hours	24 Hours	48 Hours	72 Hours	96 Hours			
Negative Control	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
130	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
216	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
360	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
600	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
1000	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0

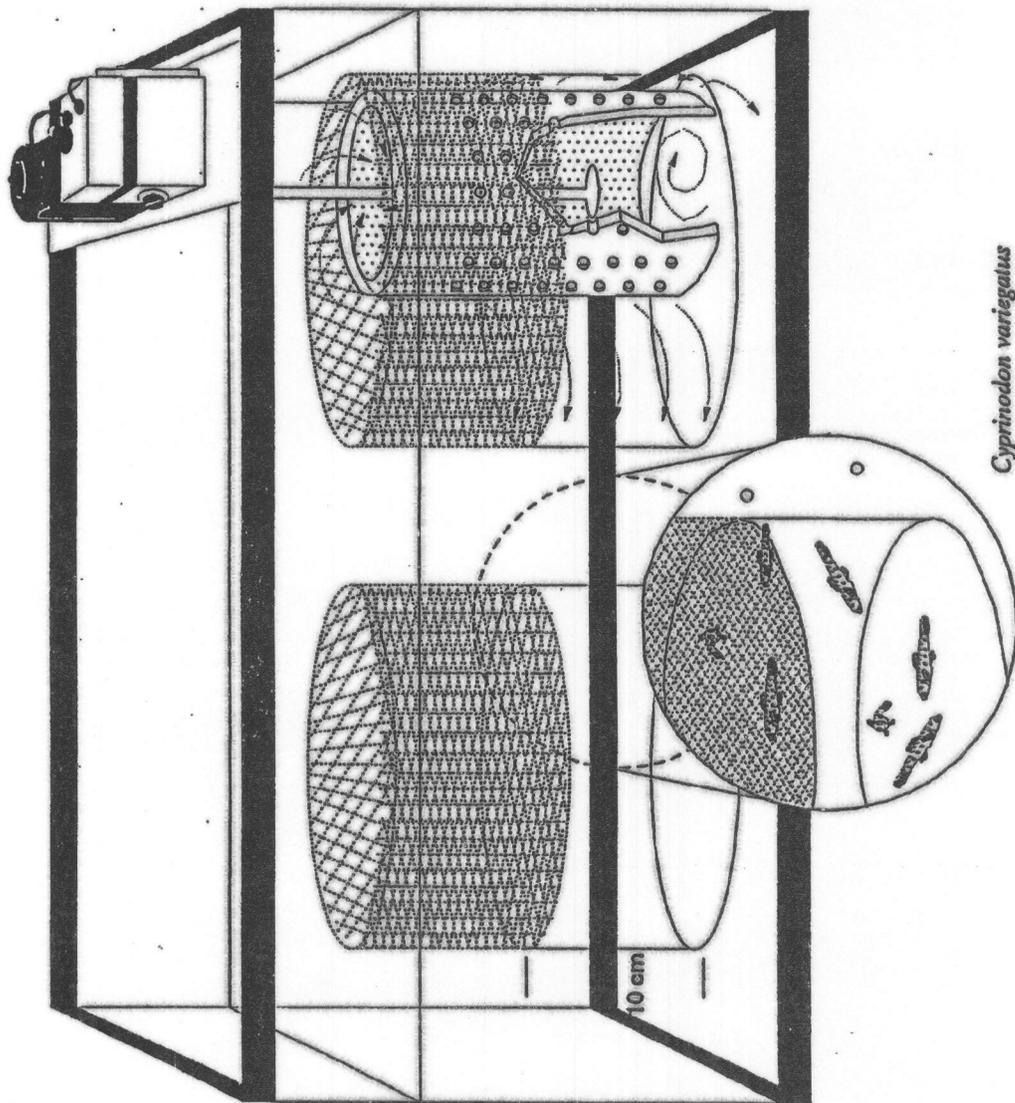
⁽¹⁾ Observed Effects: All of the organisms in the control and treatment groups appeared normal throughout the test period.

The 96-hour LC50 was >1000 mg/L.

Table 3
LC50 Values Calculated During the Test

Sponsor:	The Lubrizol Corporation			
Test Substance:	OS 87319			
Test Organism:	Sheepshead Minnow (<u>Cyprinodon variegatus</u>)			
Dilution Water:	Seawater			
Time	LC50 (mg OS 87319/L)	Lower 95% Confidence Limits	Upper 95% Confidence Limits	Method
24 Hours	>1000	N/A ⁽¹⁾	N/A	Visual Inspection
48 Hours	>1000	N/A	N/A	Visual Inspection
72 Hours	>1000	N/A	N/A	Visual Inspection
96 Hours	>1000	N/A	N/A	Visual Inspection
⁽¹⁾ N/A= Not Applicable; the data did not facilitate the calculation of 95% confidence limits.				

