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E.I. DUPONT DE NEMOURS & COMPANY INC.

[REDACTED]

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TITLE: Aquatic Toxicity and Solubility of [REDACTED] Fluorotelomer Intermediate

AUTHOR: Roland C. Hall, Jr.

CONTRIBUTORS: Thomas V. Robertson, Olver Laboratories

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ABSTRACT ONLY

[REDACTED]

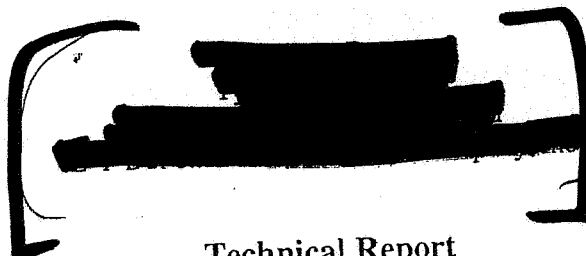
COMPLETE REPORT

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Report Number GL-2001-42
Issued December 5, 2001



Technical Report

Aquatic Toxicity and Solubility of [REDACTED] Fluorotelomer Intermediate

Report Written By

(Roland C Hall, Jr)

Period Covered

December 2000 to May 2001

Research Notebooks

NA

Work Supervised By

(J F Kmec)

Abstract

This report documents a study done by Oliver Laboratories to determine the aquatic toxicity and solubility of [REDACTED] Fluorotelomer Intermediate, which is one component of the spin agent to be used in [REDACTED]. The study was conducted in order to obtain necessary environmental permits to conduct a test of the new spin agent in the [REDACTED] pilot plant. The test in the pilot plant was subsequently canceled, however, the information in this report was forwarded to the Line 3 organization to assist in meeting their environmental requirements.

Oliver Laboratories determined that the solubility of [REDACTED] Fluorotelomer Intermediate appeared to be in the order of 6 mg/L at standard temperature and pressure. Their screening tests also indicated no acute toxicity at concentration near the apparent maximum solubility of 5 mg/L.

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INTRODUCTION

In late 2000 a decision was made to evaluate the spin agent to be used on [REDACTED] in the [REDACTED] pilot plant. The spin agent to be tested was [REDACTED] Fluorotelomer Intermediate and trans-1,2-Dichloroethylene. In order to obtain environmental approval to run the tests in the [REDACTED] pilot plant, information was needed on the aquatic toxicity of the spin agent. There was no published data available on aquatic toxicity of [REDACTED] Fluorotelomer Intermediate, therefore a study was commissioned with Oliver Laboratories to determine the solubility in water and the aquatic toxicity. The test in the pilot plant was subsequently canceled, however, the information in this report was forwarded to the Line 3 organization to assist in meeting their environmental requirements.

OBJECTIVE

The purpose of this of the study was to determine the toxicity of [REDACTED] Fluorotelomer Intermediate at a maximum concentration in water of 6 mg/L to Ceriodaphnia and fathead minnows (Pimephales promelas).

SUMMARY AND CONCLUSIONS

The initial screening tests indicated no acute toxicity at concentrations near the apparent maximum solubility of 6 mg/L. In an effort to determine the [REDACTED] concentrations that results in acute and chronic toxicity, acute and chronic testing was conducted using an expanded test solution series prepared using [REDACTED] amended with small amounts of methanol to increase solubility. The result of these tests indicated that [REDACTED] was not acutely toxic to Ceriodaphnia and fathead minnows (Pimephales promelas) at concentrations approaching methanol-aided solubility limits. The 48-hour LC₅₀ values for Ceriodaphnia and fathead minnows was greater than 500 mg/L. The no observed effect concentration (NOEC) for the Three-Brood Ceriodaphnia Survival and Reproduction chronic test was 100 mg/L while the NOEC for the 7-Day Fathead Minnow Survival and Growth chronic test was 500 mg/L. These results suggest that there should be no direct acute or chronic toxicity due to the presence of [REDACTED] at the maximum apparent solubility in water of 6 mg/L.

PATENT SITUATION

At the present time we have no plans to file for a patent.

PROGRAM

No additional testing is planned at this time

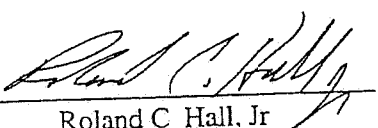
PUBLICATION STATUS

At the present time we have no plans to publish the data contained in this report

ACKNOWLEDGMENTS

Thanks to Tom Robertson and Olver Laboratories, Incorporated for their contributions to this study and report.

SIGNATURE(S)



Roland C Hall, Jr

APPENDIX

Appendix A Memo from R. Lawrence Hoffman, Vice President, Olver Laboratories to
(Thomas V. Robertson) dated May 4, 2001 subject [REDACTED] Solubility and Toxicity
Determination Program. Job Number 60175

Appendix B "Determination of solubility and aquatic toxicity of DuPont Product
[REDACTED] Fluorotelomer Intermediate for E. E. DuPont de Nemours and Company by R.
Lawrence Hoffman dated May 3, 2001



Consulting Engineers • Environmental Laboratories
1116 South Main Street Blacksburg, Virginia 24060

May 4, 2001

Mr Thomas V. Robertson
Development Associate
Environmental Affairs
E.I du Pont de Nemours and Company, Inc
P O Box 27001
Richmond, VA 23261

Re [REDACTED] Solubility and Toxicity
Determination Program, Job
Number 60175

Dear Tom

Enclosed for your review and use are three (3) copies of the report which describes the performance and results of the [REDACTED] Solubility and Toxicity Determination program performed by Olver Laboratories Incorporated for E I du Pont de Nemours and Company, Incorporated. This program was performed to support evaluations of the potential for the use of this product by examining its potential effects on wastewater toxicity. The program, as described in our December 14, 2000 proposal, consisted of an initial determination of solubility in water and subsequent determinations of acute and chronic toxicity to two common freshwater test organisms. As for the standard compliance monitoring performed by Olver Laboratories for DuPont, the toxicity testing component of this program is reported in a GLP format.

As indicated on the Material Safety Data Sheet provided with the sample, the material appeared to be only slightly soluble in water. As described in the report, the solubility of the material in water appeared to be in the order of 6 mg/L at standard temperature and pressure. The solubility in water appeared to increase to levels of 500 mg/L to 1000 mg/L by adding a small amount of methanol to the [REDACTED] prior to addition to water. For the reasons referenced in the report, the analysis of the [REDACTED] solutions provided inconsistent results and could not be used to confirm the [REDACTED] concentrations used in the toxicity evaluation component of this program.

Blacksburg, VA (540) 552-6974

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Mr Thomas V. Robertson

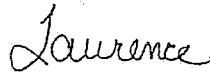
May 4, 2001

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The initial screening tests indicated no acute toxicity at concentration near the apparent maximum solubility of 6 mg/L. In an effort to determine the [REDACTED] concentrations that results in acute and chronic toxicity, acute and chronic testing was conducted using an expanded test solution series prepared using [REDACTED] amended with small amounts of methanol to increase solubility. The results of these tests indicated that [REDACTED] was not acutely toxic to *Ceriodaphnia* and fathead minnows (*Pimephales promelas*) at concentrations approaching methanol-aided solubility limits. The 48-hour LC_{50} values for *Ceriodaphnia* and fathead minnows was greater than 500 mg/L. The no observed effect concentration (NOEC) for the Three-Brood *Ceriodaphnia* Survival and Reproduction chronic test was 100 mg/L while the NOEC for the 7-Day Fathead Minnow Survival and Growth chronic test was 500 mg/L. These results suggest that there should be no direct acute or chronic toxicity due to the presence of [REDACTED] at the maximum apparent solubility in water of 6 mg/L.

I look forward to the opportunity to discuss this with you in more detail and to answer any questions that you may have. As always, please do not hesitate to contact me or David Griffiths

Sincerely,

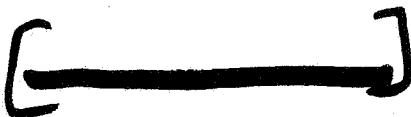


R. Lawrence Hoffman
Vice President

RLH/egl

Enclosures

cc: David W. Griffiths, Ph.D., President, Olver Laboratories Incorporated



STUDY TITLE

DETERMINATION OF SOLUBILITY AND AQUATIC TOXICITY OF
DuPONT PRODUCT [REDACTED] FLUOROTELOMER INTERMEDIATE
FOR E. I. DU PONT DE NEMOURS AND COMPANY

DATA REQUIREMENTS

DuPONT CONTRACT ORDER NUMBER [REDACTED]

AUTHOR

R. LAWRENCE HOFFMAN

STUDY COMPLETED ON

May 3, 2001

PERFORMING LABORATORY

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL LABORATORIES
AND CONSULTANTS
1116 SOUTH MAIN STREET
BLACKSBURG, VIRGINIA 24060

for

E. I. DU PONT DE NEMOURS AND COMPANY
[REDACTED]

OLVER LABORATORIES INCORPORATED JOB NUMBER

60175

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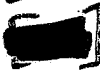
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Report Prepared by:

Susan Mirlohi 5/3/01
Susan Mirlohi Date
Bioassay Laboratory Manager

Reviewed and Approved
for Issue by

R. Lawrence Hoffman 5/3/01
R. Lawrence Hoffman Date
Vice President and
Study Director

QUALITY ASSURANCE STATEMENT

Quality Assurance Statement for final report entitled "Determination of Solubility and Aquatic Toxicity of DuPont Product [REDACTED] Fluorotelomer Intermediate for E. I. du Pont de Nemours and Company"

Audits for the study were conducted on the dates listed below:

<u>Items Audited</u>	<u>Audit Dates</u>
Sample Receipt	1/11/01
Range-Finding Test Initiation	1/25/01; 2/01/01
Range-Finding Test Termination	1/27/01; 2/03/01
Acute Test Initiation	2/14/01
Acute Test Termination	2/16/01
Chronic Test Initiation	2/13/01; 2/20/01
Chronic Test Renewal	2/14/01; 2/21/01
Chronic Test Termination	2/20/01; 2/27/01

Date findings reported to management and study director 2/27/01

The information provided within this report was generated in accordance with Good Laboratory Practice Regulations and Protocols. A final review of all data and records indicate the report submitted is an accurate reflection of the study conducted.

Reported by:


Fran C. Boltinghouse
Quality Assurance Auditor, Olver Laboratories

5/3/01

Date

GENERAL INFORMATION

Material Tested:

[REDACTED] Fluorotelomer Intermediate
[REDACTED]
[REDACTED]
[REDACTED]

Olver Sample
Designation:

[REDACTED]

Sponsor:

E. I. du Pont de Nemours and Company [REDACTED]
[REDACTED]
[REDACTED]

Study Initiated;
Completed:

1/12/01; 5/03/01

Experimental Start;
End:

1/25/01, 2/27/01

Location of Original
Raw Data and
Final Report:

Olver Laboratories Incorporated
1116 South Main Street
Blacksburg, Virginia 24060

PROJECT PERSONNEL

The study director of this project for E. I. du Pont de Nemours and Company was
R. Lawrence Hoffman.

The following Olver Laboratories personnel assisted with the various phases of the
aquatic toxicity determination study

<u>NAME</u>	<u>TITLE</u>	<u>INITIALS</u>
Susan Mirlohi	Bioassay Laboratory Manager	<u>SM</u>
Stacey Boyles	Biological Technician	<u>SLB</u>
Sheryl Newton	Biological Technician	<u>SRN</u>
Laura Dunham	Biological Technician	<u>LMD</u>
Fran Boltinghouse	Quality Assurance Officer	<u>FCB</u>

DETERMINATION OF SOLUBILITY
AND AQUATIC TOXICITY OF DuPONT
PRODUCT, [REDACTED] FLUOROTELOMER INTERMEDIATE

SUMMARY

Solubility and aquatic toxicity determination studies were performed for the DuPont product, [REDACTED] to facilitate projections and assessments of the potential for toxicity in wastewater that may contain this material. The solubility determination was performed using a procedure developed by Olver Laboratories based on the substance information contained on the Material Safety Data Sheet (MSDS) provided by DuPont. [REDACTED] solubility in water was estimated by establishing a calibration curve for GC/MS analysis. The solubility of [REDACTED] in water was determined to be on the order of 6 mg/L. However, when introduced as a methanolic solution, it proved possible to produce what appeared to be homogenous aqueous solutions containing as much as 1,000 mg/L of [REDACTED]. It is believed that these are not true solutions, but rather dispersions or micellar solutions.

The acute and chronic toxicity of [REDACTED] to *Ceriodaphnia dubia* and fathead minnows (*Pimephales promelas*) was assessed utilizing EPA/600/4-90/027F and EPA/600/4-91/002 methods. Toxicity testing consisted of initial range-finding tests which included a wide range of [REDACTED] concentrations to establish a general level of toxicity for acute and chronic testing using *Ceriodaphnia dubia* and fathead minnows. Toxicity range-finding tests were followed by 48-hour static acute *Ceriodaphnia* and fathead minnow tests, a three-brood chronic *Ceriodaphnia dubia* test, and a 7-day chronic static renewal fathead minnow test. The concentration series used in the toxicity range-finding tests ranged from 0.01 to 1,000 mg/L of [REDACTED]. For acute toxicity testing, [REDACTED] concentrations ranged from 5 to 200 mg/L, while chronic testing included concentrations which ranged from 5 to 500 mg/L.

Estimated toxicity endpoints (LC₅₀ and NOEC values) were found to be as follows.

<u>Toxicity Test Type</u>	<u>Toxicity End-points</u>
<i>Ceriodaphnia</i> Acute Test	LC ₅₀ >500 mg/L (100% survival at 500 mg/L)
Fathead Minnow Acute Test	LC ₅₀ >500 mg/L (100% survival at 500 mg/L)
<i>Ceriodaphnia</i> Chronic Test - Survival	NOEC = 100 mg/L
<i>Ceriodaphnia</i> Chronic Test - Reproduction	NOEC = 100 mg/L
Fathead Minnow Chronic Test - Survival	NOEC = 500 mg/L
Fathead Minnow Chronic Test - Growth	NOEC = 500 mg/L

INTRODUCTION

Solubility determination and aquatic toxicity testing for the DuPont product [REDACTED] were performed by Olver Laboratories Incorporated for the E.I. du Pont de Nemours and Company [REDACTED] from January 12, 2001 to February 27, 2001. The purpose of this study was to determine the solubility of [REDACTED] in water and to determine the acute and chronic toxicity of [REDACTED] to representative freshwater organisms. The toxicity of [REDACTED] was assessed through determination of median lethal concentration (LC₅₀) values for acute testing and no observed effect concentration (NOEC) values for chronic testing, using *Ceriodaphnia* and fathead minnows.

METHODS AND MATERIALS

1.0 [REDACTED] Solubility Determination

All testing was performed using a sample of [REDACTED] provided by DuPont. The sample was contained in a 3.63 kg/8 lb can bearing the label: [REDACTED]. Solubility tests were performed according to the test protocol that is provided in Appendix 2.

2.0 [REDACTED] Aquatic Toxicity Determination

The toxicity tests were conducted according to "Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms", EPA/600/4-90/027F and "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms", EPA/600/4-91/002

The toxicity testing component of this study was conducted following Good Laboratory Practice general testing and reporting guidelines and the final report was reviewed by the Olver Laboratories Quality Assurance Unit. Copies of the report and original raw data will be maintained by Olver Laboratories. The original signed report and raw data will be archived at Olver Laboratories Incorporated.

2.1 Test Organisms

Ceriodaphnia dubia and fathead minnows, originally obtained from the U.S. EPA and subsequently cultured at Olver Laboratories, were used for acute and chronic testing. Fathead minnow and *Ceriodaphnia* cultures were maintained in EPA moderately hard water and the culturing records indicated that the test organisms were in satisfactory health at test initiation. *Ceriodaphnia* cultures were fed a YCT (yeast-cerophyll-trout chow) and algae (*Selenastrum capricornutum*) food mixture prepared at Olver Laboratories. Fathead minnow cultures were fed brine shrimp nauplii (*Artemia* sp. eggs obtained from "Ocean

Star International Inc.", Snowville, VT, Lot No. 4400). Test organisms were fed for at least two hours prior to use in testing.

Larval fathead minnows used in the chronic testing (Olver Culture No. 1810) were 1-24 hours old at test initiation and the control organisms had an average dry weight of 0.429 mg at test completion. The biological loading factor in the controls was 0.02 g/L. Test organisms were fed 0.1 mL of a concentrated suspension of newly hatched (less than 24 hours old) brine shrimp nauplii (approximately 700 to 1000 nauplii) 2-3 times daily during testing. Larval fathead minnows used in the acute testing (Olver Culture No. 1809) were 3 days old at test initiation. The biological loading factor in the controls was 0.05 g/L with an average weight (not dried) of 2.4 mg at test completion.

The *Ceriodaphnia dubia* used in the chronic test were obtained from Olver Laboratories cultures produced from EPA-supplied stock. *Ceriodaphnia* used in the chronic testing (Olver Culture Nos. 1-30) were less than 24 hours old, and all within 8 hours of the same age, at test initiation, and the control organisms had an average reproduction of 25.9 (per surviving female) at test completion. Test organisms were fed 0.1 mL of YCT and 0.1 mL of a concentrated suspension of algae (approximately 3.1×10^7 cells) once daily at test solution renewal time. *Ceriodaphnia* used in acute toxicity testing were less than 24 hours old at test initiation and they were not fed during testing.

The brine shrimp and YCT used in feeding were tested to show that concentrations of metals, pesticides, and PCBs remained within acceptable limits. No chemicals were present in the brine shrimp that made them unsuitable for use as a food source in aquatic toxicity tests. Data for these analyses are included in Appendix 11.

Monthly reference tests (Appendices 8 and 9) were performed to ensure that test organism sensitivities and health were within acceptable limits. A sodium chloride reference toxicant (EM Science; CAS #7647-14-5) was used and the results showed fathead minnow and *Ceriodaphnia* sensitivities to be comparable to ranges previously established at Olver Laboratories.

2.2 Test Substance

A concentrated sample of [REDACTED] contained in a one-gallon sealed metal container was provided to Olver Laboratories by the DuPont [REDACTED] facility. The MSDS provided with the sample identified the material as [REDACTED] Fluorotelomer Intermediate with the following composition:

[REDACTED]

Based on the results of the [REDACTED] solubility determination, various concentrations of [REDACTED] were prepared for use in acute and chronic toxicity testing. The solubility level for [REDACTED] in water was estimated at 6 mg/L, therefore, the theoretical concentrations of [REDACTED] used in initial range-finding toxicity tests were selected at and below the 5 mg/L solubility level. Since no toxicity was observed at this level, higher concentrations of [REDACTED] were prepared for use in the acute and chronic toxicity testing. These ranged from 5 to 500 mg/L. To enhance the solubility of [REDACTED] in preparing test solutions, [REDACTED] was initially dissolved in methanol (Burdick & Jackson, High Purity Methanol, CAS #67-56-1) prior to being mixed in dilution water. Test solution preparation for the range-finding, acute and chronic testing is described in Section 2.4.2 through 2.4.4. In all cases, preparations of the [REDACTED] standards and test solutions was performed in a manner to minimize loss by volatilization to the extent practical.

2.3 Dilution Water

The dilution water used for culturing, toxicity testing, and [REDACTED] test solution preparation was moderately-hard synthetic freshwater prepared using EPA guidelines (EPA/600/4-90/027F and EPA 600/4-91/002). This dilution water was aerated prior to testing with oil-free compressed air. pH ranged from 7.7 to 8.0, conductivity ranged from 300 - 336 μ mhos/cm, alkalinity ranged from 60 to 64 mg/L, and hardness ranged from 84 to 100 mg/L. Annual dilution water quality analyses data are provided in Appendix 10.

2.4 Toxicity Test Procedures

2.4.1 General Procedures

The acute and chronic toxicity tests were performed at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ by placing the test chambers in a constant temperature water bath. Test organisms were exposed to ambient laboratory light (50-100 foot candles, measured at water surface) for a 16-hour day/8-hour night photoperiod. Transitional lighting (30 minutes, 30-40 foot candles) was included at the beginning and end of the 16-hour light period.

For the *Cenodaphnia dubia* acute toxicity tests, plastic beakers filled with 25 mL of test solution were used. For the fathead minnow acute toxicity tests, 600 mL glass beakers filled with 500 mL of test solution were used. Beaker positions were randomized in the water bath using random numbers. Test organisms were pooled

and placed (five at a time) into each test solution in a random order. Test organisms were less than 24 hours old for the *Ceriodaphnia* acute tests and within 1 to 14 days old and within 24 hours of age for the fathead minnow acute tests.

Routine chemical analyses were conducted. Alkalinity and hardness were measured in the control and highest test concentrations at test initiation. Conductivity was measured in all test concentrations at test initiation and termination. Dissolved oxygen, pH, and temperature were measured daily in one replicate per effluent concentration. All chemical analyses were conducted as per the EPA document "Methods for Chemical Analysis of Water and Wastes" (EPA 600/4-79-020) or APHA, "Standard Methods for the Examination of Water and Wastewater", 18th Edition. Dissolved oxygen was determined by electrode EPA Method 360.1.

PARAMETER	TEST METHOD	TEST SOLUTIONS ANALYZED	FREQUENCY
pH	EPA 150 1	All	Daily
Temperature	EPA 170 1	All	Daily
Dissolved Oxygen	EPA 360 1	All	Daily
Conductivity	EPA 120 1	All	Beginning/End
Alkalinity	EPA 310 1	Control and Highest Concentration	Beginning
Hardness	EPA 130 2	Control and Highest Concentration	Beginning

For the *Ceriodaphnia* chronic test, 30 mL plastic beakers filled with 15 mL of test solution were used. Beaker positions were randomized in a holding tray using random numbers. One *Ceriodaphnia* neonate (20-24 hours old) was placed in each of ten replicate test chambers per test solution using the assigned positions. All test chambers, contained in their holding trays, were placed in the water bath. The number of surviving *Ceriodaphnia* in each test concentration was determined prior to test solution renewal. Dead *Ceriodaphnia* were discarded. Test solutions were renewed daily by transferring each of the original *Ceriodaphnia* into freshly prepared test solutions. The number of *Ceriodaphnia* neonates, reproduced by the adult female *Ceriodaphnia* in each replicate test chamber, was counted and recorded at each daily test solution renewal time.

In the chronic test, *Ceriodaphnia* were fed 0.1 mL of a concentrated suspension (approximately 3.1×10^7 cells) of algae and 0.1 mL of YCT food mixture daily, at test solution renewal time. *Ceriodaphnia* were exposed to ambient laboratory light (50-

100 foot candles, measured at water surface) for a 16-hour day/8-hour night photoperiod with transitional lighting. Transitional lighting (30 minutes, 30-40 foot candles) was included at the beginning and end of the 16-hour light period. Temperature was maintained at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ by placing the test chambers in a constant temperature water bath.

For the fathead minnow chronic test, beakers (glass; 600 mL) filled with 250 mL of test solution were used. Ten fathead minnow larvae, 1-20 hours old, were placed in each of four replicate test chambers per test solution using random numbers. All test chambers were positioned in the water bath using randomization techniques. The number of surviving larvae in each chamber was determined prior to test solution renewal. Dead larvae were discarded. Test solutions were renewed daily by siphoning approximately 80% of the test solution out of the chamber. The original test volume was restored by adding freshly prepared [REDACTED] solutions to the test chambers.

2.4.2 Toxicity Range-Finding Tests

The initial phase of testing consisted of a series of 48-hour range-finding tests to determine the general toxicity of [REDACTED] over a wide range of test concentrations. Targeted test solutions ranged from 0.01 mg/L to 1,000 mg/L of [REDACTED]. Based on the results of the [REDACTED] solubility determination, an initial concentration series was selected to perform the toxicity range-finding tests. Since the solubility of [REDACTED] in water was estimated at 6 mg/L, the initial toxicity range-finding tests were performed using a dilution series consisting of control, 0.01 mg/L, 0.1 mg/L, 1 mg/L, and 5 mg/L of [REDACTED]. Since no evidence of toxicity was observed at these levels, the dilution series was expanded to include targeted 100 mg/L to 1,000 mg/L [REDACTED] concentrations. To enhance the solubility of [REDACTED] in water, [REDACTED] was used to prepare test concentrations greater than 6 mg/L; this mixture was initially mixed with 500 μL of reagent-grade methanol; this mixture was then added to moderately hard water to prepare the test solutions.