Figure 1. Sheephead Minnow Test Exposure System



- 20 -

Appendix I
Dilution Water Chemistry Parameters for the 4-Week Period
Immediately Preceding the Test

Sponsor: The Lubrizol Corporation
Test Substance: OS 87319
Test Organism: Sheepshead Minnow (Cyprinodon variegatus)
Dilution Water: Seawater

	Mean	Range
Salinity (‰)	23 (n = 4)	23 - 24
pH	8.0 (n = 4)	7.8 - 8.1

Appendix II
Analyses of Pesticides in Wildlife International Ltd. Saltwater⁽¹⁾

Sponsor: The Lubrizol Corporation
 Test Substance: OS 87319
 Test Organism: Sheepshead Minnow (*Cyprinodon variegatus*)
 Dilution Water: Seawater
 Date Sampled: June 13, 1990

ANALYSIS	MEASURED CONCENTRATION	LIMIT OF QUANTITATION
Total Solids	29,700 mg/L	100 mg/L
Ammonia Nitrogen	< 0.1 mg/L	0.1 mg/L
Cyanide, Total	< 0.005 mg/L	0.005 mg/L
Total Organic Carbon	0.60 mg/L	0.5 mg/L
Pesticide screen I;II;III	< 0.02 µg/L	0.02 µg/L
Alpha BHC	< 0.02 µg/L	0.02 µg/L
Beta BHC	< 0.02 µg/L	0.02 µg/L
Gamma BHC - Lindane	< 0.02 µg/L	0.02 µg/L
Delta BHC	< 0.02 µg/L	0.02 µg/L
Heptachlor Epoxide	< 0.02 µg/L	0.02 µg/L
DDE	< 0.02 µg/L	0.02 µg/L
DDD	< 0.02 µg/L	0.02 µg/L
DDT	< 0.02 µg/L	0.02 µg/L
HCB	< 0.02 µg/L	0.02 µg/L
Mirex	< 0.02 µg/L	0.02 µg/L
Methoxychlor	< 0.5 µg/L	0.5 µg/L
Dieldrin	< 0.02 µg/L	0.02 µg/L
Endrin	< 0.02 µg/L	0.02 µg/L
Telodrin	< 0.02 µg/L	0.02 µg/L
Chlordane	< 0.1 µg/L	0.1 µg/L
Toxaphene	< 2 µg/L	2 µg/L
PCB's	< 2 µg/L	2 µg/L
Ronnel	< 0.02 µg/L	0.02 µg/L
Ethion	< 0.05 µg/L	0.05 µg/L
Trithion	< 0.1 µg/L	0.1 µg/L
Diazinon	< 0.2 µg/L	0.2 µg/L
Methyl Parathion	< 0.04 µg/L	0.04 µg/L
Ethyl Parathion	< 0.04 µg/L	0.04 µg/L
Malathion	< 0.1 µg/L	0.1 µg/L
Endosulfan I	< 0.02 µg/L	0.02 µg/L
Endosulfan II	< 0.02 µg/L	0.02 µg/L
Endosulfan Sulfate	< 0.1 µg/L	0.1 µg/L
2,4-D	< 1 µg/L	1 µg/L
2,4,5-TP	< 1 µg/L	1 µg/L

⁽¹⁾ Analyses performed by Lancaster Laboratories, Inc.

- 22 -

Appendix III
Analyses of Metals in Wildlife International Ltd. Saltwater⁽¹⁾

Sponsor: The Lubrizol Corporation
 Test Substance: OS 87319
 Test Organism: Sheepshead Minnow (Cyprinodon variegatus)
 Dilution Water: Seawater
 Date Sampled: June 25, 1990

ANALYSIS	MEASURED CONCENTRATION	LIMIT OF DETECTION
Aluminum (Al)	0.018 mg/L	0.005 mg/L
Arsenic (As)	ND ⁽²⁾	0.005 mg/L
Beryllium (Be)	ND	0.005 mg/L
Cadmium (Cd)	ND	0.0005 mg/L
Calcium (Ca)	0.3 mg/L	0.1 mg/L
Chromium (Cr)	ND	0.0010 mg/L
Copper (Cu)	0.0084 mg/L	0.0010 mg/L
Iron (Fe)	ND	0.005 mg/L
Lead (Pb)	ND	0.005 mg/L
Magnesium (Mg)	ND	0.1 mg/L
Manganese (Mn)	ND	0.005 mg/L
Mercury (Hg)	ND	0.0005 mg/L
Nickel (Ni)	0.022 mg/L	0.0010 mg/L
Potassium (K)	310 mg/L	1 mg/L
Selenium (Se)	ND	0.005 mg/L
Silver (Ag)	ND	0.0010 mg/L
Zinc (Zn)	ND	0.005 mg/L
Boron (B)	2.68 mg/L	0.05 mg/L
Cobalt (Co)	ND	0.005 mg/L
Molybdenum (Mo)	ND	0.010 mg/L

⁽¹⁾ Analyses performed by Gascoyne Laboratories, Inc.

⁽²⁾ ND = Not Detected

APPENDIX IV
CHANGES IN PROTOCOL

The study was conducted in accordance with the approved Protocol with the following changes:

1. Temperature, taken daily using a hand held thermometer, ranged from 20.8 to 24.1°C. Temperature, measured continuously in Negative Control Replicate A using a Fulscope ER/C Recorder ranged from 20.6 to 23.3°C.
2. The procedure was modified so that organisms were added approximately thirty to sixty minutes after the test substance was added to the test chambers.
3. Test methods were also based on procedures outlined in Title 40 Code of Federal Regulations, Part 797.1440, Fish Acute Toxicity Test, July, 1989.
4. The study was conducted according to GLP standards described in Title 40 CFR, Part 792, rather than in Part 160.
5. The test apparatus design was modified to include a mesh net around the mixing propeller.
6. Actual nominal test concentrations varied slightly from nominal concentrations stated in the protocol. Actual nominal concentrations for the 1000 mg/L replicates ranged from 1000 to 1002 mg/L. Actual nominal concentrations for the 600 mg/L replicates ranged from 600 to 602 mg/L.
7. Analyses of the concentrations of metals and pesticides in the dilution water were not conducted twice annually.
8. A 30-minute transition period of low light intensity was not provided at dawn and dusk.

In the opinion of the Study Director, the above changes in the approved protocol did not adversely affect the results of this study.

- 24 -

APPENDIX V
PERSONNEL INVOLVED IN THE STUDY

The following key personnel were involved in the conduct or management of this study:

Aquatic Toxicology Laboratory

1. Gregory J. Smith, Ph.D., Director, Laboratory Programs
2. Steven P. Lynn, Ph.D., Wildlife Toxicologist
3. Catherine M. Holmes, Aquatic Toxicology Laboratory Coordinator
4. Daniel Murphy, Senior Research Biologist
5. Cynthia Roberts, Senior Research Biologist
6. Carol Pacey, Research Biologist

Study Title

Acute Toxicity of Lubricant Additive OS# 87319
To The Mysid, *Mysidopsis bahia*

Authors

Timothy J. Ward
Robert L. Boeri

Study Initiated

January 10, 1992

Study Completed

March 4, 1992

Sponsor

The Lubrizol Corporation
29400 Lakeland Boulevard
Wickliffe, Ohio 44092

Testing Facility

EnviroSystems Division
Resource Analysts, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

I. GOOD LABORATORY PRACTICE STATEMENT

This study was performed following Good Laboratory Practices as defined in 40 CFR part 792 and the OECD (1981). Neither the Study Director nor the sponsor are aware of any circumstances that would affect the integrity of this study. The screening test was not conducted with duplicate test vessels at each concentration and adult rather than juvenile mysids were used for the test. The photoperiod was 16 hours light/8 hours dark rather than 14 hours light/10 hours dark. Temperature was recorded continuously in a representative beaker rather than a test vessel. No other deviations were made from the protocol.

Timothy J. Ward 3/4/92
Timothy J. Ward
Study Director and Author

Robert L. Boeri 3-4-92
Robert L. Boeri
Coauthor

Jeanne P. Magazu 3-4-92
Jeanne P. Magazu
Aquatic Toxicologist

Peter L. Kowalski 3-4-92
Peter L. Kowalski
Aquatic Toxicologist

Ellen J. Stanford 3-4-92
Ellen J. Stanford
Aquatic Toxicologist

II. QUALITY ASSURANCE STATEMENT

Submitted by: EnviroSystems Division
Resource Analysts, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

Certification: This study meets requirements of 40 CFR part 792 and the OECD (1981). Data presented in this report were derived by methods and with materials identified in the section of the report entitled "Methods and Materials." The test was performed in accordance with EnviroSystems Protocol 91179-LU and the Product Registration Aquatic Toxicology Laboratory Standard Operating Procedures Manual with the exceptions noted on page 2. The toxicity test was performed by Ellen Stanford, Peter Kowalski, Jeanne Magazu, Robert Boeri, and Timothy Ward. The final report and raw data will be archived at EnviroSystems.