2.4.3 Acute Toxicity Testing

The second phase of testing involved the performance of 48-hour static acute *Ceriodaphnia dubia* and fathead minnow tests. Based on the results of the toxicity range-finding tests, a concentration series consisting of control, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L of [REDACTED] was used. The highest targeted [REDACTED] concentration used in testing was at 500 mg/L since, above 500 mg/L, [REDACTED] did not appear to remain in solution. At concentrations greater than 500 mg/L small droplets of [REDACTED] were observed adhering to the glassware used to prepare the test solutions. An additional control solution consisting of 500 μL methanol/L of dilution water was included in testing to identify any potential toxicity associated with the presence of methanol in solution. Based on the available toxicity data on methanol

(EPA documents: EPA-600/6-91/005 and EPA/600/6-91/003), the concentration of methanol in the test solutions (0.05%) was well below the expected toxic levels (i.e., 2% to 5% for acute toxicity and 0.3% to 0.5% for chronic toxicity)

[REDACTED] test solutions were prepared by first dissolving the pre-measured volume of [REDACTED] concentrate in 500 μ L of methanol prior to mixing in the dilution water. The measured concentrations of [REDACTED] in test solutions were based on a specific gravity of 1.4 as noted on the material safety data sheet provided with the sample. The acute test dilution series and [REDACTED] test solution preparations are summarized below.

Test Solution (mg/L [REDACTED])	Volume of [REDACTED]/1000 mL Dilution Water
Control	None
Control with Methanol	None
200	142 μ L
300	213 μ L
400	284 μ L
500	355 μ L

2.4.4 Chronic Toxicity Testing

The third phase of testing involved the performance of the static renewal *Ceriodaphnia dubia* and fathead minnow tests. Based on the results of the acute toxicity tests, a dilution series consisting of control, 5 mg/L, 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, and 500 mg/L of targeted [REDACTED] concentrations were used for the chronic testing. An additional control solution consisting of 500 μ L of methanol/L of dilution water was included in testing to serve as a second control for determinations of potential toxicity associated with the presence of methanol in test solutions. Methanol was used as a solvent medium for [REDACTED] prior to mixing of [REDACTED] with dilution water and preparation of test solutions.

Test solutions were prepared at test initiation and daily chronic test solution renewals. A 100 mg/L stock solution of [REDACTED] was used to prepare the 5 mg/L, 10 mg/L, 25 mg/L, and 50 mg/L [REDACTED] test solutions. [REDACTED] stock solutions were measured volumetrically, dissolved in 500 μ L of pure methanol, and added to premeasured volumes of dilution water. The solutions were gently mixed in a separatory funnel prior to use.

Test Concentration (mg/L [REDACTED])	Volume of [REDACTED] 1000 mL dilution water
Control	None
Control with Methanol	None
5	5 mL of 100 mg/L [REDACTED] solution
10	10 mL of 100 mg/L [REDACTED] solution
25	25 mL of 100 mg/L [REDACTED] solution
50	50 mL of 100 mg/L [REDACTED] solution
100	71 μ L
500	355 μ L

2.5 Statistical Analysis

In light of the analytical difficulties presented by [REDACTED] all statistical analyses of acute and chronic test data were based on targeted [REDACTED] test concentrations.

Insufficient mortalities occurred in the acute toxicity tests to calculate an LC_{50} . Mortality per test concentration was reported, and the LC_{50} values were approximated based on the highest test solution used.

Statistical analysis procedures described in EPA/600/4-91/002 were used with Toxstat Version 3.5 to perform statistical analyses for the *Ceriodaphnia* and fathead minnow chronic tests. For the *Ceriodaphnia* chronic test, survival was analyzed first using Fisher's Exact Test. Normality of distribution and homogeneity of variance for the reproduction data were next checked with the Shapiro Wilk's or Chi-Square Tests and Bartlett's Test, respectively. ANOVA and Dunnett's Test or Steel's Many-One Rank Test were used to evaluate reproduction. Any *Ceriodaphnia* test replicates identified as males were excluded from the reproduction data analyses as per EPA guidelines.

For the fathead minnow chronic test, data were transformed using the arc sine [square root (y)] transformation. Survival was analyzed first. Normality of distribution and homogeneity of variance were next checked with Shapiro-Wilks and Bartlett's Test, respectively. ANOVA and Dunnett's Test or Steel's Many-One Rank Test were used to evaluate mortality and growth. If any test concentrations showed statistically significant mortalities, they were excluded from the growth data analyses. For statistical analyses of growth data, average growth of the organisms was based on the original number of larvae, as per EPA method guidelines.

Two sets of data analyses were performed for the chronic toxicity tests. Test organism survival and reproduction or growth in the [REDACTED] test solutions were statistically compared to both the standard control group (with no added methanol) and the methanol control group.

RESULTS

1.0 [REDACTED] Solubility Determination

Attempts to determine the water solubility of [REDACTED] followed the approach given in the test protocol that is included in Appendix 2. In brief, excess [REDACTED] as received from DuPont - was equilibrated with deionized water in a separatory funnel. After phase separation, an aliquot of the aqueous phase was analyzed for [REDACTED] content by purge and trap gas chromatographic/mass spectrometric (GC/MS) techniques.

The analytical system used for all determinations consisted of a Tekmar Model 3000 purge and trap concentrator interfaced to a Hewlett Packard Model 5890 GC, and a VG Trio I mass spectrometer. Calibration standards consisted of samples of [REDACTED] in laboratory pure deionized water containing [REDACTED] at concentrations of 80, 40, 20, 8.4, 4.2, and 2.1 $\mu\text{g/L}$. The standards were prepared by carefully adding to 25.0 mL of water appropriate quantities of a stock solution containing [REDACTED] at a concentration of 40 $\mu\text{g/mL}$ in methanol. To minimize loss of [REDACTED] by evaporation, the standards were prepared in purge and trap test tubes which were secured to the Tekmar purge and trap unit immediately after preparation.

Based on the chromatographic results obtained during calibration, [REDACTED] appeared to consist of a mixture of three poorly resolved constituents eluting with a retention time of between 4.0 and 4.1 minutes. Mass spectra for each of the constituents were virtually identical in that they exhibited prominent peaks, in decreasing order of intensity, at mass to charge ratios of 77 (100% intensity), 51 (26% intensity), and 69 (14% intensity). Raw data illustrating a typical chromatogram and mass spectrum are included in Appendix 3. Also included in Appendix 3 is the derived calibration curve, which proved to be linear with a coefficient of determination of 0.991.

Samples of [REDACTED] saturated water were analyzed for [REDACTED] content on 11 separate occasions, over the course of three separate days. Each run was preceded by the analysis of a mid-point calibration standard containing [REDACTED] at a concentration of 0.040 mg/L. Recoveries of the standard ranged from a low of 0.037 mg/L to a high of 0.052 mg/L, and averaged 0.046 mg/L (115% of theory).

For reason that are not immediately obvious, the results for the [REDACTED] saturated water solutions proved to be poorly reproducible, ranging from a minimum value of 1.15 mg/L to a maximum of 16.7 mg/L. For the entire set of 11 determinations, the average was found to be 6.14 mg/L, with a standard deviation of ± 5.33 mg/L.

It is equally puzzling to note that the chromatogram obtained for the [REDACTED] saturated water solutions was different from that obtained during the analysis of the calibration standards. Whereas the [REDACTED] peak had originally appeared as a poorly resolved triplet, the peak for the saturated water solutions took on the appearance of a well-resolved doublet, with one peak eluting at a retention time of approximately 3.6 minutes, and the second with a retention time of 4.1 minutes. Again, mass spectra for the peaks were virtually identical.

Although it amounts to little more than speculation, possible explanations for the observed behavior of [REDACTED] in water include the following:

1. [REDACTED] may possess sufficient surface activity to form micellar solutions. If so, [REDACTED] may tend to form monomolecular layers at the air/water interface and/or at the water/glass interface, making it difficult to withdraw representative samples from the bulk "solutions" for analysis.
2. [REDACTED] may be sufficiently reactive to undergo degradation (or polymerization) in the heated inlet of the gas chromatograph. If this were the case, it would not be surprising to see multiple peaks of variable intensity.
3. Focusing finally on the different peak patterns, and noting that the standards (which appeared as triplets) were analyzed immediately after preparation, whereas the [REDACTED] saturated water samples (which appeared as well-resolved doublets) had aged for up to several days prior to analysis, it may be that [REDACTED] is undergoing some unspecified sort of time dependent chemical transformation when allowed to equilibrate with water.

Despite the unusual properties exhibited by [REDACTED] in water, it can be concluded with a high degree of certainty that its solubility is extremely low, most likely on the order of 10 mg/L or less.

2.0 [REDACTED] Toxicity Determination

2.1 Toxicity Range-Finding

All data generated for the toxicity range-finding tests are included in Appendix 4. The results indicated no acute toxicity to fathead minnows and *Ceriodaphnia* at targeted [REDACTED] concentrations ranging from 10 mg/L to 1,000 mg/L (actual concentration may be less than 1,000 mg/L due to apparent accumulation of [REDACTED] on glassware). Toxicity data for these tests are summarized in Table 1.

2.2 Acute Toxicity Testing

All data generated for the acute toxicity tests are included in Appendix 5. The results indicated no acute toxicity to fathead minnows and *Ceriodaphnia* at [REDACTED].

TABLE 1

TOXICITY RANGE-FINDING DATA SUMMARY

Test Concentration ⁽¹⁾ (mg/L [REDACTED])	Percent Survival			
	<i>Ceriodaphnia</i>		Fathead Minnow	
	24-Hour	48-Hour	24-Hour	48-Hour
Control ⁽²⁾	100	100	100	100
0.01	100	100	100	100
0.1	100	100	100	100
1.0	100	100	100	100
5.0	100	100	100	100
100	100	100	100	100
1,000 ⁽³⁾	100	100	100	100
Toxicity End-Point ⁽⁴⁾	LC ₅₀ >1,000 mg/L		LC ₅₀ >1,000 mg/L	

Notes

- 1 Targeted test concentrations.
- 2 Control consisted of EPA moderately hard water and contained no [REDACTED]
- 3 Actual [REDACTED] concentration may be less than 1,000 mg/L based on appearance of [REDACTED] residual on glassware used to prepare test solution.
- 4 LC₅₀ values estimated based on test organism response in highest targeted test solution.

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 [REDACTED]

concentrations ranging from 200 mg/L to 500 mg/L. The survival of *Ceriodaphnia* in the methanol control (70% survival) was reduced relative to the standard control (100% survival) and [REDACTED] test solutions (90% - 100% survival). No reason for this reduction in survival was identified. The results are summarized in Table 2. As depicted, the LC₅₀ values for both tests were estimated based on the highest [REDACTED] test solution used and are reported as greater than (>) 500 mg/L.

2.3 Chronic Toxicity Testing

All data generated for the chronic toxicity tests are included in Appendices 6 and 7 and the test results are summarized in Tables 3 and 4. Statistically, fathead minnow survival and growth were not significantly reduced by exposure to [REDACTED] test concentrations ranging from 5 to 500 mg/L. This was consistent for comparisons to standard laboratory water (no methanol) controls as well as comparisons to control water spiked with 500 µL methanol. The no observed effect concentration (NOEC) value for this test was based on the highest test concentration used and is reported as NOEC = 500 mg/L. No unusual behavior was observed by control larvae in the dilution water controls and dilution water with methanol controls.

In the *Ceriodaphnia* chronic test, survival was significantly reduced in the 500 mg/L [REDACTED] concentration (40% survival). *Ceriodaphnia* survival and reproduction data were statistically analyzed by comparing the survival and reproduction in each test concentration to the standard control group (with no methanol added) (Table 3) and to the methanol control group (Table 4). A statistically significant reduction in reproduction was observed at the 25 mg/L [REDACTED] test concentration when the data were compared to the standard control group, resulting in a non-continuous dose-dependent toxicity response. In accordance with standard EPA chronic test data evaluation procedures, the NOEC value for this test may be defined as 10 mg/L, the highest [REDACTED] concentration under the lowest statistically significant concentration (25 mg/L). Alternatively, test organism reproduction at the 25 mg/L concentration may be considered aberrant and the NOEC may be defined as 100 mg/L, based on improved reproduction (relative to the 25 mg/L concentration) in the 50 mg/L and 100 mg/L test concentrations. The reproduction data analyses showed no statistically significant reduction in reproduction in the 100 mg/L and lower [REDACTED] test concentrations when the data were compared to the methanol-spiked control group (Table 4). The NOEC value for the test when compared to the methanol-spiked control was 100 mg/L.

In light of test organism reproduction in the 5 mg/L, 10 mg/L, 50 mg/L, and 100 mg/L test concentrations, the NOEC for *Ceriodaphnia* is believed to be 100 mg/L.

TABLE 2
ACUTE TOXICITY DATA SUMMARY

Test Concentration ⁽¹⁾ (mg/L)	Percent Survival			
	<i>Ceriodaphnia</i>		Fathead Minnow	
	24-Hour	48-Hour	24-Hour	48-Hour
Control ⁽²⁾	100	100	95	95
Control with Methanol ⁽³⁾	100	70	100	100
200	100	95	100	100
300	100	100	100	100
400	100	90	100	100
500	100	95	100	100
Toxicity End- Point ⁽⁴⁾	LC ₅₀ >500 mg/L	LC ₅₀ >500 mg/L	LC ₅₀ >500 mg/L	LC ₅₀ >500 mg/L

Notes.

1. Targeted test concentrations.
2. Control consisted of EPA moderately hard water and contained no [REDACTED]
3. Control consisted of EPA moderately hard water and contained 500 µL methanol/L and no [REDACTED]
4. LC₅₀ values estimated based on test organism response in highest targeted test solution.

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[REDACTED]

TABLE 3

**CHRONIC TOXICITY DATA SUMMARY
COMPARISON TO STANDARD CONTROLS**

Test Concentration ⁽¹⁾ (mg/L)	Toxicity Test Results			
	<i>Ceriodaphnia</i>		Fathead Minnow	
	Percent Survival	Average Reproduction	Percent Survival	Average Growth (mg)
Control ⁽²⁾	100	18.0	98	0.420
5	100	16.6	98	0.468
10	90	17.1	95	0.416
25	90	13.9 ⁽³⁾	95	0.430
50	70	15.2	100	0.442
100	70	17.2	100	0.399
500	40 ⁽³⁾	NA ⁽⁴⁾	98	0.422
Toxicity End-Points	NOEC - Survival = 100 mg/L NOEC - Reproduction = 100 mg/L ⁽⁵⁾ IC ₂₅ - Reproduction >500 mg/L ⁽⁶⁾		NOEC - Survival = 500 mg/L NOEC - Growth = 500 mg/L IC ₂₅ - Growth >500 mg/L ⁽⁵⁾	

Notes:

1. Targeted test concentrations.
2. Control consisted of EPA moderately hard water and contained no [REDACTED]
3. Asterisks (*) indicates a statistically significant reduction in survival and/or reproduction as compared to the control group.
4. Not Applicable. Since test organism survival at this concentration was significantly reduced relative to the control group, reproduction data was not included in the reproduction analysis.
5. Reduction test organism reproduction at the 25 mg/L concentration considered aberrant. NOEC reproduction value based on test organism reproduction in 100 mg/L concentration.
6. IC₂₅ values estimated based on test organism response in highest targeted test solution.

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[REDACTED]

TABLE 4

**CHRONIC TOXICITY DATA SUMMARY
COMPARISON TO METHANOL-SPIKED CONTROLS**

Test Concentration ⁽¹⁾ (mg/L)	Toxicity Test Results			
	<i>Cenodaphnia</i>		Fathead Minnow	
	Percent Survival	Average Reproduction	Survival	Average Growth (mg)
Control with Methanol ⁽²⁾	90	14.3	95	0.407
5	100	16.6	98	0.468
10	90	17.1	95	0.416
25	90	13.9	95	0.430
50	70	15.2	100	0.442
100	70	17.2	100	0.399
500	40 ⁽³⁾	NA ⁽⁴⁾	98	0.422
Toxicity End-Points	NOEC - Survival = 100 mg/L NOEC - Reproduction = 100 mg/L IC ₂₅ - Reproduction >500 mg/L ⁽⁵⁾		NOEC - Survival = 500 mg/L NOEC - Growth = 500 mg/L IC ₂₅ - Growth >500 mg/L	

Notes:

1. Targeted test concentrations.
2. Control consisted of EPA moderately hard water and contained 500 µL methanol/L and no [REDACTED]
3. Asterisks (*) indicates a statistically significant reduction in survival and/or reproduction as compared to the control group.
4. Not Applicable. Since test organism survival at this concentration was significantly reduced relative to the control group, reproduction data was not included in the reproduction analysis.
5. IC₂₅ values estimated based on test organism response in highest targeted test solution.

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[REDACTED]

APPENDIX 1
MATERIAL SAFETY DATA SHEET

The MSDS format adheres to the standards and regulatory requirements of the United States and may not meet regulatory requirements in other countries.

DuPont
Material Safety Data Sheet

Page 1

DU004604 [REDACTED] FLUOROTELOMER INTERMEDIATE
Revised 12-JAN-1999

CHEMICAL PRODUCT/COMPANY IDENTIFICATION

Material Identification

[REDACTED] is a registered trademark of DuPont.

Company Identification

MANUFACTURER/DISTRIBUTOR

DuPont
1007 Market Street
Wilmington, DE 19898

PHONE NUMBERS

Product Information : 1-800-441-7515
Transport Emergency : CHEMTREC 1-800-424-9300
Medical Emergency : 1-800-441-3637

COMPOSITION/INFORMATION ON INGREDIENTS

[REDACTED]

HAZARDS IDENTIFICATION

Potential Health Effects

Eye contact may cause eye irritation with discomfort, tearing or blurring of vision.

Based on related products, inhalation of spray or mist may cause nasal, throat, or lung irritation. Inhalation of large amounts of respirable particles may be toxic to the lungs. Symptoms may be modest initially, followed in hours by severe shortness of breath requiring prompt medical attention.

Carcinogenicity Information

None of the components present in this material at concentrations equal to or greater than 0.1% are listed by IARC, NTP, OSHA or ACGIH as a carcinogen.

FIRST AID MEASURES

First Aid**INHALATION**

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.

SKIN CONTACT

Flush skin with water after contact. Wash contaminated clothing before reuse.

EYE CONTACT

In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.

INGESTION

If swallowed, do not induce vomiting. Immediately give 2 glasses of water. Never give anything by mouth to an unconscious person. Call a physician.

Notes to Physicians

Activated charcoal mixture may be administered. To prepare activated charcoal mixture, suspend 50 grams activated charcoal in 400 mL water and mix thoroughly. Administer 5 mL/kg, or 350 mL for an average adult.

FIRE FIGHTING MEASURES

Flammable Properties

Flash Point : Does not ignite
Method : PMCC

Hazardous decomposition products including carbon dioxide, carbon monoxide, hydrogen fluoride, toxic gases or particles may be formed during combustion. These products may cause severe eye, nose, throat, and lung irritation or toxic effects.

Extinguishing Media

Water Spray, Foam, Dry Chemical, CO₂.

Fire Fighting Instructions

Wear self-contained breathing apparatus. Wear full protective equipment.

(FIRE FIGHTING MEASURES - Continued)

Avoid breathing decomposition products.

ACCIDENTAL RELEASE MEASURES

Safeguards (Personnel)

NOTE: Review FIRE FIGHTING MEASURES and HANDLING (PERSONNEL) sections before proceeding with clean-up. Use appropriate PERSONAL PROTECTIVE EQUIPMENT during clean-up.

Spill Clean Up

Soak up with sawdust, sand, oil dry or other absorbent material. Shovel or sweep up.

Accidental Release Measures

Flush spill area with water.

HANDLING AND STORAGE

Handling (Personnel)

Avoid contact with eyes. Wash thoroughly after handling. Do not store or consume food, drink, or tobacco in areas where they may become contaminated with this material. Avoid circumstances that produce respirable particles unless suitable ventilation and respirator are used.

Storage

Keep container tightly closed.

Keep away from open flames and heated surfaces above 200 degrees C (392 deg F).

EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Controls

Keep container tightly closed. Use only with adequate ventilation. Vent heated extruder or dryer fumes outside work area. Do not aerosolize. In spray applications, use airless type pressure spray equipment at less than 60 psi, and exhaust ducts, drip pans, or other design features to minimize worker exposure to mists and overspray.

Keep away from open flames and heated surfaces above 200 degC (392 degF).

(EXPOSURE CONTROLS/PERSONAL PROTECTION - Continued)

Personal Protective Equipment

EYE/FACE PROTECTION

Wear safety glasses or coverall chemical splash goggles.

RESPIRATORS

Wear NIOSH approved respiratory protection as appropriate.

PROTECTIVE CLOTHING

Where there is potential for skin contact have available and wear as appropriate, impervious gloves, apron, pants, and jacket.

Exposure Guidelines

Applicable Exposure Limits

Perfluorobutylethylene

PEL (OSHA)	: None Established
TLV (ACGIH)	: None Established
AEL * (DuPont)	: 100 ppm, 8 Hr. TWA

* AEL is DuPont's Acceptable Exposure Limit. Where governmentally imposed occupational exposure limits which are lower than the AEL are in effect, such limits shall take precedence.

Exposure Guideline Comments

No AEL has been established for this product. Other products with fluorinated material components have an AEL of 0.1 mg/m³ to 1 mg/m³ (8 hour TWA) for respirable size aerosol particles.

Air monitoring studies conducted at customer sites indicates that the use of the recommended low pressure (less than 60 psi) airless type, garden type or deck specific hand pump sprayer with spray tip orifice minimum of 0.031 inches in diameter does not produce respirable size aerosol particle concentrations near the AEL.

PHYSICAL AND CHEMICAL PROPERTIES

Physical Data

Boiling Point	: 58 C (136 F) @ 760 mm Hg
% Volatiles	: 100 WT%
Solubility in Water	: Negligible
Odor	: Ethereal
Form	: Liquid, Clear
Color	: Colorless
Specific Gravity	: 1.4

STABILITY AND REACTIVITY

Chemical Stability

Stable at normal temperatures and storage conditions.

Incompatibility with Other Materials

None reasonably foreseeable.

Decomposition

Hazardous decomposition products including carbon dioxide, carbon monoxide, hydrogen fluoride, toxic gases or particles may be formed during combustion. These products may cause severe eye, nose, throat, and lung irritation or toxic effects.

Polymerization

Homopolymerization will not occur. Copolymerization can occur in the presence of free radical sources, heat or UV light.

TOXICOLOGICAL INFORMATION

Animal Data

Inhalation 1 hour ALC: >7,178 ppm in mice
Oral LD50: >25,000 mg/kg in rats

The product is not a skin irritant, is a mild eye irritant, and is untested for skin sensitization in animals. Repeated exposure to high levels (5 vol%) produced blood changes (decreased platelets and increased monocyte). Tests in animals demonstrate no developmental toxicity. The product does not produce genetic damage in bacterial cell cultures but has not been tested in animals. Tests for carcinogenic and reproductive activity have not been performed.

DISPOSAL CONSIDERATIONS

Waste Disposal

Treatment, storage, transportation, and disposal must be in accordance with applicable Federal, State/Provincial, and Local regulations.

TRANSPORTATION INFORMATION

Shipping Information

Not Regulated as a hazardous material by DOT, IMO, or IATA.

REGULATORY INFORMATION

U.S. Federal Regulations

TSCA Inventory Status : Reported/Included.

TITLE III HAZARD CLASSIFICATIONS SECTIONS 311, 312

Acute : Yes
Chronic : No
Fire : No
Reactivity : No
Pressure : No

OTHER INFORMATION

NFPA, NPCA-HMIS

NPCA-HMIS Rating

Health : 1
Flammability : 0
Reactivity : 0

Personal Protection rating to be supplied by user depending on use conditions.

The data in this Material Safety Data Sheet relates only to the specific material designated herein and does not relate to use in combination with any other material or in any process.

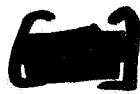
Responsibility for MSDS : MSDS Coordinator
Address : DuPont Chemical Solutions Enterprise
Wilmington, De. 19898
Telephone : 800-441-7515

Indicates updated section.

This information is based upon technical information believed to be reliable. It is subject to revision as additional knowledge and experience is gained.