All data transcribed from the raw data to the report were checked for accuracy and all data were verified by Quality Assurance Auditors. The following quality assurance audits were performed:

	Audit Date	Reported to Study Director	Reported to Management
Protocol	10/24/91	10/24/91	--
In-Life	1/15/92	1/15/92	3/ 4/92
Draft Report	2/26/92	2/26/92	3/ 4/92
	3/ 4/92	3/ 4/92	3/ 4/92


Quality Assurance Representative

III. TABLE OF CONTENTS

SECTION:	PAGE
I. Good Laboratory Practice Statement	2
II. Quality Assurance Statement	3
III. Table of Contents	4
IV. Index of Tables	5
V. Summary	6
VI. Methods and Materials	7
VII. Results	11
VIII. References	14
APPENDIX:	
Appendix A. Water Quality Data from Toxicity Test	15

IV. INDEX OF TABLES

	PAGE
Table 1. Chemical characterization of a representative sample of natural seawater used as dilution water for toxicity test	8
Table 2. Survival and sublethal effect data from toxicity test	12
Table 3. Median lethal concentrations (LC50s) from toxicity test	13
Table A.1. Salinity, dissolved oxygen concentration, pH, and temperature measured during toxicity test	16

V. SUMMARY

The acute toxicity of OS# 87319 to the mysid, *Mysidopsis bahia* is described in this final report. The test was conducted for Lubrizol Corporation for 96 hours during January 13 to 17, 1992, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Ellen Stanford, Peter Kowalski, Jeanne Magazu, Robert Boeri, and Timothy Ward according to EnviroSystems Protocol Number 91179-LU.

The test was performed under static renewal conditions with five concentrations of test substance and a dilution water control at a temperature of $24 \pm 1^\circ\text{C}$. The dilution water was filtered natural seawater collected from the Atlantic Ocean at Hampton, New Hampshire and adjusted to a salinity of 20 ± 1 ppt (parts per thousand). Nominal concentrations of the test substance were: 0 mg/L (control), 150, 250, 400, 600, and 1,000 mg/L. A mixer (approximately 1,000 rpm) was used to continuously draw floating test substance from the surface down into the dilution water in each test vessel. Nominal concentrations were used for all calculations.

Mysids used in the test were less than 24 hours old at the start of the test. They were produced at EnviroSystems from a culture originally procured from a commercial supplier (Aquatic Research Organisms, Hampton, New Hampshire). After 96 hours of exposure the control organisms had an average wet weight (blotted dry) of 1.5 mg. All mysids were in good condition at the beginning of the study.

Exposure of test organisms to the test substance resulted in a 96 hour LC50 greater than 1,000 mg/L OS# 87319. The 96 hour no observed effect concentration is greater than 1,000 mg/L OS# 87319.

VI. METHODS AND MATERIALS

TEST SUBSTANCE:

OS# 87319 (EnviroSystems Samples Number 5097E) was delivered to EnviroSystems on October 15, 1991. The sample was contained in a 250 ml plastic bottle that was labelled with the following information: "OS# 87319A, Order: 114382-003, 07 Oct. 91". The test substance (a clear yellow liquid) was shipped from Lubrizol Corporation, 29400 Lakeland Boulevard, Wickliffe, Ohio 44092. Prior to use the test material was stored in the dark at room temperature. Reserve samples will be retained at EnviroSystems for a minimum of 10 years, and unused test substance is returned to the sponsor.

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 20 ± 1 ppt and stored in a 500-gallon polyethylene tank where it was aerated. Results of chemical analysis of a representative sample of water are presented in Table 1.

TEST ORGANISM

Juvenile mysids (less than 24 hours old) employed as test organisms were from a single source and were identified using an appropriate taxonomic key. They were produced at EnviroSystems from a culture originally procured from a commercial supplier (the Aquatic Research Organisms Division of Resource Analysts, Inc., Hampton, New Hampshire). Control organisms were weighed at the conclusion of the toxicity test. Mysids were not treated for disease and they were apparently free of sickness, injuries, and abnormalities at the beginning of the test. Mysids were fed newly hatched *Artemia salina* nauplii (EnviroSystems lot number BS02) once or twice daily before and during the test. During the 14 days immediately preceding the start of the definitive toxicity test the temperature of the culture was 24.2 to 25.0°C and the dissolved oxygen concentration was always at least 7.3 mg/L.

TOXICITY TESTING:

A screening test with the test substance was conducted during January 8 to 12, 1992. Nominal concentrations of test substance were 0 mg/L (control), 0.1, 1, 10, 100, and 1,000 mg/L. After 96 hours of exposure there was 100% survival at all concentrations.

Table 1. Chemical characterization of a representative sample of natural seawater used as dilution water for toxicity test.

Parameter	Unit of Measurement	Reporting Limit		Measured Value
pH	pH units	--		8.0
Salinity	parts per thousand	--		20
Total organic carbon	mg/L	1.0	0 hr 96 hr	ND ND
Particulate matter	mg/L	10	0 hr 96 hr	34 31
Organochlorine pesticides	ug/L	2		ND
Polychlorinated biphenyls	ug/L	1		ND

- Notes: 1. ND = Not detected above the reporting limit.
 2. pH and salinity were measured in dilution water collected from the control test vessel at the beginning of the test. Total organic carbon and particulate matter (total suspended solids) were measured in dilution water from a control test vessel collected at the beginning and end of the test. Pesticide and PCB data is collected during routine biannual water quality testing.



The definitive toxicity test was performed during January 13 to 17, 1992 according to EnviroSystems Test Protocol 91179-LU (Acute Toxicity of Lubricant Additive OS# 87319 to the Mysid, *Mysidopsis bahia*). The protocol, which was signed by the Study Director on January 10, 1992, is based on procedures of the U.S. Environmental Protection Agency (1985, 1989) and the OECD (1984a, 1984b). The test was conducted at a target temperature of $24 \pm 1^\circ\text{C}$ with five concentrations of test material and a dilution water control. No stock solution was prepared and test substance was added directly to dilution water without the use of a solvent (because test substance adhered well to the weigh boats, the boats were submerged in the test vessels after an attempt had been made to transfer the material from the boats to the dilution water in the test vessels). Nominal concentrations were: 0 mg/L (control), 150, 250, 400, 600, and 1,000 mg/L OS# 87319.

Ten test organisms were distributed to each of two replicates of each treatment. The test was performed in 20 liter glass aquaria that contained 15 liters of media (water depth was approximately 18 cm). Each test vessel was equipped with a cylinder (approximately 8 cm in diameter) with two openings. One opening extended from approximately 1 cm above the water surface to approximately 1 cm below the surface and the second opening was located approximately 1 to 4 cm above the bottom. A three bladed propeller inside this cylinder revolved at a nominal speed of approximately 1,000 rpm to draw water in through the surface openings and discharge it through the bottom openings. The test substance was added into the cylinder after the mixer was switched on. Mysids were contained in a cage that consisted of a glass cylinder closed at the bottom with Nitex screen. The cage was suspended in the test vessels and extended from the surface approximately one third of the way to the bottom.

Media was renewed in each test vessel at approximately 24 hour intervals. Test vessels were randomly arranged in a walk-in incubator during the 96 hour test (a random numbers table was used to select the location of each vessel). A 16 hour light and 8 hour dark photoperiod was automatically maintained during the entire test. Cool-white fluorescent lights provided a light intensity of $30 \text{ uEs}^{-1}\text{m}^{-2}$.

The number of surviving organisms and the occurrence of sublethal effects (immobilization, 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 pH 12 meter; instrument number PRL-4), salinity (refractometer, instrument number PRL-7), and temperature (ASTM mercury thermometer; thermometer number 1686) were measured and recorded daily in each test chamber that contained live animals. The temperature was continuously recorded in a representative beaker placed with the test in the incubator.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques, when warranted. Computer methods (Stephan, 1983) were used to calculate the median lethal concentrations (LC50s). All calculations were performed by the author using nominal concentrations of test substance. The no observed effect concentration is the highest concentration of test substance at and below which at least 90% survival of exposed organisms occurred and sublethal effects were not observed.

VII. RESULTS

All non-control test vessels containing OS# 87319 had insoluble droplets of test material on the bottom of the test vessel throughout the test. After 24 hours of exposure the test vessels also had an oily surface slick that remained throughout the test. Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Table A.1, respectively. Ninety five percent survival occurred in the control exposure. Control organisms had an average wet weight (blotted dry) of 1.5 mg at the end of the test, resulting in a loading rate of 0.001 g/L.

The 24, 48, 72, and 96 hour LC50s for mysids exposed to OS# 87319 are presented in Table 3. The 96 hour LC50 is greater than 1,000 mg/L OS# 87319. The no observed effect concentration is greater than 1,000 mg/L OS# 87319.

Table 2. Survival and sublethal effect data from toxicity test.

Nominal concentration of test substance	Rep	Number of Survivors					Number Affected				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 mg/L (control)	1	10	10	9	9	9	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
150 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
250 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
400 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
600 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
1,000 mg/L	1	10	10	10	10	10	0	0	0	0	0
	2	10	9	9	9	9	0	0	0	0	0



Table 3. Median lethal concentrations (LC50s) from toxicity test.