End of MSDS

APPENDIX 2



SOLUBILITY DETERMINATION -
TEST PROTOCOL



Test Protocol for
Determining the Solubility of
[REDACTED] in Water

- 1 Obtain sample of authentic product from DuPont. No more than 100 mL should be required
- 2 Calibrate the GC/MS system using samples of the authentic product. At the same time, determine a RRF for [REDACTED] in relation to an internal standard having a retention time similar to that of [REDACTED]. Since [REDACTED] is claimed to exhibit negligible solubility in water, prepare calibration standards by dissolving carefully weighed quantities of the product as received in a suitable solvent. Methanol is suggested. The calibration curve should be constructed using a minimum of four, and preferably five, calibration standards
- 3 Equilibrate [REDACTED] with laboratory pure water as follows
 - a Place approximately 25 mL of the product as received in a clean 2L separatory funnel.
 - b Add 1L of laboratory pure water
 - c Shake vigorously to intimately mix the two phases, venting to atmosphere as needed to relieve pressure. Allow the phases to separate, then repeat both steps a minimum of five (5) times
 - d After mixing, allow the funnel and its contents to stand undisturbed overnight (to ensure complete phase separation)
 - e Remove a portion of the aqueous phase (upper phase) using a clean transfer pipet. Place the sample in a 40 mL VOA vial equipped with a Teflon®-lined cap. Make the transfer quickly to avoid loss of [REDACTED] to the atmosphere. When full, seal the vial with its cap such that zero headspace exists after the cap is secured. Prepare three such vials for subsequent analysis
 - f After removing analytical samples, measure and record. i) temperature of the aqueous phase, and ii) atmospheric pressure
- 4 Analyze all three samples for the presence of [REDACTED]. Quantify concentration in units of mg/L relative to the calibration curve and RRF, as determined in Step 2

Safety Considerations

The toxicity of [REDACTED] although not believed to be high, has not been well-defined. All preparative steps should take place in a hood. Standard and customary measures should be taken to guard against skin and eye contact. Should contact occur, follow the procedures outlined in the attached MSDS

[REDACTED]

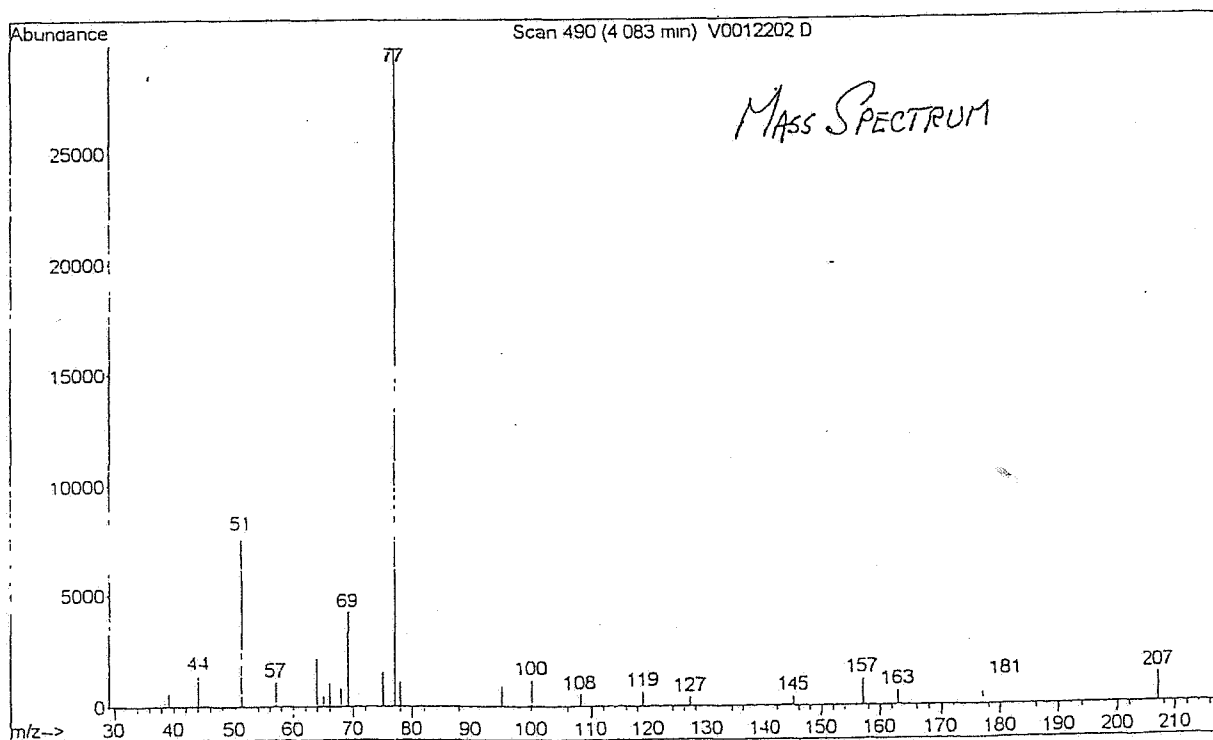
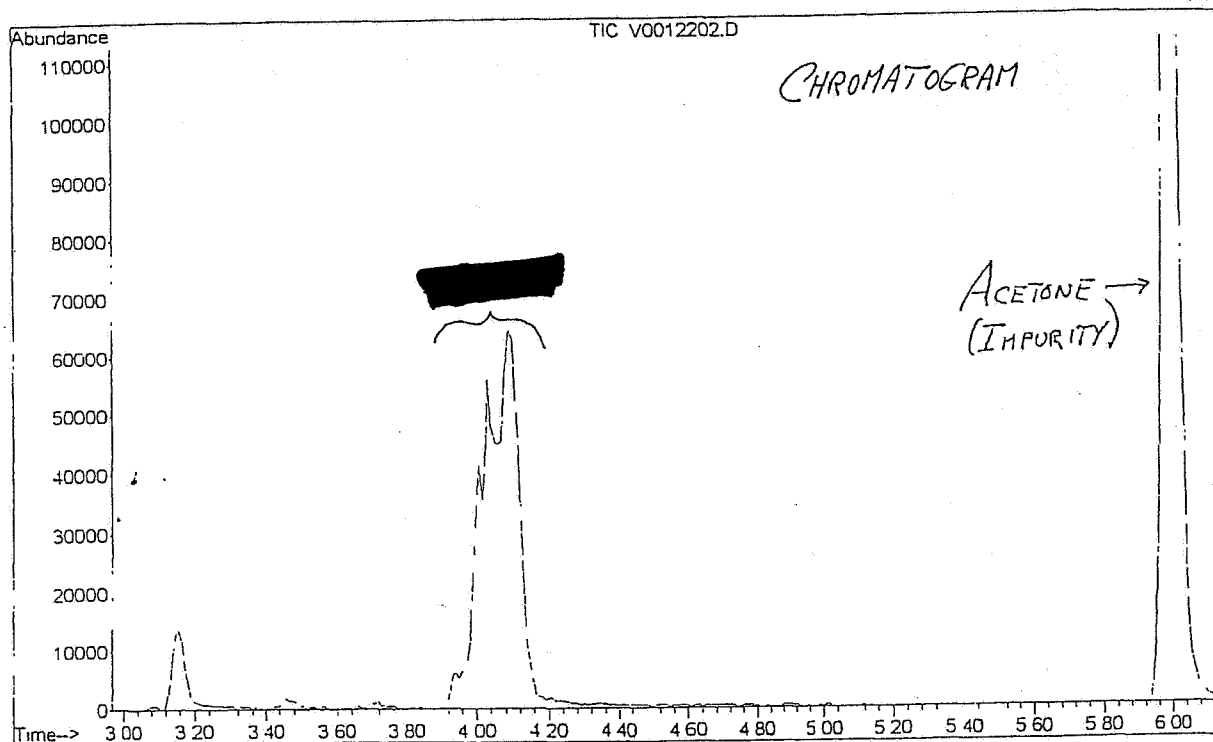
APPENDIX 3



SOLUBILITY DETERMINATION -
ANALYTICAL DATA

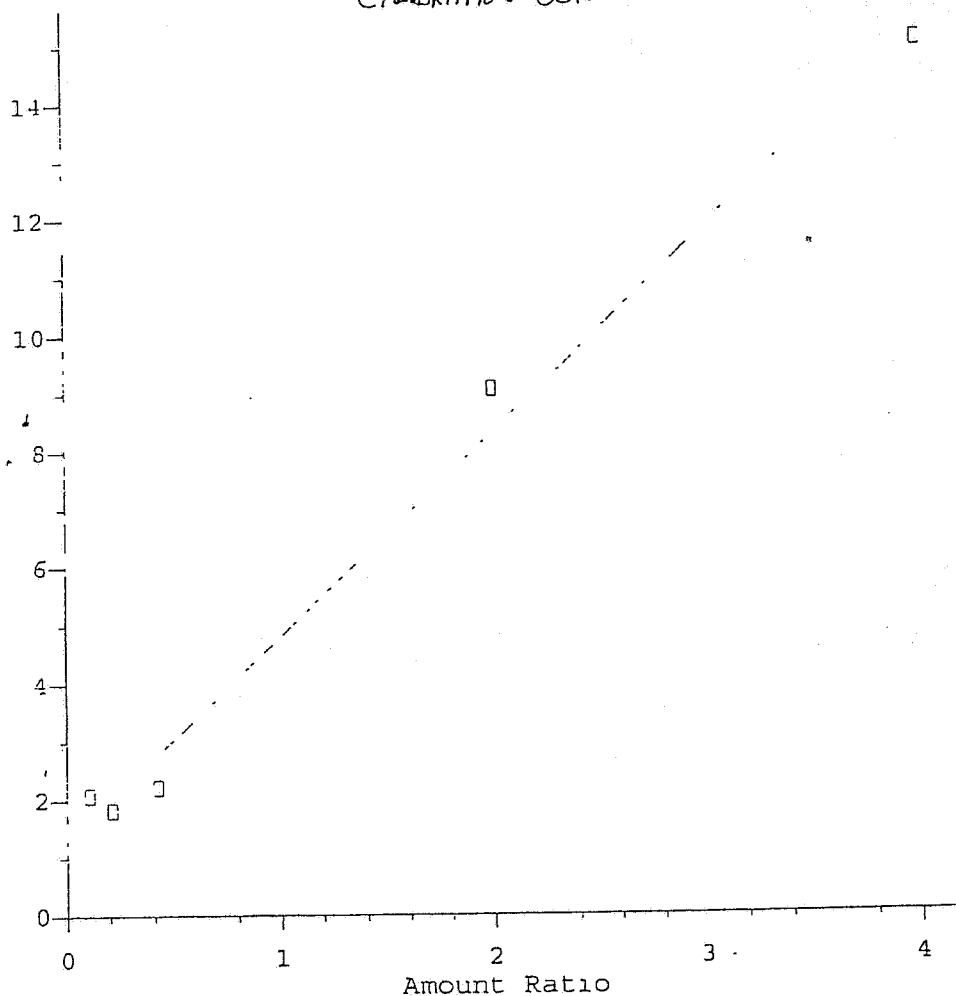
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Operator : BP
Acquired : 22 JAN 1 9.02 using AcqMethod
Instrument : Trio-1
Sample Name: 1000 NG STD
Misc Info : 25mls
Vial Number: 0

CALIBRATION STANDARD



Response Ratio

[REDACTED]
CALIBRATION CURVE



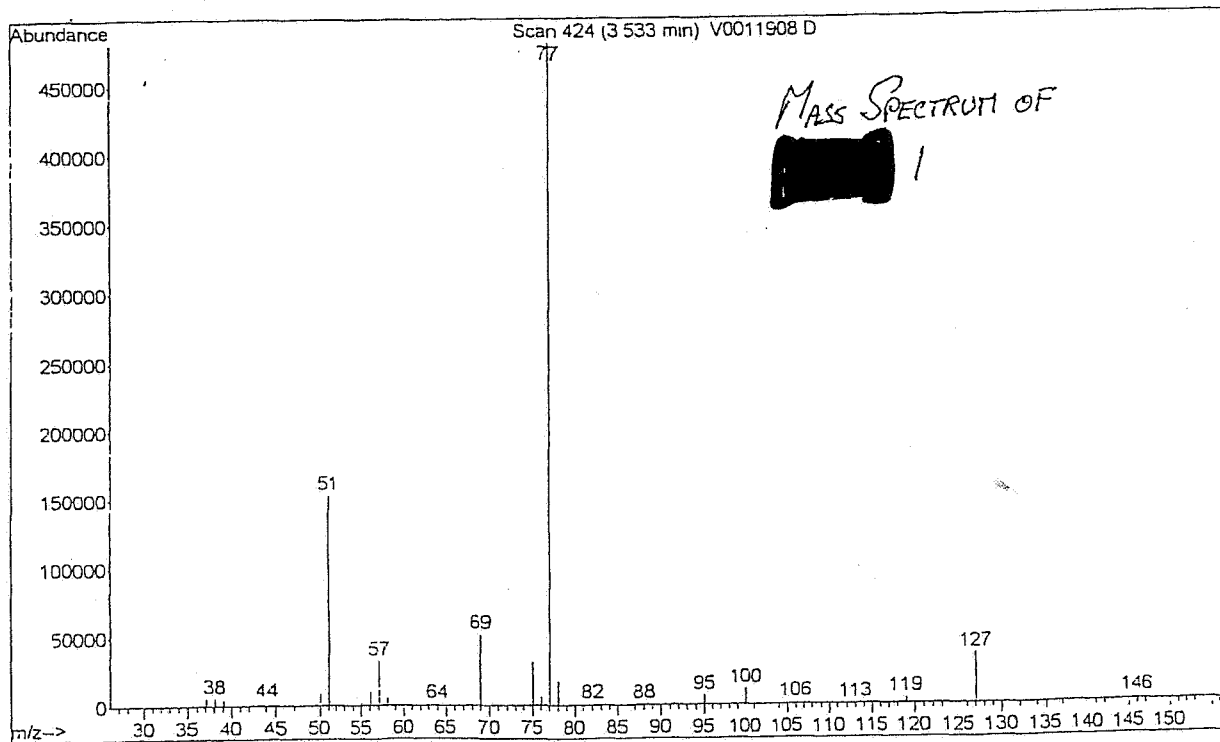
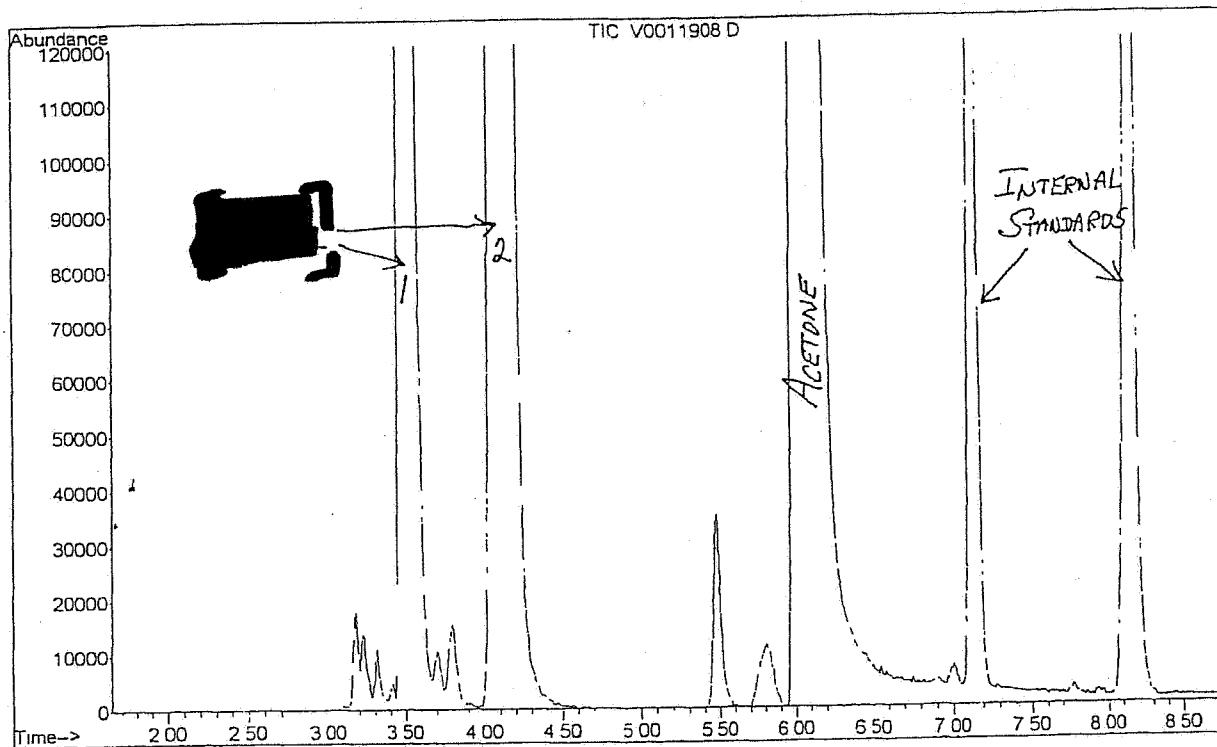
Resp Ratio = $3.49e+000 * \text{Amt} + 1.33e+000$
Coef of Det (r^2) = 0.991 Curve Fit: Linear

Method Name: C:\V012501**[REDACTED]**2.M
Calibration Table Last Updated: Wed Jan 24 07:52:06 2001

Company Sanitized. Does not contain TSCA CBI

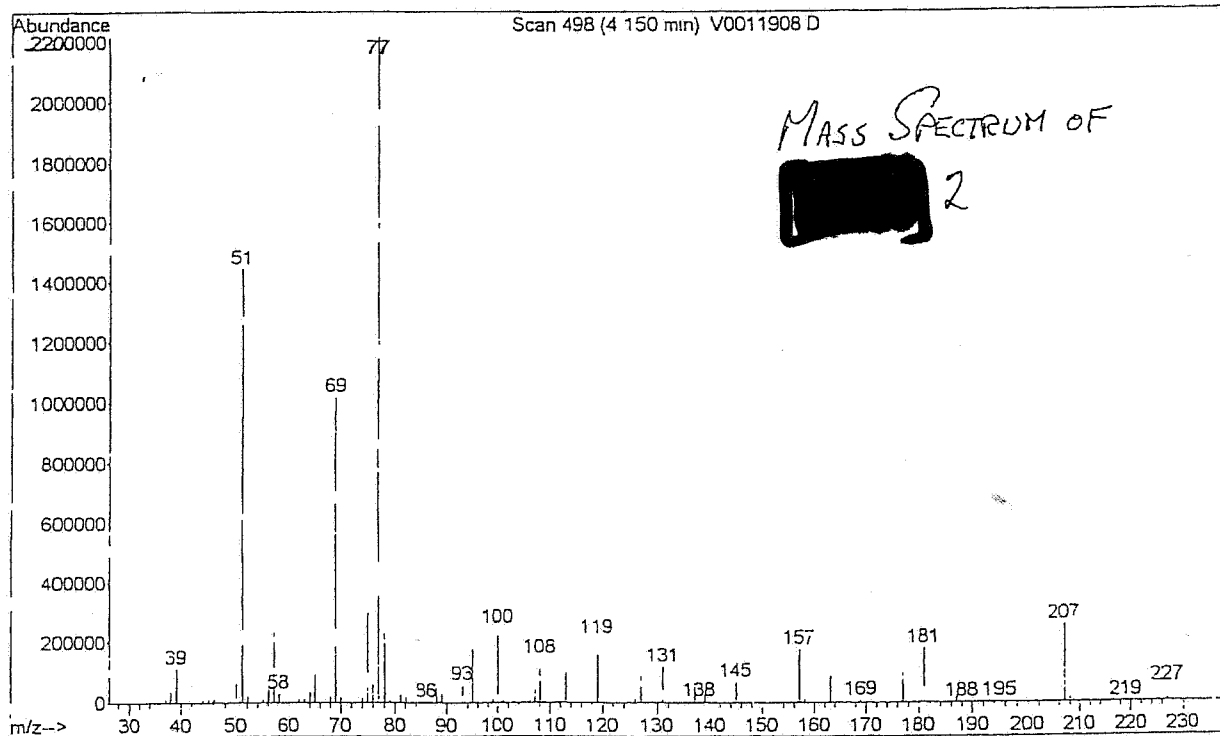
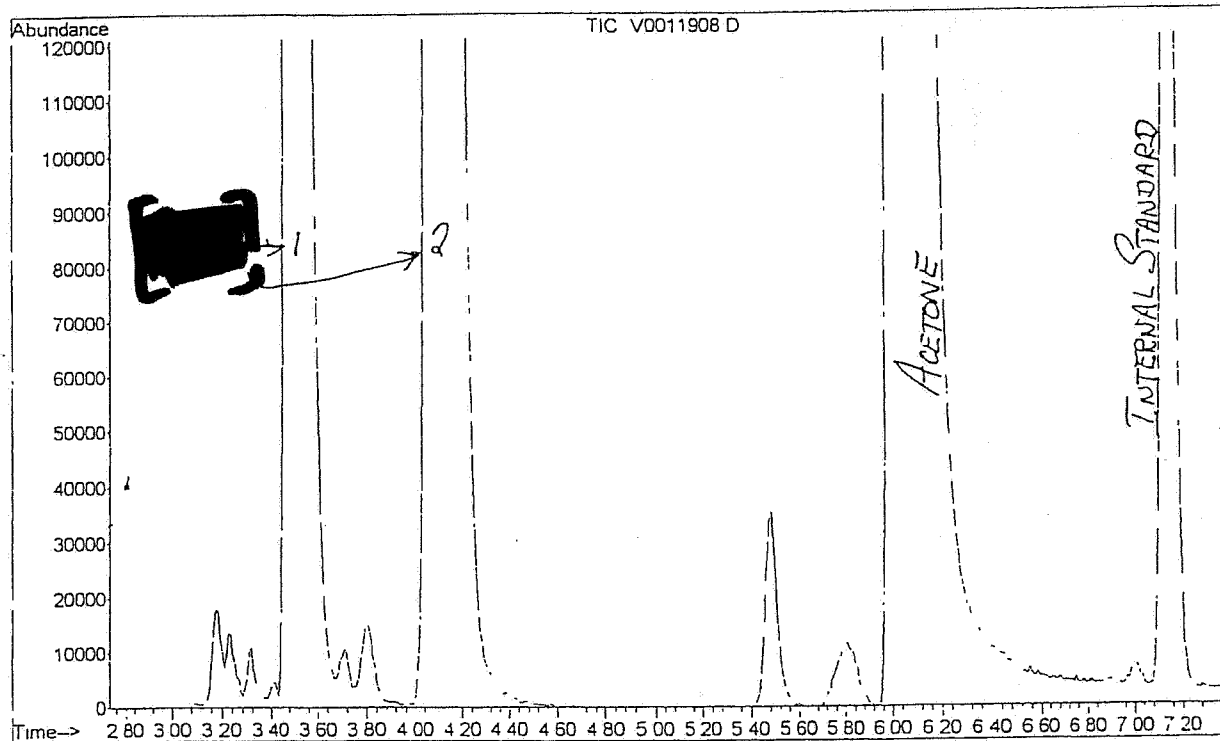
File : C:\HPCHEM\1\DATA\V011901\V0011908.D
Operator : BP
Acquired : 19 JAN 1 13:45 using AcqMethod
Instrument : Trio-1
Sample Name: 25 ML [REDACTED] H2O EXTRACT
Misc Info : 25mls
Vial Number: 0

SATURATED SOLUTION OF
[REDACTED] in H₂O



File : C:\HPCHEM\1\DATA\VG11901\V0011908.D
Operator : BP
Acquired : 19 JAN 1 13:45 using AcqMethod
Instrument : Trio-1
Sample Name: 25 ML [REDACTED] H2O EXTRACT
Misc Info : 25mls
Vial Number: 0

SATURATED SOLUTION
OF [REDACTED] IN H₂O



APPENDIX 4

TOXICITY RANGE-FINDING TEST DATA

TEST SUMMARY SHEET

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY

CLIENT Dyport- JOB NO 60175 PAGE 1 OF 1
TOXICANT/EFFLUENT [REDACTED] PERMIT NO N/A

Initial Range-Finding Tests

ACUTE TOXICITY TEST RESULTS			CHRONIC TOXICITY TEST RESULTS		
TEST DATE(S)	Ceriodaphnia	Fathead Minnow		Ceriodaphnia	Fathead Minnow
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	<u>1/25 - 1/27/01</u> <u>< 24 hours</u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	<u>1/25 - 1/27/01</u> <u>2 days</u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	<u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	<u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)	<u>100%</u>	<u>100%</u>	CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES % CONTROL FEMALES WITH 3 BROODS (≥60%) AVE NO YOUNG/SURVIVING FEMALES (≥15)		
STATISTICAL RESULTS SURVIVAL IN 100% (Highest Conc.) LC ₅₀ 95% CI METHOD	<u>100%</u> <u>> 5 mg/L</u> <u>ND *</u> <u>ND</u>	<u>100%</u> <u>> 5 mg/L</u> <u>ND</u> <u>ND</u>	AVE WT /SURV CONTROL (≥0.25 mg) SURVIVAL IN HIGHEST () CONC NUMBER OF REPLICATES NUMBER OF ORGANISM/REPLICATE NORMAL DISTRIBUTION? HOMOGENEOUS VARIANCE? NOEC METHOD NOEC SURVIVAL NOEC REPRODUCTION/GROWTH		<u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u>

COMMENTS * ND = Not Determinable (due to insufficient mortality)

TEST SUMMARY SHEET

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY

CLIENT Dupont  JOB NO 60175 PAGE 1 OF 1
TOXICANT/EFFLUENT PERMIT NO N/A

Follow-up Range-Finding Tests

ACUTE TOXICITY TEST RESULTS		CHRONIC TOXICITY TEST RESULTS	
	Ceriodaphnia	Fathead Minnow	
TEST DATE(S)	2/1-2/3/01	2/1-2/3/01	
TEST CONDITIONS			
TEST ORGANISM AGE	24 hours	2 days	TEST ORGANISM AGE
DILUTION WATER	EPA-MHW	EPA-MHW	DILUTION WATER
SOURCE	OLVER LABS	OLVER LABS	SOURCE
AERATION	continuous	continuous	AERATION
TEST ACCEPTABILITY			
CONTROL SURVIVAL (≥90%)	100%	100%	CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES
			% CONTROL FEMALES WITH 3 BROODS (≥60%)
			AVE NO YOUNG/SURVIVING FEMALES (≥15)
			AVE WT /SURV CONTROL (±0.25 mg)
STATISTICAL RESULTS			
SURVIVAL IN 100% (Highest Conc.)	100%	100%	SURVIVAL IN HIGHEST () CONC
LC ₅₀	> 1000 mg/L	> 1000 mg/L	NUMBER OF REPLICATES
95% CI	ND*	ND	NUMBER OF ORGANISMS/REPLICATE
METHOD	ND	ND	NORMAL DISTRIBUTION?
			HOMOGENEOUS VARIANCE?
			NOEC METHOD
			NOEC SURVIVAL
			NOEC REPRODUCTION/GROWTH

COMMENTS # ND = Not Determinable (due to insufficient mortality)

pg 1 of 1

ACUTE TOXICITY TEST

Sample Information
Client DuPont

Client Dejean
Permit No N/A
Toxicant/Effluent [REDACTED] (5 mg/L) *

Sample Type	N/A
Grab Collected	AM/PM / /

Composite Collected (Date)
From AM/PM / (Date)

To AM/PM / / (Date)

Job No 60175

Sample No

TRC	N/A	mg/L
-----	-----	------

Initial pH N/AInitial DO 2.1 mg/L

Adjustments

Test Information

Analysl(s)	sm, JkN
------------	---------

Test Species P. promelas

Culture No 1789

Age 2 days

Length ($\bar{x} \pm \text{SD}$)

Weight ($\bar{x} \pm \text{SD}$) 2.41 $\pm$ 0.1

Test Mode	Static
1.1	1.1
1.2	1.2
1.3	1.3
1.4	1.4
1.5	1.5
1.6	1.6
1.7	1.7
1.8	1.8
1.9	1.9
1.10	1.10
1.11	1.11
1.12	1.12
1.13	1.13
1.14	1.14
1.15	1.15
1.16	1.16
1.17	1.17
1.18	1.18
1.19	1.19
1.20	1.20
1.21	1.21
1.22	1.22
1.23	1.23
1.24	1.24
1.25	1.25
1.26	1.26
1.27	1.27
1.28	1.28
1.29	1.29
1.30	1.30
1.31	1.31
1.32	1.32
1.33	1.33
1.34	1.34
1.35	1.35
1.36	1.36
1.37	1.37
1.38	1.38
1.39	1.39
1.40	1.40
1.41	1.41
1.42	1.42
1.43	1.43
1.44	1.44
1.45	1.45
1.46	1.46
1.47	1.47
1.48	1.48
1.49	1.49
1.50	1.50
1.51	1.51
1.52	1.52
1.53	1.53
1.54	1.54
1.55	1.55
1.56	1.56
1.57	1.57
1.58	1.58
1.59	1.59
1.60	1.60
1.61	1.61
1.62	1.62
1.63	1.63
1.64	1.64
1.65	1.65
1.66	1.66
1.67	1.67
1.68	1.68
1.69	1.69
1.70	1.70
1.71	1.71
1.72	1.72
1.73	1.73
1.74	1.74
1.75	1.75
1.76	1.76
1.77	1.77
1.78	1.78
1.79	1.79
1.80	1.80
1.81	1.81
1.82	1.82
1.83	1.83
1.84	1.84
1.85	1.85
1.86	1.86
1.87	1.87
1.88	1.88
1.89	1.89
1.90	1.90
1.91	1.91
1.92	1.92
1.93	1.93
1.94	1.94
1.95	1.95
1.96	1.96
1.97	1.97
1.98	1.98
1.99	1.99
2.00	2.00
2.01	2.01
2.02	2.02
2.03	2.03
2.04	2.04
2.05	2.05
2.06	2.06
2.07	2.07
2.08	2.08
2.09	2.09
2.10	2.10
2.11	2.11
2.12	2.12
2.13	2.13
2.14	2.14
2.15	2.15
2.16	2.16
2.17	2.17
2.18	2.18
2.19	2.19
2.20	2.20
2.21	2.21
2.22	2.22
2.23	2.23
2.24	2.24
2.25	2.25
2.26	2.26
2.27	2.27
2.28	2.28
2.29	2.29
2.30	2.30
2.31	2.31
2.32	2.32
2.33	2.33
2.34	2.34
2.35	2.35
2.36	2.36
2.37	2.37
2.38	2.38
2.39	2.39
2.40	2.40
2.41	2.41
2.42	2.42
2.43	2.43
2.44	2.44
2.45	2.45
2.46	2.46
2.47	2.47
2.48	2.48
2.49	2.49
2.50	2.50
2.51	2.51

Dilution Water 5 PM MHW

Start of Test 4:10 AM PM 1/25/01 (Date)

End of Test 430 AMPM 12701 (Date)

Test Container Size 600 mL

Test Solution Volume 100 mL

Dissolved Oxygen (Time/ Test Soln/ Conc)

6w

Aeration N/A
 Drying None
 Date/Time/Place 10/10/2014

Beginning Date/Time/Rate
Observed 16 hour day/8-hour night

Conc or %	Rep	Number of Live Organisms			% Survival	Dissolved Oxygen (mg/L)			pH			Temperature (°C)			Initial Alkalinity (mg/L as CaCO ₃)	Initial Hardness (mg/L as CaCO ₃)	Conductivity (μmhos/cm) Initial/Final
		0	24	48		0	24	48	0	24	48	0	24	48			
5.0 mg/L	A	10	10	10	100%	7.8	7.5	7.0	7.7	7.7	7.7	25	25	25	60	100	318 / 324
1.0 mg/L	A	10	10	10	100%	7.8	7.4	7.0	7.7	7.7	7.7	25	25	25			317 / 327
0.1 mg/L	A	10	10	10	100%	7.8	7.5	7.0	7.8	7.7	7.7	25	25	25			316 / 325
0.01 mg/L	A	10	10	10	100%	7.8	7.5	7.0	7.8	7.7	7.7	25	25	25			316 / 321
Control	A	10	10	10	100%	7.8	7.6	7.0	7.8	7.7	7.7	25	25	25	60	88	316 / 326

Analyst's Initials/Time

Notes

Carbon #3 : I- 1/21/01

Analysis	Amount	Time	Temp
Comments	* 69 mL	50 mL	Methanol / 2L MHW

Condition of Organisms at Test Initiation/End

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#2

Pg 1 of 1

ACUTE TOXICITY TEST

Sample Information

Client Dupont
 Permit No N/A
 Toxicant/Effluent [REDACTED] (5 mg/L) *
 Sample Type N/A
 Grab Collected N/A AM/PM / / (Date)
 Composite Collected N/A AM/PM / / (Date)
 From AM/PM / / (Date)
 To AM/PM / / (Date)

Job No. 60175

Sample No N/A mg/L
 T R C N/A mg/L
 Initial pH N/A mg/L
 Initial DO N/A mg/L
 Adjustments

Test Information

Analyst(s) SM / SPN
 Test Species C. dubia
 Culture No Individual Culture
 Age 24 hrs
 Length ($\pm$ SD) N/A
 Weight ($\pm$ SD) N/A
 Test Mode Static
 Dilution Water EPH mHW
 Aeration N/A
 Beginning Date/Time/Rate 16-hour day/8-hour night
 Photoperiod

Start of Test 4:10 AM/PM 1/25/01 (Date)
 End of Test 4:15 AM/PM 1/27/01 (Date)
 Test Container Size 30 mL
 Test Solution Volume 25 mL
 Dissolved Oxygen (Time/Test Soln/Conc) / / /

Conc or %	Rep	Number of Live Organisms			% Survival	Dissolved Oxygen (mg/L)			pH			Temperature (°C)			Initial Alkalinity (mg/L as CaCO ₃)	Initial Hardness (mg/L as CaCO ₃)	Conductivity (µmhos/cm) Initial/Final
		0	24	48		0	24	48	0	24	48	0	24	48			
5.0 mg/L	A	5	5	5	100%	7.8	7.6	7.4	7.7	7.7	7.8	25	25	25	60	100	318 / 332
	B	5	5	5													317 / 332
1.0 mg/L	A	5	5	5	100%	7.8	7.6	7.4	7.7	7.7	7.8	25	25	25			316 (SM) / 332
	B	5	5	5													
0.1 mg/L	A	5	5	5	100%	7.8	7.6	7.4	7.8	7.7	7.8	25	25	25			316 / 355
	B	5	5	5													
0.01 mg/L	A	5	5	5	100%	7.8	7.6	7.4	7.8	7.6	7.8	25	25	25			316 / 340
	B	5	5	5													
Control	A	5	5	5	100%	7.8	7.6	7.4	7.8	7.6	7.7	25	25	25	60	88	316 / 311
	B	5	5	5													
Analyst's Initials/Time		SM	SPN	SPN	SPN	SPN	SPN	SPN	SPN	SPN	SPN	SPN	SPN	SPN	Notes Carboy #3 I: 1/21/01		
		6.9 mL/50 mL methanol / 22 mHW															

Condition of Organisms at Test Initiation/End Healthy, normal; All organisms appeared healthy, normal at the end of the test

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ACUTE TOXICITY TEST

Sample Information

Client DurPoat
 Permit No 6075
 Toxinant/Effluent
 Sample Type
 Grab Collected AM/PM (Date)
 Composite Collected AM/PM (Date)
 From AM/PM (Date)
 To AM/PM (Date)

Test Information

Analyst(s) SM SPN
 Test Species C. dubia
 Culture No Individual Culture
 Age 4-4 hours
 Length ($\bar{x} \pm SD$) N/A
 Weight ($\bar{x} \pm SD$) N/A
 Test Mode Static
 Dilution Water EPA muw
 Start of Test 8:00 AM 2/01/01 (Date)
 End of Test 4:00 AM 2/3/01 (Date)
 Test Container Size 30 mL
 Test Solution Volume 25 mL
 Dissolved Oxygen (Time/Test Soln/Conc)
 Aeration N/A
 Beginning Date/Time/Rate N/A
 Photoperiod 16-hour day/8-hour night

Conc or %	Rep	Number of Live Organisms			% Survival	Dissolved Oxygen (mg/L)			pH			Temperature (°C)			Initial Alkalinity (mg/L as CaCO ₃)	Initial Hardness (mg/L as CaCO ₃)	Conductivity (µmhos/cm) Initial/Final
		0	24	48		0	24	48	0	24	48	0	24	48			
1000 mg/L	A	5	5	5	100%	7.8	7.8	7.8	7.7	7.9	7.8	25	25	25	64	100	339
	B	5	5	5													349
100 mg/L	A	5	5	5	100%	7.8	7.8	7.8	7.7	7.9	7.7	25	25	25			336
	B	5	5	5													342
Control	A	5	5	5	100%	7.8	7.8	7.8	7.8	7.8	7.6	25	25	25	64	92	331
	B	5	5	5													334
Analyst's Initials/Time		SM	SPN	SPN	SPN	SM	SPN	SPN	SM	SPN	SPN	SM	SPN	SPN	Notes Carbonyl #3 1/26/01		

APPENDIX 5
ACUTE TOXICITY TEST DATA

TEST SUMMARY SHEET

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY

CLIENT DuPont JOB NO 60175 PAGE 1 OF 1
TOXICANT/EFFLUENT [REDACTED] PERMIT NO N/A

Acute Toxicity Testing

ACUTE TOXICITY TEST RESULTS		CHRONIC TOXICITY TEST RESULTS		
TEST DATE(S)	Ceriodaphnia	Fathead Minnow		
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	2/14 - 2/16/01 24 hours EPA-MHW OLVER LABS continuous	2/14 - 2/16/01 3 days EPA-MHW OLVER LABS continuous	TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	Ceriodaphnia Fathead Minnow
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)	100%	95%	CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES % CONTROL FEMALES WITH 3 BROODS (≥60%) AVE NO YOUNG/SURVIVING FEMALES (≥15)	EPA-MHW OLVER LABS continuous
STATISTICAL RESULTS SURVIVAL IN 100% (Highest Conc) LC ₅₀ 95% CI METHOD	95% > 500 mg/L ND* ND	100% > 500 mg/L ND ND	AVE WT /SURV CONTROL (±0.25 mg) SURVIVAL IN HIGHEST () CONC NUMBER OF REPLICATES NUMBER OF ORGANISM/REPLICATE NORMAL DISTRIBUTION? HOMOGENEOUS VARIANCE? NOEC METHOD NOEC SURVIVAL NOEC REPRODUCTION/GROWTH	S S S G G G

COMMENTS * ND = Not Determinable (due to insufficient mortality)

ACUTE TOXICITY TEST

Sample Information

Client: Dupont
 Permit No: N/A
 Toxicant/Effluent: [redacted]
 Sample Type: N/A
 Grab Collected: AM/PM (Date) 1/1
 Composite Collected: AM/PM (Date) 1/1
 From: AM/PM (Date) 1/1
 To: AM/PM (Date) 1/1

Job No: 60175
 Sample No: N/A
 TRC: N/A
 Initial pH: N/A
 Initial DO: N/A
 Adjustments: ↓

Test Information

Analyst(s): SLB, JRN
 Test Species: Ceriodaphnia dubia
 Culture No: indiv. dup 1
 Age: ≤ 24 hrs
 Length (x ± SD): N/A
 Weight (x ± SD): N/A
 Test Mode: static
 Dilution Water: EPA MHW
 Start of Test: 130 AM/PM 2/14/01 (Date)
 End of Test: 1230 AM/PM 2/16/01 (Date)
 Test Container Size: 30 mL
 Test Solution Volume: 25 mL
 Dissolved Oxygen (Time/Test Soln/Conc): 1/1/1
 Aeration: N/A
 Beginning Date/Time/Rate: 16-hour day/8-hour night
 Photoperiod: 16-hour day/8-hour night

Conc or %	Rep	Number of Live Organisms			% Survival	Dissolved Oxygen (mg/L)			pH			Temperature (°C)			Initial Alkalinity (mg/L as CaCO ₃)	Initial Hardness (mg/L as CaCO ₃)	Conductivity (μmhos/cm) Initial/Final
		0	24	48		0	24	48	0	24	48	0	24	48			
500 ppm	A	5	5	5	95%	7.8	7.3	7.0	7.8	7.8	7.8	25	25	25			302
	B	5	5	5													327
	C	5	5	5													
	D	5	5	5													
400 ppm	A	5	5	5	90%	7.8	7.3	7.0	7.8	7.8	7.8	25	25	25			302
	B	5	5	5													335
	C	5	5	5													
	D	5	5	5													
300 ppm	A	5	5	5	100%	7.8	7.3	7.0	7.8	7.8	7.8	25	25	25			302
	B	5	5	5													333
	C	5	5	5													
	D	5	5	5													
Analyst's Initials/Time		SLB/130	SLB/200	SLB/1330	SLB	SLB	SLB	SLB	SLB	SLB	SLB	SLB	SLB	SLB	Notes: <u>Friday #14 - I-2/8/01</u>		

Comments

Less than 500 mg/L

Condition of Organisms at Test Initiation/End healthy, normal/only the controls without methanol appeared healthy, normal

ACUTE TOXICITY TEST

Sample Information
 Client Dupont
 Permit No N/A
 Toxicant/Effluent [redacted]
 Sample Type AM/PM (Date) 1/1
 Grab Collected N/A
 Composite Collected 1/1 (Date) 1/1
 From AM/PM (Date) 1/1
 To AM/PM (Date) 1/1

Test Information
 Analyst(s) SLB, JRN
 Test Species Ceriodaphnia dubia
 Culture No indiv. culture
 Age 5-24 hrs.
 Length ($\bar{x} \pm SD$) N/A
 Weight ($\bar{x} \pm SD$) N/A
 Test Mode 3/LHC
 Dilution Water EPA MHW
 Start of Test 1:30 AM/PM 2/1/01 (Date)
 End of Test 12:30 AM/PM 2/1/01 (Date)
 Test Container Size 30 ml
 Test Solution Volume 25 ml
 Dissolved Oxygen (Time/Test Soln/Conc) 1/1/1
 Aeration N/A
 Beginning Date/Time/Rate 16-hour day/8-hour night
 Photoperiod 16-hour day/8-hour night

Job No 60135
 Sample No N/A
 TRC N/A
 Initial pH N/A
 Initial DO N/A
 Adjustments N/A

Conc or %	Rep	Number of Live Organisms			% Survival	Dissolved Oxygen (mg/L)			pH			Temperature (°C)			Initial Alkalinity (mg/L as CaCO ₃)	Initial Hardness (mg/L as CaCO ₃)	Conductivity (µmhos/cm) Initial/Final
		0	24	48		0	24	48	0	24	48	0	24	48			
200 ppm	A	5	5	5	95%	7.8	7.2	7.0	7.8	7.7	7.7	25	25	25			302 / 344
	B	5	5	5													
	C	5	5	5													
	D	5	5	5													
Control w/ 500 µL M.E.O.H.	A	5	5	5		7.8	7.3	7.0	7.8	7.7	7.7	25	25	25			302 / 324
	B	5	5	5	70%												
	C	5	5	5													
	D	5	5	5													
Control	A	5	5	5		7.8	7.4	7.0	7.8	7.7	7.7	25	25	25	60	84	302 / 333
	B	5	5	5	100%												
	C	5	5	5													
	D	5	5	5													
Analyst's Initials/Time		SLB/1:30	SLB/2:00	SLB/2:30	SPN	SLB	SLB	SLB	SLB	SLB	SLB	SLB	SLB	SLB	Notes: Monday #4 - I-2/8/01		

Comments * accidentally wrote in the wrong column
 Condition of Organisms at Test Initiation/End healthy, normal / only the controls without methanol appeared healthy, normal

123 1 of 2

ACUTE TOXICITY TEST

Sample Information
 Client Dupont
 Permit No. 100175
 Toxicant/effluent N/A
 Sample Type Grab Collected
 Composite Collected AM/PM (Date) 1/1
 From AM/PM (Date) 1/1
 To AM/PM (Date) 1/1

Job No. 60175
 Sample No. N/A
 TRC 1
 Initial pH 7.8
 Initial DO 7.8
 Adjustments ↓

Test Information
 Analyst(s) sm LMD
 Test Species P. promelas
 Culture No. 1809
 Age 3 days
 Length (x ± SD) 0.49 ± 0.06 cm
 Weight (x ± SD) 0.41 ± 0.01 mg
 Test Mode Static
 Dilution Water EPA MHW
 Aeration None
 Beginning Date/Time/Rate 16-hour day/8-hour night
 Photoperiod 16-hour day/8-hour night

Start of Test 2:00 AM PM 2/14/01 (Date)
End of Test 1:30 AM PM 2/14/01 (Date)
Test Container Size 600 mL
Test Solution Volume 400 mL
Dissolved Oxygen (Time/Test Soln/Conc) 1/1/1

Conc or %	Rep	Number of Live Organisms			% Survival	Dissolved Oxygen (mg/L)			pH			Temperature (°C)			Initial Alkalinity (mg/L as CaCO ₃)	Initial Hardness (mg/L as CaCO ₃)	Conductivity (µmhos/cm) Initial/Final
		0	24	48		0	24	48	0	24	48	0	24	48			
500 ppm	A	10	10	10	100%	7.8	7.0	6.3	7.8	7.5	7.7	25	25	25	62	92	302/308
	B	10	10	10	100%												302/310
400 ppm	A	10	10	10	100%	7.8	7.0	6.2	7.8	7.5	7.7	25	25	25			302/309
	B	10	10	10	100%												
300 ppm	A	10	10	10	100%	7.8	7.2	6.6	7.8	7.5	7.7	25	25	25			
	B	10	10	10	100%												302/308
200 ppm	A	10	10	10	100%	7.8	7.0	5.8	7.8	7.5	7.7	25	25	25			302/308
	B	10	10	10	100%												302/308
Control	A	10	9	9	90%	7.8	7.2	6.6	7.8	7.5	7.7	25	25	25	60	84	302/307
	B	10	10	10	100%												302/307
Analyst's Initials/Time		MEH	3:48 PM	3:48 PM	LMD	sm	LMD	LMD	LMD	LMD	LMD	sm	LMD	LMD	Notes Carboy #4. 2/18/01		

Comments

Condition of Organisms at Test Initiation/End Healthy, normal/Healthy, normal

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ACUTE TOXICITY TEST

Test Information

Analys(s)	5m / Lmd	Start of Test	3:00	AM/PM	2 / 14 / 01	(Date)
Test Species	P. promelas	End of Test	1:30	AM/PM	2 / 16 / 01	(Date)
Culture No	1809	Test Container Size	600 mL			
Age	3 days	Test Solution Volume	400 mL			
Length ($\bar{x} \pm SD$)	0.49 $\pm$ 0.06 cm	Dissolved Oxygen (Time/Test Soln/Conc)	/ / / / /			
Weight ($\bar{x} \pm SD$)	2.4 $\pm$ 0.21 mg	Aeration	/ / / / /			
Test Mode	Static	Beginning Date/Time/Rate	/ / / / /			
Dilution Water	EPA mH ₂ O	Photoperiod	16-hour day/8-hour night			

Conc or %	Rep	Number of Live Organisms			% Survival	Dissolved Oxygen (mg/L)			pH			Temperature (°C)			Initial Alkalinity (mg/L as CaCO ₃)	Initial Hardness (mg/L as CaCO ₃)	Conductivity (μmhos/cm) Initial/Final
		0	24	48		0	24	48	0	24	48	0	24	48			
Control	A	10	10	10	100	7.8	7.2	6.3	7.8	7.5	7.7	25	25	25			302
(me OH) 500 μL	B	10	10	10	100												312
Analyst's Initials/Time	MEH 1/20	10	10	10	100	Sm	LMD	LMD	Sm	LMD	LMD	Sm	LMD	LMD			
Notes	Carboy # 4. 2/8/01																

Condition of Organisms at Test Initiation/End	Healthy, normal / Healthy, normal

OLVER
LABORATORIES

APPENDIX 6

CHRONIC TOXICITY TEST DATA -
Pimephales promelas

TEST SUMMARY SHEET

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY

CLIENT Dupont  JOB NO 60175 PAGE 1 OF 1
TOXICANT/EFFLUENT N/A PERMIT NO N/A

ACUTE TOXICITY TEST RESULTS		CHRONIC TOXICITY TEST RESULTS	
	Ceriodaphnia	Fathead Minnow	
TEST DATE(S)			Fathead Minnow <u>2/13-2/20/01</u>
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	<u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	<u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	<u>1-24 hours</u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>
TEST ACCEPTABILITY CONTROL SURVIVAL ($\geq 90\%$)			<u>98%</u>
STATISTICAL RESULTS SURVIVAL IN 100%			<u>0 429</u>
LC ₅₀			<u>98%</u>
95% CI METHOD			<u>4</u> <u>10</u> <u>S NO G Yes</u> <u>S NO G Yes</u> <u>S Steel's G D₅₀ 4000</u> <u>500 mg/L</u> <u>500 mg/L</u> <u>> 500 mg/L</u>
TEST ORGANISM AGE DILUTION WATER SOURCE AERATION		TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	
CONTROL SURVIVAL ($\geq 80\%$) NO OF CONTROL FEMALES		CONTROL SURVIVAL ($\geq 80\%$) NO OF CONTROL FEMALES	
% CONTROL FEMALES WITH 3 BROODS ($\geq 60\%$)		% CONTROL FEMALES WITH 3 BROODS ($\geq 60\%$)	
AVE NO YOUNG/SURVIVING FEMALES (≥ 15)		AVE NO YOUNG/SURVIVING FEMALES (≥ 15)	
AVE WT /SURV CONTROL (≥ 0.25 mg)		AVE WT /SURV CONTROL (≥ 0.25 mg)	
SURVIVAL IN HIGHEST (<u>500 mg/L</u>) CONC		SURVIVAL IN HIGHEST (<u>500 mg/L</u>) CONC	
NUMBER OF REPLICATES		NUMBER OF REPLICATES	
NUMBER OF ORGANISM/REPLICATE		NUMBER OF ORGANISM/REPLICATE	
NORMAL DISTRIBUTION?		NORMAL DISTRIBUTION?	
HOMOGENEOUS VARIANCE?		HOMOGENEOUS VARIANCE?	
NOEC METHOD		NOEC METHOD	
NOEC SURVIVAL		NOEC SURVIVAL	
NOEC REPRODUCTION/GROWTH		NOEC REPRODUCTION/GROWTH	
IC ₂₅ : Reproduction/Growth		IC ₂₅ : Reproduction/Growth	