Exposure period	LC50	95 percent confidence limit	LC50 calculation method
24 hours	>1,000 mg/L	--	--
48 hours	>1,000 mg/L	--	--
72 hours	>1,000 mg/L	--	--
96 hours	>1,000 mg/L	--	--

VIII. REFERENCES

- Stephan, C.E. 1983. Computer Program for Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
- OECD. 1981. OECD Guidelines for Testing of Chemicals. Annex 2. OECD Principles of Good Laboratory Practice. Adopted 1 June 1981.
- OECD. 1984a. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 202, Daphnia sp., Acute Immobilisation Test and Reproduction Test. Adopted 4 April 1984.
- OECD. 1984b. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 203, Fish Acute Toxicity Test. Adopted 4 April 1984.
- U.S. EPA. 1985. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Federal Register, Friday, September 27, 1985.
- U.S. EPA. 1987. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Amendments. Federal Register, Wednesday, May 20, 1987.
- U.S. EPA. 1989. 40 CFR Part 792. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Final Rule. Federal Register, Thursday, August 17, 1989.

Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Salinity, dissolved oxygen concentration, pH, and temperature measured during toxicity test.

Nominal concentration of test substance	Rep.	Salinity (ppt)						Dissolved oxygen (% saturation)					
		0 hr	24 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	24 hr	48 hr	72 hr	96 hr
0 mg/L (control)	1	20	20	20	20	20	20	90	86	89	88	90	87
	2	20	20	20	20	20	20	90	87	89	87	89	88
150 mg/L	1	20	20	20	20	20	20	90	86	89	88	89	88
	2	20	20	20	20	20	20	90	84	89	87	88	87
250 mg/L	1	20	20	20	20	20	20	90	86	89	90	89	86
	2	20	20	20	20	20	20	90	84	89	87	89	88
400 mg/L	1	20	20	20	20	20	20	90	86	89	86	90	88
	2	20	20	20	20	20	20	90	86	89	86	89	87
600 mg/L	1	20	20	20	20	20	20	90	87	89	88	88	87
	2	20	20	20	20	20	20	90	86	89	89	90	88
1,000 mg/L	1	20	20	20	20	-	-	90	86	89	89	89	86
	2	20	20	20	20	20	20	90	86	89	90	89	87

- Notes:
1. All measurements were made before media renewal except the second 24 hour measurement which was made after renewal.
 2. Dissolved oxygen saturation is 8.4 mg/L at 24°C. Percent saturation is calculated as the actual dissolved oxygen concentration in mg/L divided by 8.4 and multiplied by 100.

Table A.1. Continued.

Nominal concentration of test substance	Rep.	Temperature (°C)						pH					
		0 hr	24 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	24 hr	48 hr	72 hr	96 hr
0 mg/L (control)	1	23.3	24.9	24.8	24.6	23.6	23.2	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.4	24.8	24.7	24.4	23.7	23.1	8.0	8.1	8.0	8.5	8.0	7.9
150 mg/L	1	23.4	24.8	24.7	23.7	23.3	23.3	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.3	24.9	24.8	24.5	23.5	23.2	8.0	8.1	8.0	8.5	8.0	7.9
250 mg/L	1	23.3	24.8	24.7	24.0	23.3	23.2	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.3	24.7	24.7	24.4	23.4	23.3	8.0	8.1	8.0	8.5	8.0	7.9
400 mg/L	1	23.4	24.7	24.6	24.8	24.6	23.2	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.4	24.8	24.7	24.8	24.6	23.1	8.0	8.1	8.0	8.5	8.0	7.9
600 mg/L	1	23.3	24.9	24.7	23.5	23.4	23.3	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.5	24.8	24.8	23.6	23.5	23.3	8.0	8.1	8.0	8.5	8.0	7.9
1,000 mg/L	1	23.4	24.9	24.8	23.7	23.2	23.2	8.0	8.1	8.0	8.5	8.0	7.9
	2	23.3	24.7	24.8	24.2	23.2	23.3	8.0	8.1	8.0	8.5	8.0	7.9

Note: All measurements were made before media renewal except the second 24 hour measurement which was made after renewal.



WARF INSTITUTE, INC.

MADISON, WISCONSIN

Lubrizol 5301

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REPORT

Sample:

OS 26198E

Submitted by:

C. B. Swan
The Lubrizol Corp
Cleveland, Ohio

Testing requested:

Oral LD 50
Skin irritation
Eye irritation

Date Received:

12/16/71

Conclusion:

Under the conditions specified the product has an estimated oral LD 50 in excess of 20 grams per kilogram of body weight, a primary skin irritation index of 0, and eye irritation scores of 0, 0, and 0 at 24, 48 and 72 hours respectively.

Within the meaning of the F.H. S.A. the product is not toxic orally, not irritating to the eye and Not irritating to the skin.

Signed

By and for WARF Institute Inc.

Date: January 11, 1972

WARF NO. 1121858

I U C L I D

D a t a S e t

Existing Chemical ID: 61788-76-9
CAS No. 61788-76-9
EINECS Name Alkanes, chloro
EC No. 263-004-3
TSCA Name Alkanes, chloro

Producer Related Part

Company: The Lubrizol Corporation
Creation date: 01-NOV-2006

Substance Related Part

Company: The Lubrizol Corporation
Creation date: 01-NOV-2006

Printing date: 01-NOV-2006
Revision date:
Date of last Update: 01-NOV-2006

Number of Pages: 18

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK
(DE), TA-Luft (DE), Material Safety Dataset, Risk
Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

-

1.0.2 Location of Production Site, Importer or Formulator

-

1.0.3 Identity of Recipients

-

1.0.4 Details on Category/Template

-

1.1.0 Substance Identification

-

1.1.1 General Substance Information

Substance type: organic
Physical status: liquid
Purity: 99 - % w/w
Colour: Clear light amber
Odour: mild

01-NOV-2006

1.1.2 Spectra

-

1.2 Synonyms and Tradenames

-

1.3 Impurities

-

1.4 Additives

-

1.5 Total Quantity

-

1.6.1 Labelling

-

1.6.2 Classification

1.6.3 Packaging

1.7 Use Pattern

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

1.8.4 Major Accident Hazards

1.8.5 Air Pollution

1.8.6 Listings e.g. Chemical Inventories

1.9.1 Degradation/Transformation Products

1.9.2 Components

1.10 Source of Exposure

-

1.11 Additional Remarks

-

1.12 Last Literature Search

-

1.13 Reviews

-

2.1 Melting Point

-

2.2 Boiling Point

-

2.3 Density

-

2.3.1 Granulometry

-

2.4 Vapour Pressure

-

2.5 Partition Coefficient

-

2.6.1 Solubility in different media

-

2.6.2 Surface Tension

-

2.7 Flash Point

-

2.8 Auto Flammability

-

2.9 Flammability

-

2.10 Explosive Properties

-

2.11 Oxidizing Properties

-

2.12 Dissociation Constant

-

2.13 Viscosity

-

2.14 Additional Remarks

-

3.1.1 Photodegradation

3.1.2 Stability in Water

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

3.3.2 Distribution

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

3.8 Additional Remarks

AQUATIC ORGANISMS**4.1 Acute/Prolonged Toxicity to Fish**

Type: other: Static renewal
Species: Cyprinodon variegatus (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
NOEC: 1000
LC50: > 1000

Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: 1992
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Sheepshead minnow were exposed to geometric series of five test concentrations and a negative control under static renewal conditions for 96 hours. Two replicate test chambers were maintained in each treatment and control group with 10 fish in each test chamber. Nominal test concentrations were 130, 216, 360, 600, and 1000 mg/l. The no mortality concentration and no effect concentration were determined by visually examining results of mortality and clinical observations.

After the test substance was added to the vortex a cloudy solution was formed. The degree of cloudiness varied according to concentration and decreased over time. Oil-like droplets were noted on the water surface and on the bottom of the test tanks, and the test solution adhered to glass.

The fish appeared normal at all treatment groups with no clinical signs of toxicity. The 96 hour LC50 was greater than 1000 mg/L, the highest concentration tested. The no observed effect concentration was 1000 mg/L.

Reliability: (1) valid without restriction
01-NOV-2006

(4)

Type: static
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
NOEC: 3299.99
LC50: > 3299.99 measured/nominal

Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: 1997
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: The test was conducted under static conditions using oil-water dispersion method. Five concentrations of the test material and a control were evaluated. Target concentrations of the test substance were 0, 0.33, 3.30, 33, 330, and 3330 mg/L. Actual concentrations were 0, 0.67, 3.37, 32.97, 330.02, and 3299.9 mg/L.

Exposure of the test organisms to the test substance resulted in a 96 hour LC50 of > 3299.99 mg/L. The NOEC was 3299.99 mg/L.