COMMENTS

TEST SUMMARY SHEET

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY

CLIENT DuPont JOB NO 60175 PAGE 1 OF 1
TOXICANT/EFFLUENT [REDACTED] PERMIT NO N/A

ACUTE TOXICITY TEST RESULTS		CHRONIC TOXICITY TEST RESULTS	
TEST DATE(S)	Ceriodaphnia	Fathead Minnow	Fathead Minnow
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	<u> </u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	<u> </u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	<u>2/13-2/20/01</u> <u>1-24 hrs</u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)	<u> </u>	<u> </u>	<u>95%</u>
STATISTICAL RESULTS SURVIVAL IN 100%	<u> </u>	<u> </u>	<u>0 428</u>
LC ₅₀	<u> </u>	<u> </u>	<u>98%</u>
95% CI METHOD	<u> </u>	<u> </u>	<u>4</u> <u>10</u> <u>S NO G Yes</u> <u>S NO G Yes</u> <u>S Stat's G Durdh's</u> <u>500 mg/L</u> <u>500 mg/L</u> <u>>500 mg/L</u>

COMMENTS * Control water with added methanol (500 µL of methanol per 1 liter of dilution water)



AQUATIC TOXICITY TEST SAMPLE USE SHEET
FOR STANDARD SYNTHETIC DILUTION WATER

Client: Dupont
Effluent/Toxicant: [REDACTED]
Synthetic Dilution Water⁽¹⁾: EPA Moderately Hard Water (MHW)
Testing Requirements:

Job Number: 60175
Permit No: N/A

Page 1 of 1

☒ 7-Day Chronic Fathead Minnow Test
☐ 3-Brood Chronic Ceriodaphnia Test
☐ Other: _____

☐ 48-Hour Acute Ceriodaphnia Test
☐ 48-Hour Acute Fathead Minnow Test
☐ 96-Hour Acute Fathead Minnow Test

TEST DAY	1	2	3	4	5	6	7
Carboy No / Identification	#4	#4	#3	#3	#4	#4	#3
Preparation Date	2/8/01	2/8/01	2/13/01	2/13/01	2/16/01	2/16/01	2/19/01
Time & Day of Use	2:15 am 2/13/01	2:00 am 2/14/01	2:00 am 2/15/01	2:00 am 2/16/01	2:30 am 2/17/01	3:00 am 2/18/01	2:30 am 2/19/01
Age of Dilution Water ⁽²⁾	5 days	6 days	2 days	3 days	1 day	2 days	0 days
Alkalinity (mg/L) (Acceptable Range 60-70 mg/L)	60	60	60	60	60	60	64
Hardness (mg/L) (Acceptable Range 80- 100 mg/L)	84	84	96	96	92	92	100
Conductivity (μ mhos/cm)	300	300	307	307	314	313	314
pH	7.8	7.8	7.7	7.8	7.9	8.0	7.9
Total Residual Cl ₂ (mg/L)	ND*	ND	ND	ND	ND	ND	ND
D O (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Aeration	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous

COMMENTS: (1) Synthetic dilution water is prepared with laboratory deionized water and reagent grade chemicals (EPA/600/4-91/002)

(2) Each batch of dilution water is not used for more than 14 days following the preparation date

* ND = Not Detectable



LARVAL FATHEAD MINNOW
SURVIVAL and GROWTH TEST
SURVIVAL DATA SHEET

CLIENT Dupont
PERMIT NO [REDACTED]
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER FDA MHW
PHOTOPERIOD 16 hr Day/8 hr Night

JOB NO 10175 PAGE 1 of 2
TEST ORGANISM AGE 24 hrs (1-24 hrs)
START of TEST 3:15 A.M.P.M. 2/13/01
END of TEST 2:15 A.M.P.M. 2/20/01

NUMBER SURVIVING PER DAY

CONC	REP	START	1	2	3	4	5	6	7	COMMENTS
Control	A	10	10	10	10	10	10	10	10	TEST SOLUTION VOLUME 250 ml
	B	10	10	10	10	10	10	10	10	TEST CONTAINER SIZE 600 ml
	C	10	10	10	10	10	10	9	9	
	D	10	10	10	10	10	10	10	10	
5 mg/L	A	10	10	10	10	10	10	10	10	FEEDING 0.1 ml BRINE SHRIMP NAUPLII 2-3 TIMES PER DAY
	B	10	10	10	10	9	9	9	9	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
10 mg/L	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	9	9	9	9	8	
25 mg/L	A	10	10	10	10	10	10	9	9	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	9	
	D	10	10	10	10	10	10	10	10	
50 mg/L	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
100 mg/L	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
ANALYST			M.E.H	SEN 300P	LMD 320	M.E.H 200P	SEN 415P	AA	LMD 220	LMD

COMMENTS Culture # 1911 Organisms appear healthy at test initiation and completion



LARVAL FATHEAD MINNOW
SURVIVAL and GROWTH TEST
SURVIVAL DATA SHEET

CLIENT Dupont
PERMIT NO [REDACTED]
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER EPA mhw
PHOTOPERIOD 16 hr Day/8 hr Night

JOB NO 60175 PAGE 2 of 2
TEST ORGANISM AGE <24 hours (1-24 hrs)
START of TEST 3 15 A M P M 2/13/01
END of TEST 2 15 A M P M 2/20/01

NUMBER SURVIVING PER DAY										
CONC	REP	START	1	2	3	4	5	6	7	COMMENTS
500 mg/L	A	10	10	10	10	10	10	10	10	TEST SOLUTION VOLUME. 250 ml
	B	10	10	10	10	10	9	9	9	TEST CONTAINER SIZE. 600 ml
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
Control w/ 500 mg/L Methanol	A	10	10	10	10	10	10	10	10	FEEDING 0.1 ml BRINE SHRIMP NAUPLII 2-3 TIMES PER DAY
	B	10	10	10	10	10	10	10	10	
	C	10	10	9	9	9	9	9	9	
	D	10	10	10	9	9	9	9	9	
	A	10								
	B	10								
	C	10								
	D	10								
	A	10								
	B	10								
	C	10								
	D	10								
	A	10								
	B	10								
	C	10								
	D	10								
	A	10								
	B	10								
	C	10								
	D	10								
ANALYST		MEH	SRN	LMD	MEH	SRN	AA			
			200P	220D						

COMMENTS Culture #1810 - organisms appear healthy at test initiation + completion.



LARVAL FATHEAD MINNOW
SURVIVAL and GROWTH TEST
GROWTH DATA SHEET

CLIENT Dupont [REDACTED] JOB NO 10175 PAGE 1 of 2
 PERMIT NO [REDACTED] ANALYST LMD
 EFFLUENT/TOXICANT [REDACTED] DATE WEIGHED 2/23/01
 INITIAL BALANCE CALIBRATION CHECK Expected Weight 50.00 mg / Actual Weight 49.99 mg

CONC	REP	FOIL WEIGHT (mg)	FOIL WEIGHT + FISH	WEIGHT of FISH	NO of SURVIVING FISH	AVERAGE * WEIGHT PER FISH	PERCENT SURVIVAL
Control	A	106.016	110.48	4.42	10	.442	100%
	B	106.868	111.79	5.11	10	.511	100%
	C	112.70	115.92	3.22	9	.322	90%
	D	81.36	85.40	4.04	10	.404	100%
Control (MeOH)	A	97.99	103.13	5.14	10	.514	100%
	B	90.38	93.88	3.50	10	.350	100%
	C	86.02	89.55	3.53	9	.353	90%
	D	102.62	106.72	4.10	9	.410	90%
5 mg/L	A	103.96	108.96	5.00	10	.500	100%
	B	69.12	73.42	4.30	9	.430	90%
	C	81.60	86.82	5.22	10	.522	100%
	D	80.54	84.75	4.21	10	.421	100%
10 mg/L	A	85.02	89.56	4.54	10	.454	100%
	B	78.57	82.83	4.26	10	.426	100%
	C	68.04	72.45	4.41	10	.441	100%
	D	77.66	81.09	3.43	8	.343	80%
25 mg/L	A	80.06	83.88	3.82	9	.382	90%
	B	97.74	102.77	5.03	10	.503	100%
	C	89.09	93.28	4.19	9	.419	90%
	D	98.10	102.34	4.14	10	.414	100%
50 mg/L	A	75.51	80.45	4.94	10	.494	100%
	B	82.24	86.34	4.10	10	.410	100%
	C	89.18	92.96	3.78	10	.378	100%
	D	74.70	79.54	4.84	10	.484	100%

FINAL BALANCE CALIBRATION CHECK. Expected Weight 50.00 mg / Actual Weight 49.98 mg

COMMENTS Culture #1810

* Average Weight based on the original number of larvae.



LARVAL FATHEAD MINNOW
SURVIVAL and GROWTH TEST
GROWTH DATA SHEET

CLIENT Dupont
PERMIT NO
EFFLUENT/TOXICANT
INITIAL BALANCE CALIBRATION CHECK

JOB NO 60175 PAGE 2 of 2
ANALYST MD
DATE WEIGHED 2/23/01
Expected Weight 50.00 mg / Actual Weight 49.99 mg

CONC	REP	FOIL WEIGHT (mg)	FOIL WEIGHT + FISH	WEIGHT of FISH	NO of SURVIVING FISH	AVERAGE WEIGHT PER FISH *	PERCENT SURVIVAL
100 mg/L	A	86.77	91.59	4.82	10	.482	100%
	B	76.82	80.65	3.83	10	.383	100%
	C	77.19	81.17	3.98	10	.398	100%
	D	76.05	79.36	3.31	10	.331	100%
500 mg/L	A	100.12	105.05	4.93	10	.493	100%
	B	67.87	71.02	3.15	9	.315	90%
	C	89.16	93.74	4.58	10	.458	100%
	D	84.59	88.80	4.21	10	.421	100%
	A						
	B						
	C						
	D						
	A						
	B						
	C						
	D						
	A						
	B						
	C						
	D						
	A						
	B						
	C						
	D						

FINAL BALANCE CALIBRATION CHECK Expected Weight 50.00 mg / Actual Weight 49.98 mg

COMMENTS culture #

* Average weight based on the original number of larvae



OLIVER
LABORATORIES

CLIENT DuPont
JOB NO 160175
PERMIT NO [REDACTED]
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER EPA MHW

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

Page 1 of 4

TEST INITIATION DATE 2/13/01
TEST COMPLETION DATE 2/26/01
TEST ORGANISM P promelas
PHOTOPERIOD 16-Hour Day/8-Hour Night
LIGHT INTENSITY 50-100 ft-c

CONCENTRATION Control (w/ 500 uL Methanol)

INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	COMMENTS
pH	7.8	7.8	7.7	7.8	7.9	8.0	7.9	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	300	300	307	307	314	313	314	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	SM	SRN	LMD	LMD	SRN	SRN	LMD	
FINAL								
pH	7.5	7.3	7.45	7.5	7.5	7.3	7.5	* 7.7 was written in wrong concentration 2/16/01
D.O. (mg/L)	6.0	3.9	3.8	5.8	3.8	4.3	4.2	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	312	312	313	332	296	325	318	
Analyst's Initials	SRN	LMD	LMD	SRN	AA	LMD	LMD	

CONCENTRATION Control

INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	COMMENTS
pH	7.8	7.8	7.7	7.8	7.9	8.0	7.9	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	300	300	307	307	314	313	314	
Hardness (mg/L as CaCO ₃)	84	84	96	96	92	92	96	
Alkalinity (mg/L as CaCO ₃)	60	60	60	60	60	60	60.64	SRN 2/20/01 (data was transcribed incorrectly)
Analyst's Initials	LMD	SRN	LMD	LMD	SRN	SRN	LMD	
FINAL								
pH	7.5	7.4	7.7	7.5	7.8	7.5	7.6	
D.O. (mg/L)	6.0	5.0	5.2	5.9	5.2	6.0	6.0	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	315	330	320	342	338	339	328	
Analyst's Initials	SRN	LMD	LMD	SRN	AA	LMD	LMD	



OLIVER
LABORATORIES

CLIENT Dupont
JOB NO. 120175
PERMIT NO. [REDACTED]
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER. EPA MHW

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

Page 2 of 4

TEST INITIATION DATE 2/13/01
TEST COMPLETION DATE 2/26/01
TEST ORGANISM P. promelas
PHOTOPERIOD 16-Hour Day/8-Hour Night
LIGHT INTENSITY 50-100 ft-c

CONCENTRATION <u>10 ppm</u>								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.8	7.8	7.7	7.8	7.9	8.0	7.9	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	300	300	307	307	314	313	314	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	LMD	SRN	LMD	LMD	SRN	SRN	LMD	
FINAL								COMMENTS
pH	7.5	7.3	7.4	7.5	7.3	7.3	7.4	
D.O. (mg/L)	6.0	4.9	4.1	5.0	4.8	4.8	5.3	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	287	326	321	331	336	332	333	
Analyst's Initials	SRN	LMD	LMD	SRN	AA	LMD	LMD	

CONCENTRATION <u>5 ppm</u>								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.8	7.8	7.7	7.8	7.9	8.0	7.9	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	300	300	307	307	314	313	314	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	LMD	SRN	LMD	LMD	SRN	SRN	LMD	
FINAL								COMMENTS
pH	7.5	7.3	7.4	7.5	7.3	7.3	7.4	
D.O. (mg/L)	6.0	4.1	3.5	5.0	3.8	4.3	4.0	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	311	309	315	332	332	325	319	
Analyst's Initials	SRN	LMD	LMD	SRN	AA	LMD	LMD	



CHRONIC TOXICITY TEST CHEMICAL ANALYSIS DATA SHEET

Page 3 of 4

CLIENT DuPont
JOB NO 60175
PERMIT NO 6
EFFLUENT/TOXICANT 6
DILUTION WATER EPA MHW

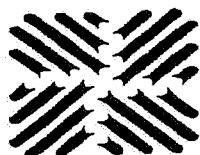
TEST INITIATION DATE 2/13/01
TEST COMPLETION DATE 2/20/01
TEST ORGANISM P promelas
PHOTOPERIOD 16-Hour Day/8-Hour Night
LIGHT INTENSITY 50-100 ft-c

CONCENTRATION 50 Ppm

INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	COMMENTS
pH	7.8	7.8	7.7	7.8	7.9	8.0	7.9	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	300	300	307	307	314	313	314	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	LMD	SRN	LMD	LMD	SRN	SRN	LMD	
FINAL								
pH	7.5	7.3	7.4	7.4	7.3	7.3	7.4	
D.O. (mg/L)	6.0	3.8	3.8	5.0	3.2	4.0	4.2	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	317	313	313	331	336	329	326	
Analyst's Initials	SRN	LMD	LMD	SRN	AA	LMD	LMD	

CONCENTRATION 25 ppm

INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	COMMENTS
pH	7.8	7.8	7.7	7.8	7.9	8.0	7.9	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	300	300	307	307	314	313	314	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	LMD	SRN	LMD	LMD	SRN	SRN	LMD	
FINAL								
pH	7.5	7.3	7.4	7.4	7.4	7.3	7.4	
D.O. (mg/L)	6.0	4.0	3.9	5.0	3.9	4.0	3.9	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	309	310	311	326	331	321	320	
Analyst's Initials	SRN	LMD	LMD	SRN	AA	LMD	LMD	



OLVER
LABORATORIES

CLIENT Dupont
JOB NO 60175
PERMIT NO [REDACTED]
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER: EPA MW

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

Page 4 of 4

TEST INITIATION DATE 2/13/01
TEST COMPLETION DATE 2/20/01
TEST ORGANISM P. promelas
PHOTOPERIOD 16-Hour Day/8-Hour Night
LIGHT INTENSITY 50-100 ft-c

CONCENTRATION 500 ppm

INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	COMMENTS
pH	7.8	7.8	7.7	7.8	7.9	8.0	7.9	
D.O (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	300	300	307	307	314	313	314	
Hardness (mg/L as CaCO ₃)	92	92	104	104	92	92	100	
Alkalinity (mg/L as CaCO ₃)	62	62	62	62	60	60	64	
Analyst's Initials	LMD	SRN	LMD	LMD	SRN	SRN	LMD	
FINAL								
pH	7.5	7.2	7.3	7.3	7.3	7.3	7.3	
D.O (mg/L)	6.0	3.8	3.7	3.8	3.8	4.2	3.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	317	312	312	331	330	325	320	
Analyst's Initials	SRN	LMD	LMD	SRN	AA	LMD	LMD	

CONCENTRATION 100 ppm

INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	COMMENTS
pH	7.8	7.8	7.7	7.8	7.9	8.0	7.9	
D.O (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	300	300	307	307	314	313	314	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	LMD	SRN	LMD	LMD	SRN	SRN	LMD	
FINAL								
pH	7.5	7.2	7.3	7.3	7.3	7.3	7.4	
D.O (mg/L)	6.0	3.2	3.2	4.0	3.4	4.8	4.7	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	283	309	310	328	331	323	322	
Analyst's Initials	SRN	LMD	LMD	SRN	AA	LMD	LMD	

Data Analysis- Comparison to standard Controls

A. TEST FOR NORMALITY OF SURVIVAL DATA

Title Dupont [REDACTED] Toxicity-P promelas Survival-2/13/01
File [REDACTED] Transform ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.1560
W = 0.8283

Critical W = 0.8960 (alpha = 0.01, N = 28)
W = 0.9240 (alpha = 0.05, N = 28)

Data FAIL normality test (alpha = 0.01) Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality
and should not be performed with this data as is

B. TEST FOR HOMOGENEITY OF VARIANCE

Title Dupont [REDACTED] Toxicity-P promelas Survival-2/13/01
File [REDACTED] Transform ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has
zero variance

Data FAIL to meet homogeneity of variance assumption
Additional transformations are useless



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Blacksburg, Virginia 24060
Phone (540) 552-5548 FAX (540) 552-1715

Data Analysis - Comparison to standard Controls

C. STEEL'S MANY-ONE RANK TEST

Title Dupont [REDACTED] Toxicity-P promelas Survival-2/13/01
 File [REDACTED] Transform ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test			Ho Control < Treatment			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Control	1 3713				
2	5 mg/L	1 3713	18 00	10 00	4 00	
3	10 mg/L	1 3358	17 50	10 00	4 00	
4	25 mg/L	1 3305	16 00	10 00	4 00	
5	50 mg/L	1 4120	20 00	10 00	4 00	
6	100 mg/L	1 4120	20 00	10 00	4 00	
7	500 mg/L	1 3713	18.00	10.00	4 00	

Critical values are 1 tailed (k = 6)



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Data Analysis - Comparison to Standard Controls

A. TEST FOR NORMALITY OF GROWTH DATA

Title Dupont ~~XXXXXXXXXX~~ Toxicity-P promelas Growth-2/13/01
File ~~XXXXXXXXXX~~ Transform NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0 0809
W = 0 9714

Critical W = 0 8960 (alpha = 0 01 , N = 28)
W = 0 9240 (alpha = 0 05 , N = 28)

Data PASS normality test (alpha = 0 01) Continue analysis

B. TEST FOR HOMOGENEITY OF VARIANCE

Title Dupont ~~XXXXXXXXXX~~ Toxicity-P promelas Growth-2/13/01
File ~~XXXXXXXXXX~~ Transform NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 1 2700 (p-value = 0 9733)

Data PASS B1 homogeneity test at 0 01 level. Continue analysis

Critical B = 16 8119 (alpha = 0 01, df = 6)
= 12 5916 (alpha = 0 05, df = 6)



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Data Analysis- Comparison to Standard Controls

C. ANALYSIS OF VARIANCE OF DRY WEIGHT DATA

Title Dupont Toxicity-P promelas Growth-2/13/01
File Transform NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	0 0117	0 0020	0 5065
Within (Error)	21	0 0809	0 0039	
Total	27	0 0926		

(p-value = 0.7965)

Critical F = 3 8117 (alpha = 0 01, df = 6,21)
= 2 5727 (alpha = 0 05, df = 6,21)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 All equal (alpha = 0.05)

D. DUNNETT'S TEST

Title Dupont Toxicity-P promelas Growth-2/13/01
File Transform NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 H_0 Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0 05
1	Control	0.4198	0 4198		
2	5 mg/L	0.4683	0.4683	-1 1053	
3	10 mg/L	0.4160	0.4160	0 0855	
4	25 mg/L	0 4295	0 4295	-0.2222	
5	50 mg/L	0 4415	0 4415	-0 4957	
6	100 mg/L	0 3985	0 3985	0 4843	
7	500 mg/L	0 4217	0.4217	-0 0456	

Dunnett critical value = 2 4600 (1 Tailed, alpha = 0 05, df [used] = 6,20)

(Actual df = 6,21)



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Data Analysis - Comparison to Standard Controls

Title Dupont-~~XXXXXX~~ Toxicity-P promelas Growth-2/13/01
 File ~~XXXXXX~~ Transform NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Control	4			
2	5 mg/L	4	0 1079	25 7	-0.0485
3	10 mg/L	4	0 1079	25 7	0 0038
4	25 mg/L	4	0 1079	25.7	-0 0097
5	50 mg/L	4	0.1079	25 7	-0 0217
6	100 mg/L	4	0 1079	25 7	0 0213
7	500 mg/L	4	0 1079	25 7	-0 0020

INHIBITION CONCENTRATION (IC₂₅) ESTIMATE

Title Dupont-~~XXXXXX~~ Toxicity-P promelas Growth-2/13/01
 File ~~XXXXXX~~ Transform NO TRANSFORMATION

GRP	IDENTIFICATION	MEAN	SMOOTHED MEAN	CONCENTRATION
1	Control	0 4198	0.4440	0 0000
2	5 mg/L	0 4683	0.4440	5 0000
3	10 mg/L	0 4160	0 4290	10 0000
4	25 mg/L	0 4295	0 4290	25 0000
5	50 mg/L	0 4415	0.4290	50.0000
6	100 mg/L	0 3985	0 4101	100.0000
7	500 mg/L	0.4217	0 4101	500 0000

ICp estimate with p = 25 is > 500 0000



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 Blacksburg, Virginia 24060
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Data Analysis - Comparison to Methanol Controls

A. TEST FOR NORMALITY OF SURVIVAL DATA

Title Dupont ~~XXXX~~ Toxicity-P promelas Survival-2/13/01
File ~~XXXX~~ Transform ARC SINE(SQUARE ROOT(I))

Shapiro - Wilk's Test for Normality

D = 0.1627
W = 0.8625

Critical W = 0.3960 (alpha = 0.01, N = 28)
W = 0.9240 (alpha = 0.05, N = 28)

Data FAIL normality test (alpha = 0.01) Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality
and should not be performed with this data as is

B. TEST FOR HOMOGENEITY OF VARIANCE

Title Dupont ~~XXXX~~ Toxicity-P promelas Survival-2/13/01
File ~~XXXX~~ Transform ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has
zero variance

Data FAIL to meet homogeneity of variance assumption
Additional transformations are useless.



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Blacksburg, Virginia 24060
Phone (540) 552-5548 FAX (540) 552-1715

Data Analysis- Comparison to methanol Controls

C. STEEL'S MANY-ONE RANK TEST

Title Dupont XXXXXXXXXX Toxicity-P promelas Survival-2/13/01
 File XXXXXXXXXX Transform ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT VALUE	DF	SIG 0 05
1	Control w/ MeOH	1 3305				
2	5 mg/L	1 3713	20 00	10 00	4 00	
3	10 mg/L	1 3358	19 00	10 00	4 00	
4	25 mg/L	1 3305	18 00	10 00	4 00	
5	50 mg/L	1 4120	22 00	10 00	4 00	
6	100 mg/L	1 4120	22 00	10 00	4 00	
7	500 mg/L	1 3713	20 00	10 00	4 00	

Critical values are 1 tailed (k = 6)



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Data Analysis- Comparison to methanol Controls

A. TEST FOR NORMALITY OF GROWTH DATA

Title Dupont  Toxicity-P promelas Growth-2/13/01
File  Transform NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0 0799

W = 0 9786

Critical W = 0.8960 (alpha = 0 01 , N = 28)

W = 0.9240 (alpha = 0 05 , N = 28)

Data PASS normality test (alpha = 0 01) Continue analysis

B. TEST FOR HOMOGENEITY OF VARIANCE

Title Dupont  Toxicity-P promelas Growth-2/13/01
File  Transform NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 1 1848 (p-value = 0 9776)

Data PASS B1 homogeneity test at 0 01 level Continue analysis

Critical B = 16 8119 (alpha = 0 01, df = 6)

= 12 5916 (alpha = 0 05, df = 6)



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Data Analysis - Comparison to methanol Controls

C. ANALYSIS OF VARIANCE OF DRY WEIGHT DATA

Title Dupont [REDACTED] Toxicity-P promelas Growth-2/13/01
File [REDACTED] Transform NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	0 0131	0 0022	0 5754
Within (Error)	21	0 0799	0 0038	
Total	27	0 0930		

(p-value = 0 7456)

Critical F = 3 8117 (alpha = 0.01, df = 6,21)
= 2 5727 (alpha = 0 05, df = 6,21)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 . All equal (alpha = 0 05)

D. DUNNETT'S TEST

Title Dupont [REDACTED] Toxicity-P promelas Growth-2/13/01
File [REDACTED] Transform NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0 05
1	Control w/MeOH	0 4068	0 4068		
2	5 mg/L	0 4683	0.4683	-1 4103	
3	10 mg/L	0 4160	0 4160	-0 2121	
4	25 mg/L	0 4295	0 4295	-0 5217	
5	50 mg/L	0 4415	0.4415	-0.7969	
6	100 mg/L	0 3985	0.3985	0.1892	
7	500 mg/L	0 4217	0.4217	-0.3440	

Dunnett critical value = 2 4600 (1 Tailed, alpha = 0.05, df [used] = 6,20)

(Actual df = 6,21)



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Data Analysis - Comparison to methanol Controls

Title Dupont ~~XXXX~~ Toxicity-P promelas Growth-2/13/01
 File ~~XXXX~~ Transform NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Control w/MeOH	4			
2	5 mg/L	4	0 1073	26 4	-0 0615
3	10 mg/L	4	0 1073	26 4	-0 0093
4	25 mg/L	4	0 1073	26 4	-0 0228
5	50 mg/L	4	0 1073	26 4	-0 0348
6	100 mg/L	4	0 1073	26 4	0 0082
7	500 mg/L	4	0 1073	26 4	-0 0150

INHIBITION CONCENTRATION (IC₂₅) ESTIMATE

Title Dupont ~~XXXX~~ Toxicity-P promelas Growth-2/13/01
 File ~~XXXX~~ Transform NO TRANSFORMATION

GRP	IDENTIFICATION	MEAN	SMOOTHED MEAN	CONCENTRATION
1	Control w/MeOH	0 4068	0.4375	0 0000
2	5 mg/L	0 4683	0 4375	5.0000
3	10 mg/L	0 4160	0.4290	10.0000
4	25 mg/L	0 4295	0.4290	25.0000
5	50 mg/L	0 4415	0.4290	50.0000
6	100 mg/L	0 3985	0.4101	100.0000
7	500 mg/L	0 4217	0 4101	500.0000

ICp estimate with p = 25 is > 500 0000



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 1116 South Main Street
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 Phone (540) 552-5548 FAX (540) 552-1715

APPENDIX 7

CHRONIC TOXICITY TEST DATA -
Ceriodaphnia dubia

TEST SUMMARY SHEET

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY

CLIENT Dupont JOB NO 60175 PAGE 1 OF 1
TOXICANT/EFFLUENT [REDACTED] PERMIT NO N/A

ACUTE TOXICITY TEST RESULTS			CHRONIC TOXICITY TEST RESULTS		
TEST DATE(S)	Ceriodaphnia	Fathead Minnow		Ceriodaphnia	Fathead Minnow
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION			DILUTION WATER SOURCE AERATION	<u>2/20-2/27/01</u> <u>1-8 hrs.</u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	<u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)			CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES % CONTROL FEMALES WITH 3 BROODS (≥60%) AVE NO YOUNG/SURVIVING FEMALES (≥15) AVE WT /SURV CONTROL (±0.25 mg)	<u>100%</u> <u>10</u> <u>90%</u> <u>18.0</u>	
STATISTICAL RESULTS SURVIVAL IN 100% LC ₅₀ 95% CI METHOD			SURVIVAL IN HIGHEST (<u>500 mg/L</u>) CONC NUMBER OF REPLICATES NORMAL DISTRIBUTION? HOMOGENEOUS VARIANCE? NOEC METHOD NOEC SURVIVAL NOEC REPRODUCTION/GROWTH IC ₂₅ : Reproduction/Growth	<u>40/</u> <u>10</u> <u>1</u> <u>Yes</u> <u>Yes</u> <u>Dunneth's</u> <u>100 mg/L</u> <u>10 mg/L or 100 mg/L</u> <u>>500 mg/L</u>	<u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>#</u>

COMMENTS * Reproduction data showed a non-continuous dose-dependent toxicity response. A statistically significant effect was noted only at 25 mg/L concentration. Based on the observation of test organisms' performance and appearance in 100 mg/L and lower concentrations, the NOEC is believed to be more accurately represented at 100 mg/L.

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY

TEST SUMMARY SHEET

CLIENT DuPont JOB NO 60175 PAGE 1 OF 1
TOXICANT/EFFLUENT [REDACTED] PERMIT NO N/A

ACUTE TOXICITY TEST RESULTS		CHRONIC TOXICITY TEST RESULTS	
TEST DATE(S)	Ceriodaphnia	Fathead Minnow	Fathead Minnow
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	<u> </u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	<u> </u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>	<u> </u> <u>EPA-MHW</u> <u>OLVER LABS</u> <u>continuous</u>
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)	<u> </u>	<u> </u>	<u>90%</u> <u>10</u>
STATISTICAL RESULTS: SURVIVAL IN 100%	<u> </u>	<u> </u>	<u>80%</u>
LC ₅₀	<u> </u>	<u> </u>	<u>15.9</u>
95% CI METHOD	<u> </u>	<u> </u>	<u>40%</u> <u>10</u> <u>Yes</u> <u>No</u> <u>Stages</u> <u>100 mg/L</u> <u>100 mg/L</u> <u>>500 mg/L</u>
TEST ORGANISM AGE DILUTION WATER SOURCE AERATION		TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	
* CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES		* CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES	
% CONTROL FEMALES WITH 3 BROODS (≥60%)		% CONTROL FEMALES WITH 3 BROODS (≥60%)	
AVE NO YOUNG/SURVIVING FEMALES (≥15)		AVE NO YOUNG/SURVIVING FEMALES (≥15)	
AVE WT /SURV CONTROL (≥0.25 mg)		AVE WT /SURV CONTROL (≥0.25 mg)	
SURVIVAL IN HIGHEST (500 mg/L) CONC		SURVIVAL IN HIGHEST (500 mg/L) CONC	
NUMBER OF REPLICATES		NUMBER OF REPLICATES	
NORMAL DISTRIBUTION?		NORMAL DISTRIBUTION?	
HOMOGENEOUS VARIANCE?		HOMOGENEOUS VARIANCE?	
NOEC METHOD		NOEC METHOD	
NOEC SURVIVAL		NOEC SURVIVAL	
NOEC REPRODUCTION/GROWTH		NOEC REPRODUCTION/GROWTH	
IC ₂₅ : Reproduction/Growth		IC ₂₅ : Reproduction/Growth	

COMMENTS * Control water with added methanol (500 µL of methanol per 1 liter of dilution water).



AQUATIC TOXICITY TEST SAMPLE USE SHEET
FOR STANDARD SYNTHETIC DILUTION WATER

Client Durant - [REDACTED]
Effluent/Toxicant [REDACTED]
Synthetic Dilution Water EPA Moderately Hard Water (MHW)
Testing Requirements

Job Number 6075
Permit No N/A

Page 1 of 1

48-Hour Acute Ceriodaphnia Test
48-Hour Acute Fathead Minnow Test
96-Hour Acute Fathead Minnow Test
7-Day Chronic Fathead Minnow Test
3-Brood Chronic Ceriodaphnia Test
Other

TEST DAY	1	2	3	4	5	6	7
Carboy No/Identification	#3	#3	#3	#4	#4	#4	#3
Preparation Date	2/9/01	2/19/01	2/19/01	2/19/01	2/19/01	2/19/01	2/22/01
Time & Day of Use	1:30 am 2/20/01	11:30 am 2/21/01	11:30 am 2/22/01	11:45 am 2/23/01	11:30 am 2/24/01	11:30 am 2/24/01	2:30 am 2/26/01
Age of Dilution Water ⁽¹⁾	1 Day	2 days	3 days	4 days	5 days	6 days	4 days
Alkalinity (mg/L) (Acceptable Range 60-70 mg/L)	64	64	64	62	62	62	60
Hardness (mg/L) (Acceptable Range 80- 100 mg/L)	100	100	100	92	92	92	100
Conductivity (µmhos/cm)	336	336	336	315	315	315	325
pH	7.8	7.8	7.8	7.8	7.9	8.0	7.8
Total Residual Cl ₂ (mg/L)	ND*	ND	ND	ND	ND	ND	ND
D O (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Aeration	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous

COMMENTS (1) Synthetic dilution water is prepared with laboratory deionized water and reagent grade chemicals (EPA/600/4-91/002)

(2) Each batch of dilution water is not used for more than 14 days following the preparation date

* ND = Not Detectable



CLIENT DuPont
PERMIT NO N/A
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER EPA MHW
PHOTOPERIOD 16 hr Day/8 hr Night

Ceriodaphnia dubia
SURVIVAL AND
REPRODUCTION RECORD

JOB NO 60175 PAGE 1 of 3
TEST ORGANISM AGE 224 hrs. (within 8 hr)
START of TEST 1:36 A.M. 2/20/01
END of TEST 3:00 A.M. 2/27/01

PHOTOPERIOD 16 hr Day/8 hr Night											
CONCENTRATION	REP	REPRODUCTION COUNT/DAY									COMMENTS
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		TOTAL	
% Survival= 100											
<u>Control</u>	1 (A)	0	0	0	4	0	9	4		17	TEST SOLUTION
	2 (B)	0	0	0	5	0	8*	1*		14	VOLUME 15 ml
	3 (C)	0	0	0	5	0	8	4		17	TEST CONTAINER
	4 (D)	0	0	0	5	0	8	5		18	SIZE 30 ml
	5 (E)	0	0	0	4	6	0	5		15	
	6 (F)	0	0	0	1	7	8*	1*		17	FEEDING
	7 (G)	0	0	0	6	4	0	11		21	0.1 ml TYC +
	8 (H)	0	0	0	4	4	0	9		17	0.1 ml ALGAE/DAY
	9 (I)	0	0	0	5	5	0	12		22	* Split broods
	10 (J)	0	0	0	4	3	0	15		22	
TOTAL		0	0	0	43	29	41	67		180	

CONCENTRATION		REP	REPRODUCTION COUNT/DAY								COMMENTS
% Survival= 90			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	TOTAL	
Control (with Methanol) 500 µL	1 (A)	0	0	0	5	0	6	5		16	
	2 (B)	0	0	0	5	0	6	3		14	
	3 (C)	0/x	—	—	—	—	—	—		0	
	4 (D)	0	0	0	4	0	6	4		14	
	5 (E)	0	0	0	4	5	7	6		22	
	6 (F)	0	0	0	3	6	0	8		17	
	7 (G)	0	0	0	3	5	0	9		17	
	8 (H)	0	0	0	4	6	0	10		20	
	9 (I)	0	0	0	4	7	0	0		11	
	10 (J)	0	0	0	4	0	5	3		12	
TOTAL		0	0	0	36	29	30	48		143	

CONCENTRATION % Survival=100	REP	REPRODUCTION COUNT/DAY									COMMENTS
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		TOTAL	
<u>5 mg/L</u>	1 (A)	0	0	0	5	0	8	4		17	
	2 (B)	0	0	0	5	0	6	6		17	X = DEAD
	3 (C)	0	0	0	4	0	6	6		16	
	4 (D)	0	0	0	4	1	7	5		17	-- = DIED PREVIOUSLY
	5 (E)	0	0	0	4	0	7	6		17	
	6 (F)	0	0	0	4	2	0	9		15	#X = # OF YOUNG
	7 (G)	0	0	0	4	7	0	8		19	PRODUCED BEFORE
	8 (H)	0	0	0	4	4	0	9		17	ADULT DIED
	9 (I)	0	0	0	3	6	0	7		16	
	10 (J)	0	0	0	4	5	0	6		15	
TOTAL		0	0	0	37	25	34	66		160	
ANALYST		SLB	SLB	SLB	SRN	SRN	SLB	SLB		SRN	



CLIENT Dupont-
PERMIT NO N/A
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER EPA mhw
PHOTOPERIOD 16 hr Day/8 hr Night

Ceriodaphnia dubia
SURVIVAL AND
REPRODUCTION RECORD

JOB NO 60175 PAGE 2 of 3
TEST ORGANISM AGE <24 hrs (within 8 hrs)
START of TEST 1:30 A.M. PM 2/20/01
END of TEST 3:00 A.M. PM 2/23/01

CONCENTRATION	REP	REPRODUCTION COUNT/DAY								TOTAL	COMMENTS
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
<u>10 mg/L</u>											
	1 (A)	0	0	0	3	0	6	4		13	TEST SOLUTION
	2 (B)	0	0	0	5	5	0	6		16	VOLUME 15 ml
	3 (C)	0	0	0	3	0	6	6		15	TEST CONTAINER
	4 (D)	0	0	0	4	6	1	7		18	SIZE 30 ml
	5 (E)	0	0	0	5	0	5	8		18	
	6 (F)	0	0	0	3	5	0	11		19	FEEDING
	7 (G)	0	0	0	4	6	0	10		20	0.1 ml TVC +
	8 (H)	0	0	0	4	5	8	0		17	0.1 ml ALGAE/DAY
	9 (I)	0	0	0	3	3	0	8/X		14	
	10 (J)	0	0	0	3	6	0	12		21	
TOTAL		0	0	0	37	36	26	72		171	

CONCENTRATION	REP	REPRODUCTION COUNT/DAY								TOTAL	COMMENTS
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
<u>25 mg/L</u>											
	1 (A)	0	0	0	4	0	8	6		18	
	2 (B)	0	0	0	4	0	6	5		15	
	3 (C)	0	0	0	5	0	4	0		9	
	4 (D)	0	0	0	5	0	6	0		11	
	5 (E)	0	0	0	5	0	6	5		16	
	6 (F)	0	0	0	1	3	8	0		12	
	7 (G)	0	0	0	4	3	1	6		14	
	8 (H)	0	0	0	3	2	9	0/X		14	
	9 (I)	0	0	0	3	6	0	2		11	
	10 (J)	0	0	0	4	4	1	10		19	
TOTAL		0	0	0	38	18	44	34		139	

CONCENTRATION	REP	REPRODUCTION COUNT/DAY								TOTAL	COMMENTS
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
<u>50 mg/L</u>											
	1 (A)	0	0	0	4	0	6	3		13	
	2 (B)	0	0	0	5	0	7	5		17	X = DEAD
	3 (C)	0	0	0	6	0	5	4		15	
	4 (D)	0	0	0	4	5	7	5		16	- = DIED PREVIOUSLY
	5 (E)	0	0	0	5	0	5	3		13	
	6 (F)	0	0	0	4	0	0	6/X		10	#X = # OF YOUNG
	7 (G)	0	0	0	6	6	2	9		23	PRODUCED BEFORE
	8 (H)	0	0	0	4	7	0	3		14	ADULT DIED
	9 (I)	0	0	0	5	5	4	0/X		14	
	10 (J)	0	0	0	4	3	0	10/X		17	
TOTAL		0	0	0	47	21	36	48		152	
ANALYST		SLB	SLB	SLB	SRN	SRN	SLB	SLB		SRN	



CLIENT Dupont
PERMIT NO N/A
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER EPA MHW
PHOTOPERIOD 16 hr Day/8 hr Night

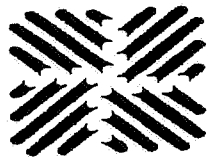
Ceriodaphnia dubia
SURVIVAL AND
REPRODUCTION RECORD

JOB NO 60175 PAGE 3 of 3
TEST ORGANISM AGE < 24 hrs (within 8 hrs)
START of TEST 1:30 A.M. PM 2/20/01
END of TEST 3:00 A.M. PM 2/27/01

CONCENTRATION	REP	REPRODUCTION COUNT/DAY								TOTAL	COMMENTS
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
<u>100 ms/L</u>	1 (A)	0	0	0	5	5	0	12		22	TEST SOLUTION
	2 (B)	0	0	0	5	5	0	9/X		19	VOLUME 15 ml
	3 (C)	0	0	0	5	4	0	7/X		16	TEST CONTAINER
	4 (D)	0	0	0	4	5	0	11		20	SIZE 30 ml
	5 (E)	0	0	0	5	5	0	9		19	
	6 (F)	0	0	0	4	5	0	10		19	FEEDING
	7 (G)	0	0	0	4	4	0	8		16	0.1 ml TYC+
	8 (H)	0	0	0	4	4	0	0/X		8	0.1 ml ALGAE/DAY
	9 (I)	0	0	0	4	5	0	6		15	
	10 (J)	0	0	0	3	6	0	9		18	
TOTAL		0	0	0	43	48	0	81		172	

CONCENTRATION	REP	REPRODUCTION COUNT/DAY								TOTAL	COMMENTS
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
<u>500 ms/L</u>	1 (A)	0	0	0	3	0	4	11		18	
	2 (B)	0	0	0	5	5	0	12		22	
	3 (C)	0	0	0	5	0	4	6		15	
	4 (D)	0	0	0	5	5	0/X	—		10	
	5 (E)	0	0	0	4	0	7	8		19	
	6 (F)	0	0	0	3	6	0	7/X		16	
	7 (G)	0	0	0	5	2	3	8/X		18	
	8 (H)	0	0	0	4	5	0	10/X		19	
	9 (I)	0	0	0	4	5	0	9/X		18	
	10 (J)	0	0	0	4	5	0	10/X		19	
TOTAL		0	0	0	42	33	18	81		174	

CONCENTRATION	REP	REPRODUCTION COUNT/DAY								TOTAL	COMMENTS
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
	1 (A)										
	2 (B)										X = DEAD
	3 (C)										
	4 (D)										— = DIED PREVIOUSLY
	5 (E)										
	6 (F)										#/X = # OF YOUNG
	7 (G)										PRODUCED BEFORE
	8 (H)										ADULT DIED
	9 (I)										
	10 (J)										
TOTAL											
ANALYST		SLB	SLB	SLB	SRN	SRN	SLB	SLB		SRN	



OLIVER
LABORATORIES

CLIENT Dupont
JOB NO 6c175
PERMIT NO N/A
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER EPA mHW

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

Page 1 of 4

TEST INITIATION DATE 2/20/01
TEST COMPLETION DATE 2/27/01
TEST ORGANISM Ceriodaphnia dubia
PHOTOPERIOD 16-Hour Day/8-Hour Night
LIGHT INTENSITY 50-100 ft-c

CONCENTRATION <u>Control (w/ methanol)</u>								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.8	7.8	7.8	7.8	7.9	8.0	7.8	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	336	336	336	315	315	315	325	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	SM	SLB	SLB	SLB	SRN	SRN	SLB	
FINAL								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.9	7.9	7.7	7.5	7.9	7.6	7.6	
D.O. (mg/L)	7.4	7.4	7.2	6.8	7.0	6.9	6.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	383	353	374	349	324	332	341	
Analyst's Initials	SLB	SLB	SLB	SRN	SRN	SLB	SLB	

CONCENTRATION <u>Control</u>								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.8	7.8	7.8	7.8	7.9	8.0	7.8	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	336	336	336	315	315	315	325	
Hardness (mg/L as CaCO ₃)	100	100	100	92	92	92	100	
Alkalinity (mg/L as CaCO ₃)	64	64	64	62	62	62	60	* Data transcribed incorrectly SM
Analyst's Initials	SM	SLB	SLB	SLB	SRN	SRN	SLB	
FINAL								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.9	7.9	7.7	7.6	7.9	7.6	7.6	
D.O. (mg/L)	7.4	7.4	7.2	6.8	7.0	6.9	6.9	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	383	356	374	349	325	329	334	
Analyst's Initials	SLB	SLB	SLB	SRN	SRN	SLB	SLB	



CHRONIC TOXICITY TEST CHEMICAL ANALYSIS DATA SHEET

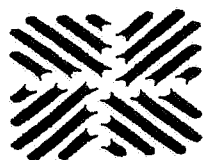
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CLIENT Dupont
JOB NO 6c175
PERMIT NO N/A
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER: EPA MHW

TEST INITIATION DATE 2/20/01
TEST COMPLETION DATE 2/27/01
TEST ORGANISM Ceriodaphnia dubia
PHOTOPERIOD 16-Hour Day/8-Hour Night
LIGHT INTENSITY 50-100 ft-c

CONCENTRATION <u>10 mg/L</u>								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.8	7.8	7.8	7.8	7.9	8.0	7.8	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	336	336	336	315	315	315	325	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	SM	SLB	SLB	SLB	SRN	SRN	SLB	
FINAL								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.9	7.9	7.7	7.4	7.8	7.6	7.6	
D.O. (mg/L)	7.4	7.4	7.1	7.2	7.0	6.8	6.9	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	384	357	377	348	327	328	335	
Analyst's Initials	SLB	SLB	SLB	SRN	SRN	SLB	SLB	

CONCENTRATION <u>5 mg/L</u>								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.8	7.8	7.8	7.8	7.9	8.0	7.8	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	336	336	336	315	315	315	325	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	SM	SLB	SLB	SLB	SRN	SRN	SLB	
FINAL								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.9	7.9	7.7	7.5	7.8	7.6	7.6	
D.O. (mg/L)	7.4	7.4	7.1	7.0	7.0	6.8	6.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	384	353	374	345	327	333	338	
Analyst's Initials	SLB	SLB	SLB	SRN	SRN	SLB	SLB	



OLIVER
LABORATORIES

CHRONIC TOXICITY TEST CHEMICAL ANALYSIS DATA SHEET

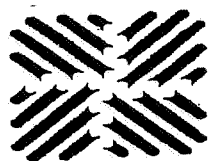
Page 3 of 4

CLIENT Dupont
JOB NO 60175
PERMIT NO N/A
EFFLUENT/TOXICANT EPA mhw
DILUTION WATER EPA mhw

TEST INITIATION DATE 2/20/01
TEST COMPLETION DATE 2/27/01
TEST ORGANISM Ceriodaphnia dubia
PHOTOPERIOD 16-Hour Day/8-Hour Night
LIGHT INTENSITY 50-100 ft-c

CONCENTRATION <u>50 mg/L</u>								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.8	7.8	7.8	7.8	7.9	8.0	7.8	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	336	336	336	315	315	315	325	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	SM	SLB	SLB	SLB	SRN	SRN	SLB	
FINAL								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.9	7.9	7.7	7.3	7.8	7.6	7.6	
D.O. (mg/L)	7.3	7.4	7.1	6.8	7.0	6.7	6.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	384	359	376	345	325	328	337	
Analyst's Initials	SLB	SLB	SLB	SRN	SRN	SLB	SLB	

CONCENTRATION <u>25 mg/L</u>								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.8	7.8	7.8	7.8	7.9	8.0	7.8	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	336	336	336	315	315	315	325	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	SM	SLB	SLB	SLB	SRN	SRN	SLB	
FINAL								COMMENTS
INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	
pH	7.9	7.9	7.7	7.4	7.8	7.6	7.6	
D.O. (mg/L)	7.4	7.4	7.1	7.2	7.0	6.8	6.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	384	358	375	349	324	330	341	
Analyst's Initials	SLB	SLB	SLB	SRN	SRN	SLB	SLB	



OLIVER
LABORATORIES

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

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CLIENT Dupont
JOB NO 6017
PERMIT NO N/A
EFFLUENT/TOXICANT [REDACTED]
DILUTION WATER: EPA MFW

TEST INITIATION DATE 2/20/01
TEST COMPLETION DATE 2/27/01
TEST ORGANISM Ceriodaphnia dubia
PHOTOPERIOD 16-Hour Day/8-Hour Night
LIGHT INTENSITY 50-100 ft-c

CONCENTRATION 500 mg/L

INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	COMMENTS
pH	7.8	7.8	7.8	7.8	7.9	8.0	7.8	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	336	336	336	315	315	315	325	
Hardness (mg/L as CaCO ₃)	96	96	96	104	104	104	104*	100
Alkalinity (mg/L as CaCO ₃)	64	64	64	64	64	64	64	60
Analyst's Initials	SM	SLB	SLB	SLB	SRN	SRN	SLB	5/27/01 * Data transcribed incorrectly
FINAL								
pH	7.9	7.9	7.8	7.3	7.7	7.6	7.6	
D.O. (mg/L)	7.3	7.4	6.9	7.0	6.6	6.6	6.2	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	346	375	375	346	317	320	328	
Analyst's Initials	SLB	SLB	SLB	SRN	SRN	SLB	SLB	

CONCENTRATION 100 mg/L

INITIAL	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	COMMENTS
pH	7.8	7.8	7.8	7.8	7.9	8.0	7.8	
D.O. (mg/L)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	336	336	336	315	315	315	325	
Hardness (mg/L as CaCO ₃)								
Alkalinity (mg/L as CaCO ₃)								
Analyst's Initials	SM	SLB	SLB	SLB	SRN	SRN	SLB	
FINAL								
pH	7.9	7.9	7.8	7.4	7.7	7.6	7.6	
D.O. (mg/L)	7.3	7.4	7.0	7.0	6.6	6.6	6.4	
Temperature (°C)	25	25	25	25	25	25	25	
Conductivity (µmhos/cm)	348	362	373	342	315	319	328	
Analyst's Initials	SLB	SLB	SLB	SRN	SRN	SLB	SLB	

Data Analysis- Comparison to the Standard Control

A. FISHER'S EXACT TEST FOR THE DETERMINATION OF SIGNIFICANT DIFFERENCES IN TEST ORGANISM MORTALITY

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
5 mg/L	10	0	10
TOTAL	20	0	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6.0 b value is 10
 Since b is greater than 6.0 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
10 mg/L	9	1	10
TOTAL	19	1	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6.0 b value is 9
 Since b is greater than 6.0 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level



Olver Laboratories Incorporated
 1116 South Main Street
 Blacksburg, Virginia 24060
 Phone (540) 552-5548 FAX (540) 552-1715

Data Analysis- Comparison to The Standard Control

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
25 mg/L	9	1	10
TOTAL	19	1	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6.0 b value is 9
 Since b is greater than 6.0 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
50 mg/L	7	3	10
TOTAL	17	3	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6.0 b value is 7
 Since b is greater than 6.0 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level



Oliver Laboratories Incorporated
 1116 South Main Street
 Blacksburg, Virginia 24060
 Phone (540) 552-5548 FAX (540) 552-1715

Data Analysis - Comparison to the standard Control

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
100 mg/L	7	3	10
TOTAL	17	3	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6.0 b value is 7
 Since b is greater than 6.0 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
500 mg/L	4	6	10
TOTAL	14	6	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6.0 b value is 4
 Since b is less than or equal to 6.0 there is a significant difference
 between CONTROL and TREATMENT at the 0.05 level.