Reliability: (1) valid without restriction (2)
01-NOV-2006

4.2 Acute Toxicity to Aquatic Invertebrates

Type: other: static renewal
Species: Mysidopsis bahia (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: > 1000

Method: OECD Guide-line 202
Year: 1992
Test substance: as prescribed by 1.1 - 1.4

Result: The test was performed under static renewal conditions with five concentrations of test substance and a dilution water control. The dilution water was filtered natural seawater collected from the Atlantic Ocean at Hampton, New Hampshire and adjusted to a salinity of 20 +/- 1 ppt. Nominal concentrations of the test substance were 0, 150, 250, 400, 600, and 1000 mg/L. A mixer was used to continuously draw floating test substance from the surface down into the dilution water in each test vessel.

Exposure of test organisms to the test substance resulted in a 96 hour LC50 greater than 1000 mg/L. The 96 hour no observed effect concentration is greater than 1000 mg/L.

Reliability: (1) valid without restriction (1)
01-NOV-2006

4.3 Toxicity to Aquatic Plants e.g. Algae

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4.4 Toxicity to Microorganisms e.g. Bacteria

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4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

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4.5.2 Chronic Toxicity to Aquatic Invertebrates

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TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

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4.6.2 Toxicity to Terrestrial Plants

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4.6.3 Toxicity to Soil Dwelling Organisms

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4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

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4.7 Biological Effects Monitoring

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4.8 Biotransformation and Kinetics

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4.9 Additional Remarks

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5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: Sprague-Dawley
Sex: male
No. of Animals: 18
Doses: 5000, 10000, and 20000 mg/kg
Value: > 20000 mg/kg bw

Year: 1972
Test substance: as prescribed by 1.1 - 1.4

Remark: Groups of 6 albino rats (6 males/group) were administered 5000, 10000, 20000 mg/kg test material with a stomach tube. There were no deaths during the two week observation period.

Reliability: (2) valid with restrictions
01-NOV-2006

(3)

5.1.2 Acute Inhalation Toxicity

5.1.3 Acute Dermal Toxicity

5.1.4 Acute Toxicity, other Routes

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Exposure Time: 24 hour(s)
No. of Animals: 6
PDII: 0
Result: not irritating
EC classificat.: not irritating

Year: 1971
Test substance: as prescribed by 1.1 - 1.4

Remark: The test material was applied to two areas on each of the six rabbits, 1 abraded area, and 1 intact area in the amount of 0.5 ml per area for liquids or 0.5 g per area for solids. The treated areas were covered with gauze patch and taped to maintain the test material in contact with the skin and decrease the rate of evaporation. The animals were fitted with collars for a 24-hour period at which time the coverings were removed, the test material was washed off and the degree of erythem and edema were recorded. A second reading was taken at 72 hours. All readings gave a score of zero.

Reliability: (2) valid with restrictions
01-NOV-2006

(3)

5.2.2 Eye Irritation

Species: rabbit
No. of Animals: 6
Result: not irritating
EC classificat.: not irritating

Year: 1971
Test substance: as prescribed by 1.1 - 1.4

Remark: One-tenth of a milliliter of test substance was instilled in one eye, and the other eye served as a control. A series of six albino rabbits was used. Any residue of the test material and accumulated discharge was flushed from the eye each time they are scored. All readings (24, 48, 72 hour) gave a score of zero.

Reliability: (2) valid with restrictions
01-NOV-2006

(3)

5.3 Sensitization

5.4 Repeated Dose Toxicity

5.5 Genetic Toxicity 'in Vitro'

5.6 Genetic Toxicity 'in Vivo'

5.7 Carcinogenicity

5.8.1 Toxicity to Fertility

5.8.2 Developmental Toxicity/Teratogenicity

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

5. Toxicity

date: 01-NOV-2006
Substance ID: 61788-76-9

5.11 Additional Remarks

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6.1 Analytical Methods

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6.2 Detection and Identification

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7. Eff. Against Target Org. and Intended Uses

7.1 Function

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7.2 Effects on Organisms to be Controlled

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7.3 Organisms to be Protected

-

7.4 User

-

7.5 Resistance

-

8.1 Methods Handling and Storing

8.2 Fire Guidance

8.3 Emergency Measures

8.4 Possib. of Rendering Subst. Harmless

8.5 Waste Management

8.6 Side-effects Detection

8.7 Substance Registered as Dangerous for Ground Water

8.8 Reactivity Towards Container Material

- (1) Unpublished study; EnviroSystems, Incorporated (1992); Study Number 91179-LU.
- (2) Unpublished study; EnviroSystems, Incorporated (1997) Reference LUB5401-96-06.
- (3) Unpublished study; WARF Institute, Inc(1972) WARF No. 1121858.
- (4) Unpublished study; Wildlife International Ltd.(1992)Project number 331A-103.

10.1 End Point Summary

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10.2 Hazard Summary

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10.3 Risk Assessment

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