Oliver Laboratories Incorporated
 1116 South Main Street
 Blacksburg, Virginia 24060

Data Analysis- Comparison to the standard Control

Summary of Fisher's Exact Tests

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG 0.05
	CONTROL	10	0	
1	5 mg/L	10	0	
2	10 mg/L	10	1	
3	25 mg/L	10	1	
4	50 mg/L	10	3	
5	100 mg/L	10	3	
6	500 mg/L	10	6	*



Olver Laboratories Incorporated
1116 South Main Street
Blacksburg, Virginia 24060
Phone (540) 552-5548 FAX (540) 552-1715

Data Analysis- Comparison to the Standard Control

B. CHI-SQUARE TEST FOR NORMALITY OF DATA

Title Dupont [REDACTED] Toxicity-C dubia Chronic-2/20/01
File. [REDACTED] Transform. NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.0200	14.5200	22.9200	14.5300	4.0200
OBSERVED	4	14	27	12	3

Chi-Square = 1.4412 (p-value = 0.8370)

Critical Chi-Square = 13.277 (alpha = 0.01, df = 4)
= 9.488 (alpha = 0.05, df = 4)

Data PASS normality test (alpha = 0.01) Continue analysis

C. TEST FOR HOMOGENEITY OF VARIANCE

Title Dupont [REDACTED] Toxicity-C dubia Chronic-2/20/01
File. [REDACTED] Transform. NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 11.0373 (p-value = 0.0506)

Data PASS B1 homogeneity test at 0.01 level Continue analysis

Critical B = 15.0863 (alpha = 0.01, df = 5)
= 11.0705 (alpha = 0.05, df = 5)



Oliver Laboratories Incorporated
1116 South Main Street
Blacksburg, Virginia 24060
Phone (540) 552-5548 FAX (540) 552-1715

Data Analysis- Comparison to the standard Control

D. SUMMARY STATISTICS ON REPRODUCTION DATA

Title Dupont  Toxicity-C dubia Chronic-2/20/01
 File  Transform NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	10	14 0000	22 0000	18 0000
2	5 mg/L	10	15 0000	19.0000	16 6000
3	10 mg/L	10	13.0000	21.0000	17.1000
4	25 mg/L	10	9 0000	19 0000	13 9000
5	50 mg/L	10	10 0000	23 0000	15.2000
6	100 mg/L	10	8.0000	22.0000	17.2000

Title Dupont  Toxicity-C dubia Chronic-2/20/01
 File  Transform NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C V %
1	Control	7 7778	2 7889	0 8819	15 4937
2	5 mg/L	2 3778	1.1738	0.3712	7 0710
3	10 mg/L	6 7667	2.6013	0.8226	15.2122
4	25 mg/L	10 3222	3.2128	1.0160	23 1138
5	50 mg/L	11 9556	3.4577	1.0934	22 7479
6	100 mg/L	14 8444	3.8528	1.2184	22 4003



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E. ANALYSIS OF VARIANCE OF REPRODUCTION DATA

Title Dupont Toxicity-C dubia Chronic-2/20/01
File Transform NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	113 9333	22 7867	2 5775
Within (Error)	54	477 4000	8.8407	
Total	59	591 3333		

(p-value = 0 0366)

Critical F = 3 3769 (alpha = 0 01, df = 5,54)

= 2 3861 (alpha = 0 05, df = 5,54)

Since F > Critical F REJECT Ho All equal (alpha = 0 05)

F. DUNNETT'S TEST

Title Dupont Toxicity-C dubia Chronic-2/20/01
File Transform NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0 05
1	Control	18.0000	18 0000		
2	5 mg/L	16 6000	16 6000	1 0529	
3	10 mg/L	17 1000	17.1000	0 6768	
4	25 mg/L	13 9000	13 9000	3 0834	*
5	50 mg/L	15 2000	15 2000	2 1057	
6	100 mg/L	17 2000	17 2000	0 6016	

Dunnett critical value = 2 3100 (1 Tailed, alpha = 0 05, df [used] = 5,40)
(Actual df = 5,54)



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Data Analysis - Comparison to the Standard Control

Title Dupont ~~XXXXXX~~ Toxicity-C. dubia Chronic-2/20/01
 File ~~XXXXXX~~ Transform. NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho-Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Control	10			
2	5 mg/L	10	3.0716	17.1	1.4000
3	10 mg/L	10	3.0716	17.1	0.9000
4	25 mg/L	10	3.0716	17.1	1.1000
5	50 mg/L	10	3.0716	17.1	2.8000
6	100 mg/L	10	3.0716	17.1	0.8000

G. INHIBITION CONCENTRATION ESTIMATE

Title. Dupont-Zonyl Toxicity-C dubia Chronic-2/20/01
 File zoncercic Transform. NO TRANSFORMATION

GRP	IDENTIFICATION	MEAN	SMOOTHED MEAN	CONCENTRATION
1	Control	18.0000	18.0000	0.0000
2	5 mg/L	16.6000	16.8500	5.0000
3	10 mg/L	17.1000	16.8500	10.0000
4	25 mg/L	13.9000	15.9250	25.0000
5	50 mg/L	15.2000	15.9250	50.0000
6	100 mg/L	17.2000	15.9250	100.0000
7	500 mg/L	17.4000	15.9250	500.0000

ICp estimate with p = .25 is > 500.0000



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Data Analysis - Comparison to Controls with added methanol (300 ml)

A. FISHER'S EXACT TEST FOR THE DETERMINATION OF SIGNIFICANT DIFFERENCES IN TEST ORGANISM MORTALITY

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	DEAD	ALIVE	TOTAL ANIMALS
CONTROL w/MeOH	1	9	10
5 mg/L	0	10	10
TOTAL	1	19	20

Critical Fisher's value (10,10,1) ($\alpha=0.05$) is negative b value is 0
no significant difference

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL w/MeOH	9	1	10
10 mg/L	9	1	10
TOTAL	18	2	20

Critical Fisher's value (10,10,9) ($\alpha=0.05$) is 4.0. b value is 9
Since b is greater than 4 0 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level



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Data Analysis- Comparison to Controls with 500 μ L added methanol

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL w/MeOH	9	1	10
25 mg/L	9	1	10
TOTAL	18	2	20

Critical Fisher's value (10,10,9) ($\alpha=0.05$) is 4.0 b value is 9
 Since b is greater than 4.0 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL w/MeOH	9	1	10
50 mg/L	7	3	10
TOTAL	16	4	20

Critical Fisher's value (10,10,9) ($\alpha=0.05$) is 4.0 b value is 7
 Since b is greater than 4.0 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level



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Data Analysis- Comparison to Controls with 500 μ L added methanol

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL w/MeOH	9	1	10
100 mg/L	7	3	10
TOTAL	16	4	20

Critical Fisher's value (10,10,9) ($\alpha=0.05$) is 4.0 b value is 7.
 Since b is greater than 4.0 there is no significant difference
 between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test

IDENTIFICATION	NUMBER OF		
	ALIVE	DEAD	TOTAL ANIMALS
CONTROL w/MeOH	9	1	10
500 mg/L	4	6	10
TOTAL	13	7	20

Critical Fisher's value (10,10,9) ($\alpha=0.05$) is 4.0. b value is 4.
 Since b is less than or equal to 4.0 there is a significant difference
 between CONTROL and TREATMENT at the 0.05 level.



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Data Analysis - Comparison to Control with 500 μ L added methanol

Summary of Fisher's Exact Tests

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG 0 05
	CONTROL w/MeOH	10	1	
1	5 mg/L	10	0	
2	10 mg/L	10	1	
3	25 mg/L	10	1	
4	50 mg/L	10	3	
5	100 mg/L	10	3	
6	500 mg/L	10	6	*



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Data Analysis - Comparison to Control with 500 µL added methanol

B. CHI-SQUARE TEST FOR NORMALITY OF DATA

Title Dupont  Toxicity-C dubia Chronic-2/20/01
File zcer Transform NO TRANSFORMATION

Chi-Square Test for Normality

Actual and Expected Frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.0200	14.5200	22.9200	14.5200	4.0200
OBSERVED	5	13	25	11	3

Chi-Square = 2.6361 (p-value = 0.6204)

Critical Chi-Square = 13.277 (alpha = 0.01, df = 4)
= 9.488 (alpha = 0.05, df = 4)

Data PASS normality test (alpha = 0.01) Continue analysis

C. TEST FOR HOMOGENEITY OF VARIANCE

Title Dupont  Toxicity-C dubia Chronic-2/20/01
File zcer Transform NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 20.1697 (p-value = 0.0012)

Data FAIL B1 homogeneity test at 0.01 level Try another transformation

Critical B = 15.0863 (alpha = 0.01, df = 5)
= 11.0705 (alpha = 0.05, df = 5)



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Data Analysis- Comparison to Controls with 500 μ L added methanol

D. SUMMARY STATISTICS ON REPRODUCTION DATA

Title Dupont  Toxicity-C dubia Chronic-2/20/01
File zcer Transform NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control w/MeOH	10	0 0000	22 0000	14 3000
2	5 mg/L	10	15 0000	19 0000	16.5000
3	10 mg/L	10	13 0000	21 0000	17 1000
4	25 mg/L	10	9.0000	19 0000	13 9000
5	50 mg/L	10	10 0000	23 0000	15 2000
6	100 mg/L	10	8.0000	22.0000	17 2000

Title Dupont  Toxicity-C. dubia Chronic-2/20/01
File zcer Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C V %
1	Control w/MeOH	36 6778	6 0562	1 9151	42.3512
2	5 mg/L	1 3778	1 1738	0.3712	7 0710
3	10 mg/L	6 7667	2 6013	0.8226	15.2122
4	25 mg/L	10.3222	3.2128	1 0160	23.1138
5	50 mg/L	11 9556	3.4577	1.0934	22.7479
6	100 mg/L	14 8444	3 8528	1 2184	22.4003



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Data Analysis- Comparison to Controls with 500 μ L added methanol

E. STEEL'S MANY-ONE RANK TEST

Title. Dupont ~~6~~ Toxicity-C dubia Chronic-2/20/01
 File zcer Transform NO TRANSFORMATION

Steel's Many-One Rank Test				Ho Control < Treatment		
GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT VALUE	DF	SIG 0 05
1	Control w/MeOH	14.3000				
2	5 mg/L	16.6000	119.00	75.00	10.00	
3	10 mg/L	17.1000	121.00	75.00	10.00	
4	25 mg/L	13.9000	96.00	75.00	10.00	
5	50 mg/L	15.2000	104.50	75.00	10.00	
6	100 mg/L	17.2000	122.00	75.00	10.00	

Critical values are 1 tailed (k = 5)

F. INHIBITION CONCENTRATION ESTIMATE

Title Dupont ~~6~~ Toxicity-C dubia Chronic-2/20/01
 File zceric Transform: NO TRANSFORMATION

GRP	IDENTIFICATION	MEAN	SMOOTHED MEAN	CONCENTRATION
1	Control w/MeOH	14.4000	16.0333	0.0000
2	5 mg/L	16.6000	16.0333	5.0000
3	10 mg/L	17.1000	16.0333	10.0000
4	25 mg/L	13.9000	15.9250	25.0000
5	50 mg/L	15.2000	15.9250	50.0000
6	100 mg/L	17.2000	15.9250	100.0000
7	500 mg/L	17.4000	15.9250	500.0000

ICp estimate with p = 25 is > 500.0000



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APPENDIX 8

ACUTE REFERENCE TOXICITY TEST DATA



REFERENCE TOXICITY TEST
DATA SUMMARY SHEET

PAGE 1 OF 1

TESTING MONTH: January 2001 REFERENCE TOXICANT: Sodium Chloride

ACUTE TOXICITY TEST RESULTS			CHRONIC TOXICITY TEST RESULTS		
TEST DATE(S)	Caridaphnia	Fathead Minnow		Caridaphnia	Fathead Minnow
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	1/19-1/21/01 424 hours EPA-MHW OLVER LABS Continuous	1/17-1/19/01 1 day EPA-MHW OLVER LABS Continuous	TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	EPA-MHW OLVER LABS continuous	EPA-MHW OLVER LABS continuous
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)	100%	100%	CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES % CONTROL FEMALES WITH 3 BROODS (≥60%) AVE NO YOUNG/SURVIVING FEMALES (≥15)		
STATISTICAL RESULTS SURVIVAL IN 100% LC ₅₀ 95% C I METHOD	0% 2.5 g/L 2.2-2.8 g/L Specimen based	0% 5.3 g/L 4.4-6.1 g/L Probit	AVE WT /SURV CONTROL (≥0.25 mg) SURVIVAL IN HIGHEST () CONC NUMBER OF REPLICATES NUMBER OF ORGANISM/REPLICATE NORMAL DISTRIBUTION? HOMOGENEOUS VARIANCE? NOEC METHOD NOEC SURVIVAL NOEC. REPRODUCTION/GROWTH		S G S G S G

COMMENTS: Reference toxicity test results were within the expected ranges



REFERENCE TOXICITY TEST
DATA SUMMARY SHEET

PAGE 1 OF 1

TESTING MONTH: February 2001 REFERENCE TOXICANT: Sodium Chloride

ACUTE TOXICITY TEST RESULTS		CHRONIC TOXICITY TEST RESULTS		
TEST DATE(S)	Ceriodaphnia	Fathead Minnow	Ceriodaphnia	Fathead Minnow
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	2/14-2/16/01 24 hours EPA-MHW OLVER LABS Continuous	2/14-2/16/01 2 days EPA-MHW OLVER LABS Continuous		EPA-MHW OLVER LABS continuous
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)	100%	100%		
STATISTICAL RESULTS SURVIVAL IN 100% (Highest Conc.) LC ₅₀ 95% CI METHOD	0% 2.1 g/L 1.8 - 2.5 g/L Probit	0% 11.1 g/L 10.2 - 12.0 g/L Spearman-Kärber		S G S G S G
			TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	
			CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES	
			% CONTROL FEMALES WITH 3 BROODS (≥60%)	
			AVE. NO. YOUNG/SURVIVING FEMALES (≥15)	
			AVE WT /SURV CONTROL (≥0.25 mg)	
			SURVIVAL IN HIGHEST () CONC NUMBER OF REPLICATES NUMBER OF ORGANISM/REPLICATE NORMAL DISTRIBUTION? HOMOGENEOUS VARIANCE? NOEC METHOD NOEC SURVIVAL NOEC REPRODUCTION/GROWTH	

COMMENTS: Reference toxicity test results were within the expected ranges



REFERENCE TOXICITY TEST
DATA SUMMARY SHEET

PAGE 1 OF 1

REFERENCE TOXICANT: Sodium Chloride

TESTING MONTH: March 2001

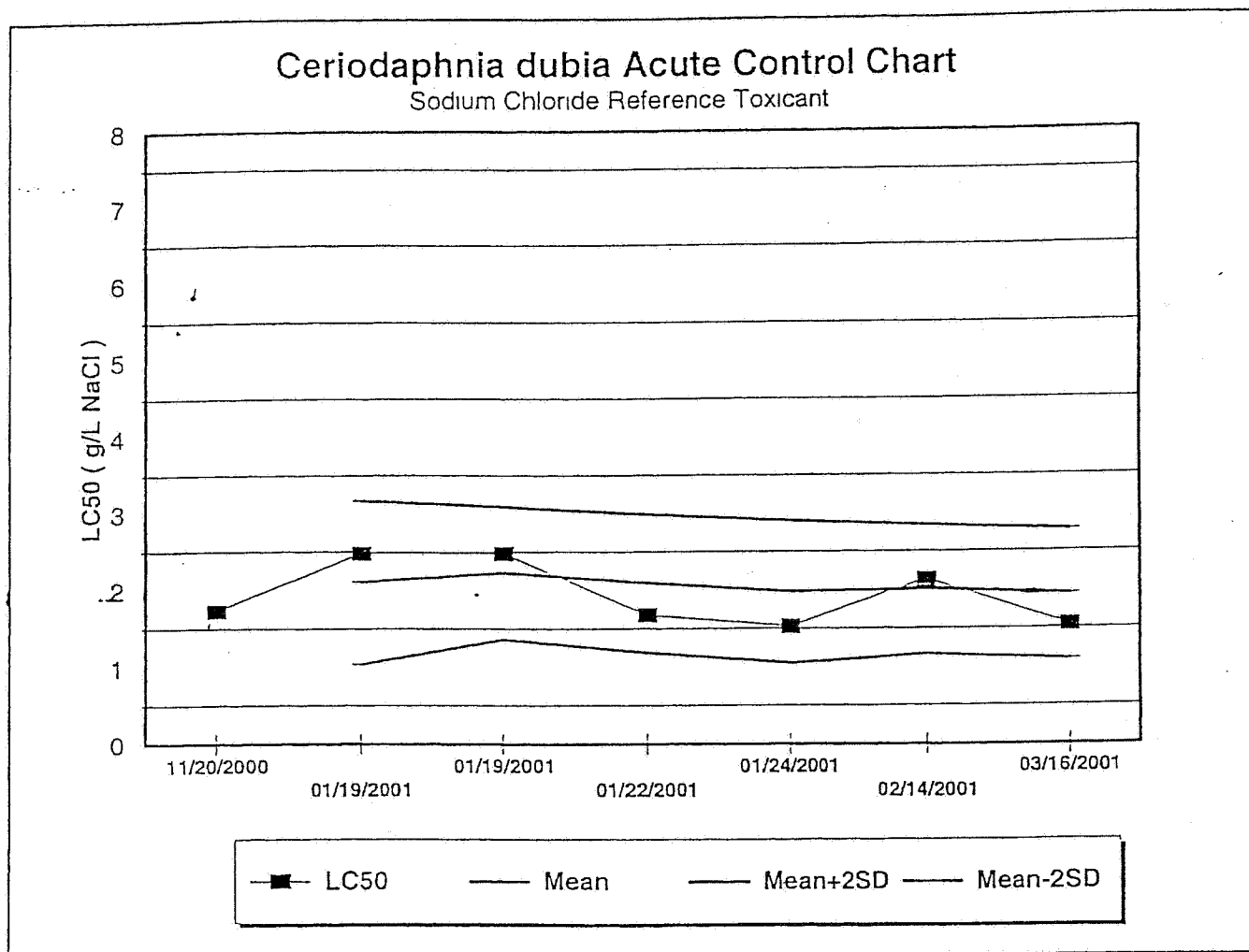
ACUTE TOXICITY TEST RESULTS			CHRONIC TOXICITY TEST RESULTS		
TEST DATE(S)	Ceriodaphnia	Fathead Minnow		Ceriodaphnia	Fathead Minnow
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	3/16-3/18/01 24 hours EPA-MHW OLVER LABS Continuous	3/29-3/31/01 2 days EPA-MHW OLVER LABS Continuous	TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	EPA-MHW OLVER LABS continuous	EPA-MHW OLVER LABS continuous
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)	100%	100%	CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES % CONTROL FEMALES WITH 3 BROODS (≥60%) AVE NO. YOUNG/SURVIVING FEMALES (±15)		
STATISTICAL RESULTS SURVIVAL IN 100% (Highest Conc.) LC ₅₀ 95% C.I. METHOD	0% 1.6 g/L 1.3-1.8 g/L Probit	0% 11.4 g/L 10.6-12.2 g/L Spearman-Kärker	AVE. WT /SURV CONTROL (±0.25 mg) SURVIVAL IN HIGHEST () CONC NUMBER OF REPLICATES NUMBER OF ORGANISM/REPLICATE NORMAL DISTRIBUTION? HOMOGENEOUS VARIANCE? NOEC METHOD NOEC SURVIVAL NOEC REPRODUCTION/GROWTH		

COMMENTS: Reference toxicity test results were within the expected ranges.

16-APR-2001

TITLE: Ceriodaphnia dubia Acute Test
Reference Toxicant Sodium Chloride

Test Date	Test Name LC50	Mean	Mean+2SD	Mean-2SD
11/20/2000	1.73			
01/19/2001	2.48	2.11	3.17	1.04
01/19/2001	2.48	2.23	3.10	1.36
01/22/2001	1.68	2.09	2.99	1.20
01/24/2001	1.53	1.98	2.90	1.06
02/14/2001	2.14	2.01	2.84	1.17
03/16/2001	1.55	1.94	2.78	1.10



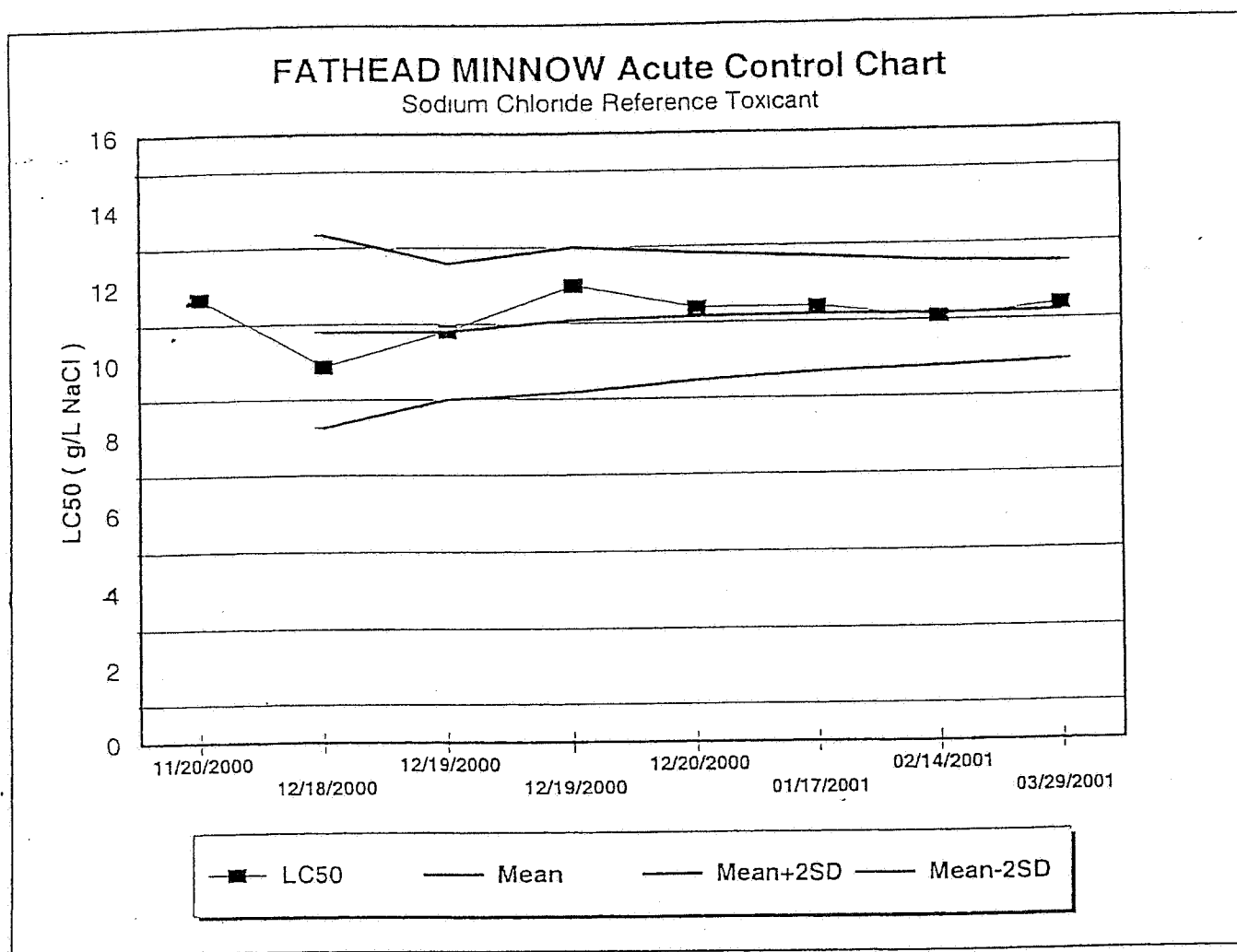
16-APR-2001

TITLE: FATHEAD MINNOW ACUTE TEST

Reference Toxicant: Sodium Chloride (g/L)

Test Date	Test Name	Mean	Mean+2SD	Mean-2SD
	LC50			
11/20/2000	11.7			
12/18/2000	9.9	10.80	13.35	8.25
12/19/2000	10.8	10.80	12.60	9.00
12/19/2000	12	11.10	13.00	9.20
12/20/2000	11.4	11.16	12.82	9.50
01/17/2001	11.4	11.20	12.70	9.70
02/14/2001	11.1	11.19	12.56	9.81
03/29/2001	11.4	11.21	12.49	9.93

16-Apr-2001



APPENDIX 9

CHRONIC REFERENCE TOXICITY TEST DATA



REFERENCE TOXICITY TEST
DATA SUMMARY SHEET

PAGE 1 OF 1

TESTING MONTH: January 2001 REFERENCE TOXICANT: Sodium Chloride

ACUTE TOXICITY TEST RESULTS			CHRONIC TOXICITY TEST RESULTS		
TEST DATE(S)	Ceriodaphnia	Fathead Minnow	Ceriodaphnia	Fathead Minnow	
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	 EPA-MHW OLVER LABS Continuous	 EPA-MHW OLVER LABS Continuous	1/3-1/10/01 18-24 hrs. EPA-MHW OLVER LABS continuous	1/22-1/29/01 1-19 hrs. EPA-MHW OLVER LABS continuous	
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)			100% 10 100% 27.7	95% 10 100% 27.7	
STATISTICAL RESULTS SURVIVAL IN 100% LC ₅₀ 95% CI METHOD			0% 10 Yes NO Steel's 2.0 g/L 0.25 g/L	53% 4 10 S No G Yes S No G Yes S Steel's G Duodithi 2.0 g/L 2.0 g/L	

COMMENTS: Reference toxicity test results were within the expected ranges



REFERENCE TOXICITY TEST
DATA SUMMARY SHEET

PAGE 1 OF 1

REFERENCE TOXICANT: Sodium Chloride

TESTING MONTH: February 2001

ACUTE TOXICITY TEST RESULTS			CHRONIC TOXICITY TEST RESULTS		
	Carlotaphnia	Fathead Minnow		Carlotaphnia	Fathead Minnow
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	 EPA-MHW OLVER LABS Continuous	 EPA-MHW OLVER LABS Continuous	TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	2/7-2/13/01 1-8 hrs. EPA-MHW OLVER LABS continuous	2/12-2/19/01 1-24 hrs. EPA-MHW OLVER LABS continuous
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)			CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES % CONTROL FEMALES WITH 3 BROODS (≥60%) AVE NO YOUNG/SURVIVING FEMALES (≥15)	100% 10 100% 27.3	100% 100%
STATISTICAL RESULTS SURVIVAL IN 100% LC ₅₀ 95% CI METHOD			AVE WT /SURV CONTROL (±0.25 mg) SURVIVAL IN HIGHEST (3.0/5.0) CONC NUMBER OF REPLICATES NUMBER OF ORGANISM/REPLICATE NORMAL DISTRIBUTION? HOMOGENEOUS VARIANCE? NOEC METHOD NOEC SURVIVAL NOEC REPRODUCTION/GROWTH	0% 10 1 Yes Yes Dunnett's 1.25 g/L 0.19 g/L	35% 10 4 S NO G Yes S NO G Yes S Shear 15G Dunnett's 1.25 g/L 1.25 g/L

COMMENTS: Reference toxicity test results were within the established ranges.



REFERENCE TOXICITY TEST
DATA SUMMARY SHEET

PAGE 1 OF 1

TESTING MONTH: March 2001 REFERENCE TOXICANT: Sodium Chloride

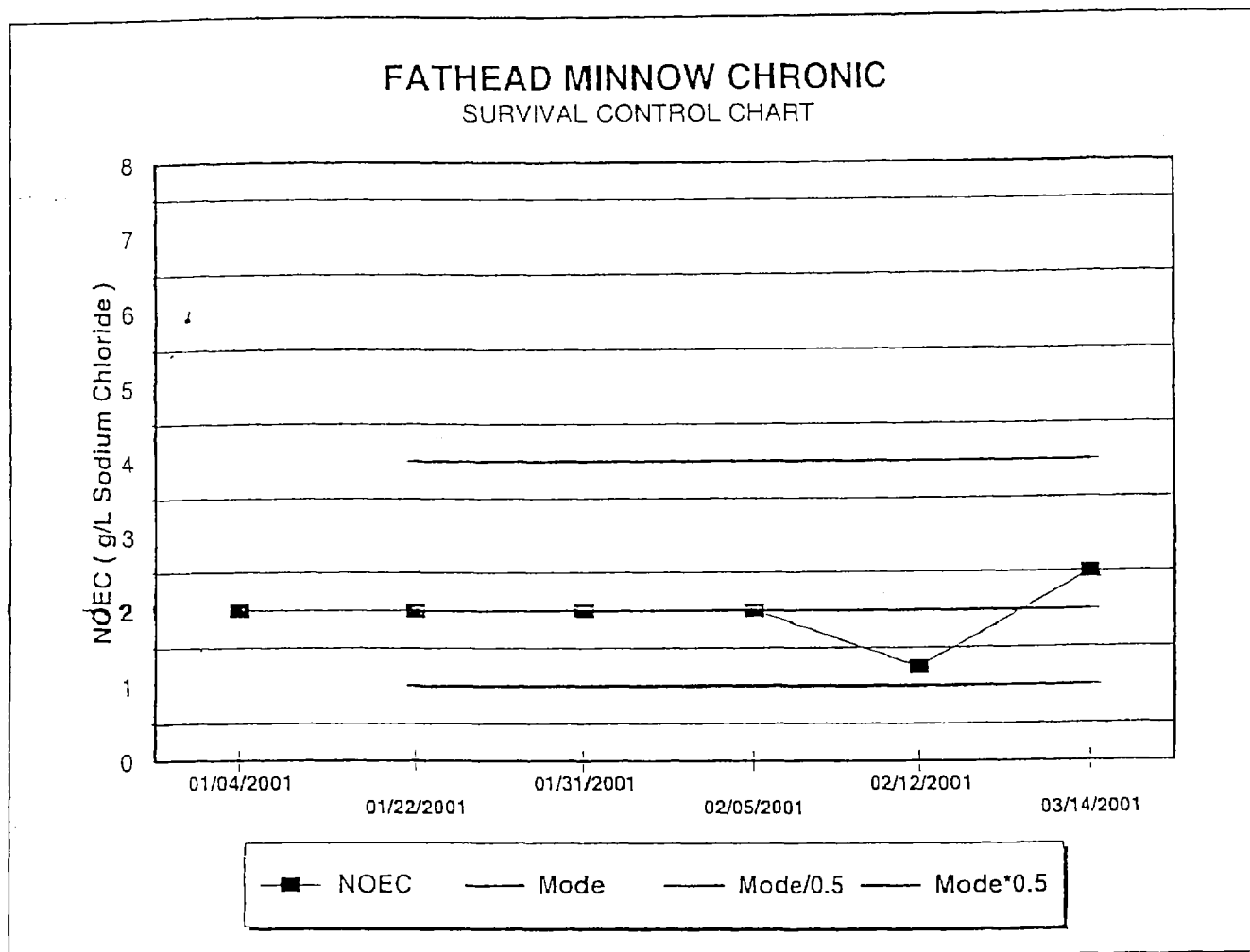
ACUTE TOXICITY TEST RESULTS			CHRONIC TOXICITY TEST RESULTS		
TEST DATA	Ceriodaphnia	Fathead Minnow		Ceriodaphnia	Fathead Minnow
TEST CONDITIONS TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	 EPA-MHW OLVER LABS Continuous	 EPA-MHW OLVER LABS Continuous	TEST ORGANISM AGE DILUTION WATER SOURCE AERATION	3/15-3/21/01 1-8 hrs. EPA-MHW OLVER LABS continuous	3/14-3/21/01 1-24 hrs. EPA-MHW OLVER LABS continuous
TEST ACCEPTABILITY CONTROL SURVIVAL (≥90%)			CONTROL SURVIVAL (≥80%) NO OF CONTROL FEMALES % CONTROL FEMALES WITH 3 BROODS (≥60%) AVE NO YOUNG/SURVIVING FEMALES (≥15)	100% 10 70% 18.5	90%
STATISTICAL RESULTS SURVIVAL IN 100% LC ₅₀ 95% CI METHOD			AVE WT /SURV CONTROL (≥0.25 mg) SURVIVAL IN HIGHEST (4.0/5.0) CONC NUMBER OF REPLICATES NUMBER OF ORGANISM/REPLICATE NORMAL DISTRIBUTION? HOMOGENEOUS VARIANCE? NOEC SURVIVAL NOEC REPRODUCTION/GROWTH IC ₂₅ : Reproduction/Growth	0% 10 1 Yes NO 5 Reps 2.0 g/L 0.5 g/L 0.37 g/L	0.603 23% 4 10 S Yes G Yes S NO G Yes S 5 Reps G Dunnett 2.5 g/L 1.25 g/L 1.75 g/L

COMMENTS: Reference toxicity test results were within the established control limits.

TITLE: FATHEAD MINNOW CHRONIC-SURVIVAL
Reference Toxicant: Sodium Chloride (g/L)

OLVER LABORATORIES, INC

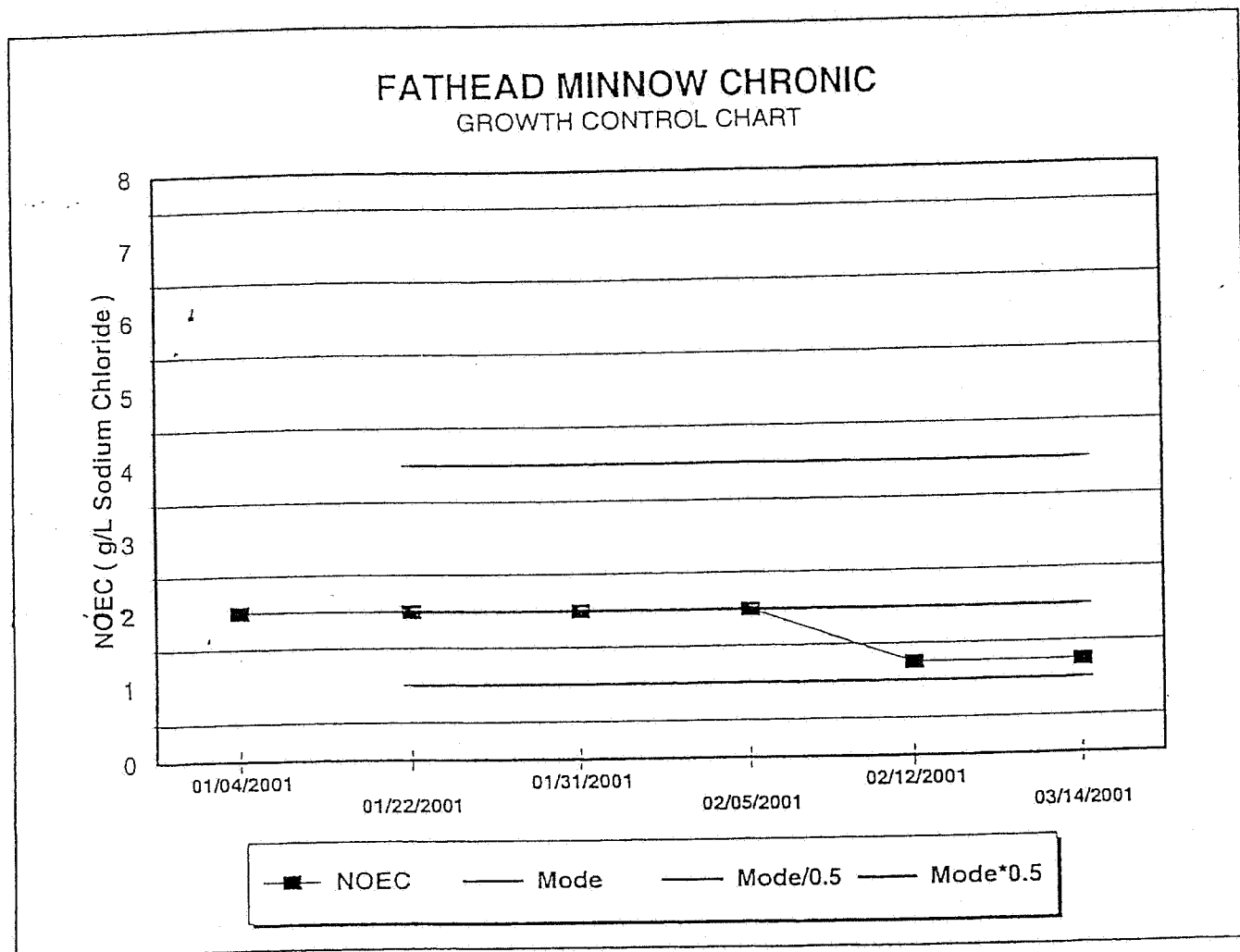
16-Apr-2001



TITLE: FATHEAD MINNOW CHRONIC-GROWTH
Reference Toxicant: Sodium Chloride (g/L)

OLVER LABORATORIES, INC

16-Apr-2001



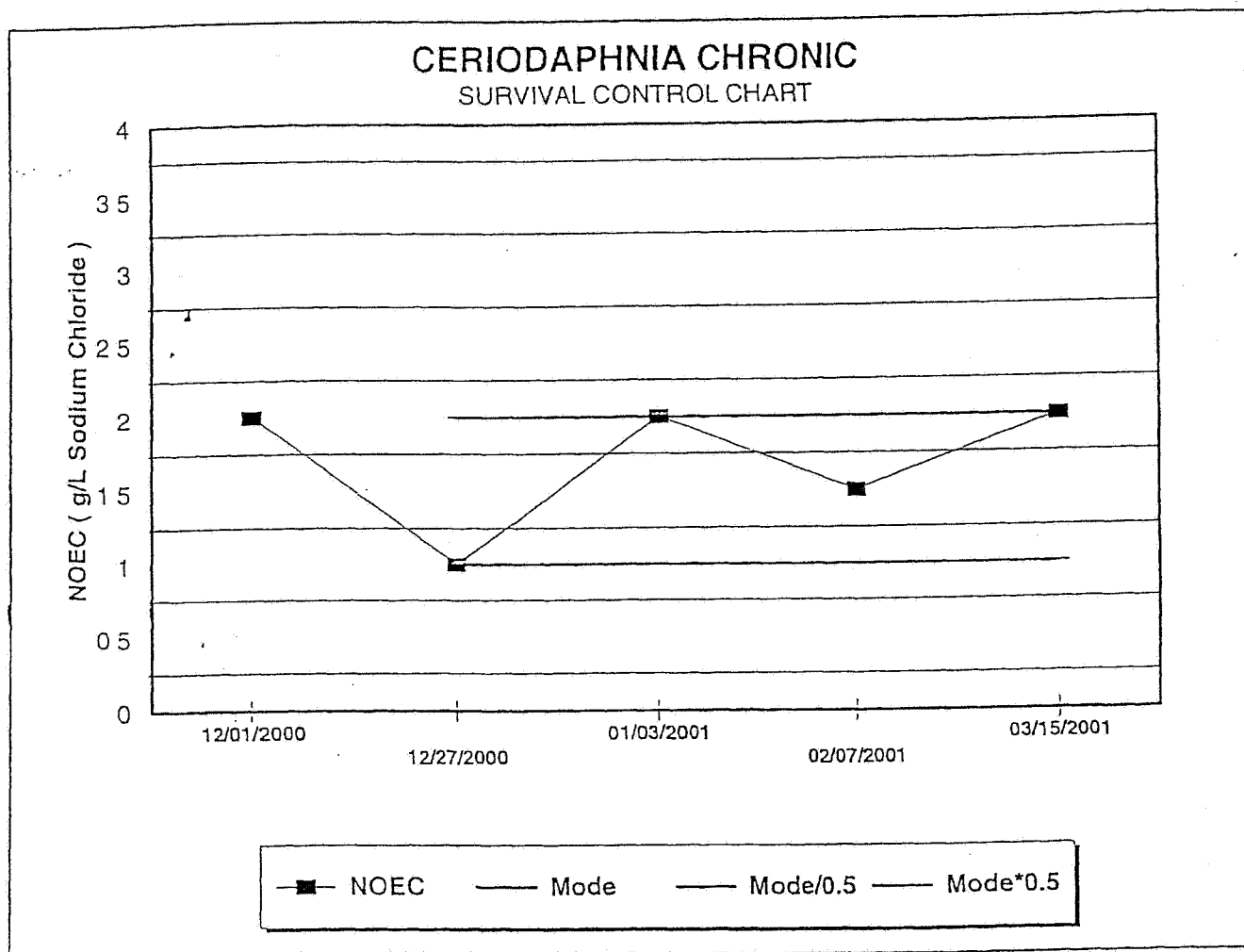
Olver Laboratories, Inc.

16-APR-2001

TITLE: CERIODAPHNIA CHRONIC TEST_SURVIVAL
Reference Toxicant: Sodium Chloride (g/L)

Test Date	Test Name NOEC	Mode	Mode/0.5	Mode*0.5
12/01/2000	2			
12/27/2000	1	2.00	4.00	1.00
01/03/2001	2	2.00	4.00	1.00
02/07/2001	1.5	2.00	4.00	1.00
03/15/2001	2	2.00	4.00	1.00

16-Apr-2001



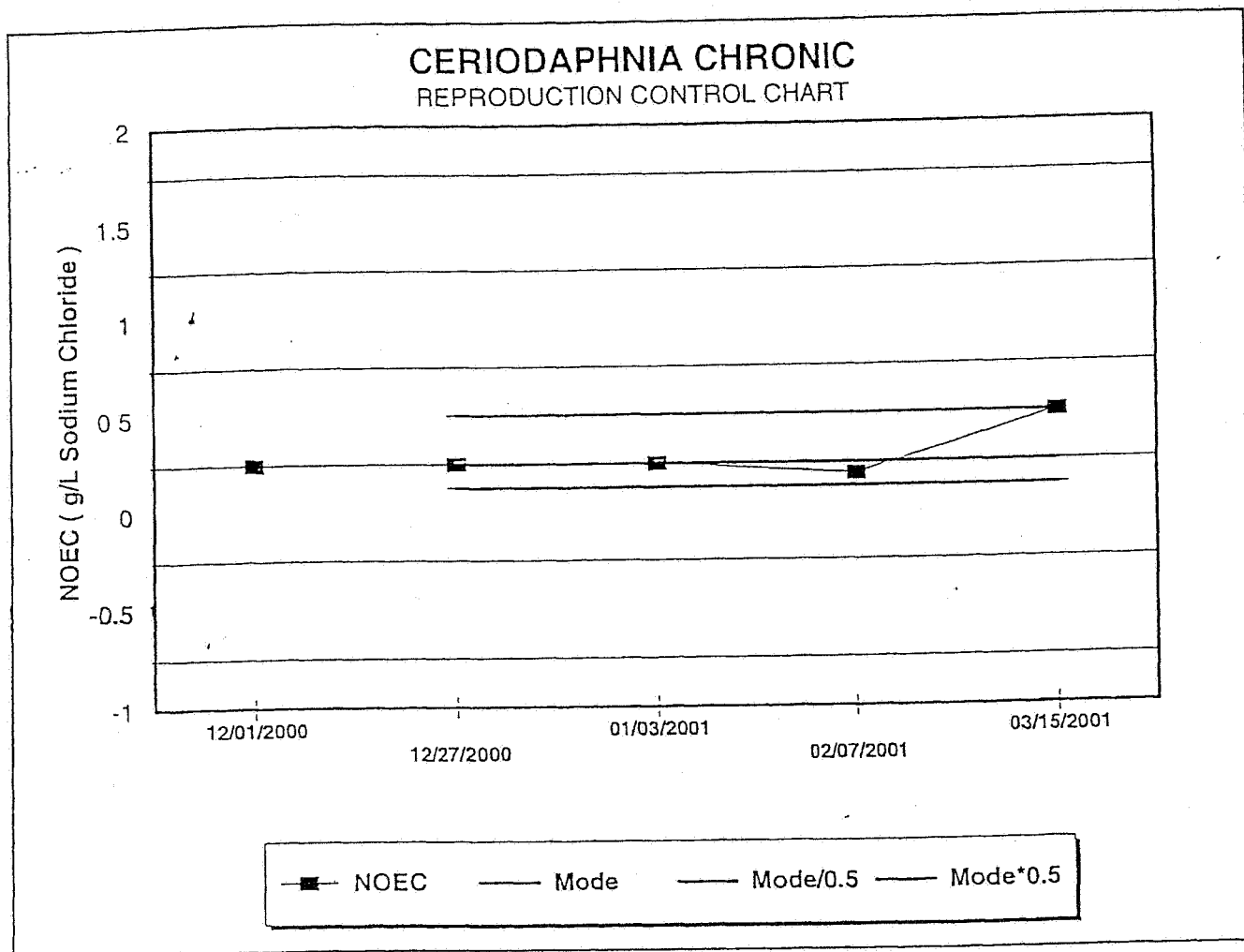
16-APR-2001

TITLE: CERIODAPHNIA CHRONIC TEST-Reproduction
Reference Toxicant: Sodium Chloride (g/L)

[illegible]

OLVER LABORATORIES INC

16-Apr-2001



APPENDIX 10
DILUTION WATER QUALITY
ANALYSES DATA



Consulting Engineers • Environmental Laboratories
1116 South Main Street Blacksburg, Virginia 24060

Client Sheet No.: 0004151 Job No.: 30001
Date: May 8, 2000 Date Received: 4/12/00
Client: Olver Laboratories Incorporated
Source: Blacksburg, Virginia
Shipping Information: Delivered to Olver Laboratories Incorporated by Olver Personnel

Sample No.: 123511
Date Collected: 4/12/00
Time Collected: 9 45 a m and 3:00 p m.
Description: EPA Moderately Hard Dilution Water

Analysis	Result	MDL	Date/Time Analyzed	Analyst
pH (Laboratory) (EPA 150 1)	8 09	N/A	4/12/00, 7 45 p m.	SAH
Alkalinity as CaCO ₃ (EPA 310 1)	60 mg/L	10 mg/L	4/20/00, 11 00 a.m	ACC
Conductivity (EPA 120.1)	317 μ mhos/cm	1 0 μ mhos/cm	5/02/00, 10 45 a m	ACC
Total Cyanide (EPA 335 2)	ND	0 005 mg/L	4/20/00, 8 15 a m	DF
Ammonia as N (SM 4500 NH ₃ ,F)	ND	0.1 mg/L	4/17/00, 6 00 p m.	SAH
Nitrate as N (EPA 352.1)	ND	0 1 mg/L	4/14/00, 3 00 p m.	BCW
Nitrite as N (EPA 354 1)	ND	0 01 mg/L	4/14/00, 1 30 p m.	SAH
Hardness as CaCO ₃ (EPA 200 7)	100 mg/L	1 mg/L	4/18/00, 5 00 p m	HT
Total Phenols (EPA 420 1)	ND	0 005 mg/L	4/24/00, 1 10 p m.	DF

MDL = Minimum Detection Limit
N/A = Not Applicable
ND = Not Detectable

All tests according to Standard Methods for the Examination of Water and Wastewater, 18th Edition, Test Methods for Evaluating Solid Waste (Physical/Chemical), 2nd and 3rd Editions, and Methods for the Chemical Analysis of Water and Wastes, EPA

By Brt Ritchey
Brt Ritchey
Laboratory Manager

Unless otherwise indicated, this report sets forth the results of our analysis of samples delivered to our laboratory and shall not be construed to be a representation by Olver Laboratories Incorporated as to the source or method of procuring such samples. All reports are submitted as the confidential property of clients and authorization for publication of any statements contained in our reports is reserved pending our written approval.

OLVER LABORATORIES

Client Sheet No
Client
Date

0004151
Olver Laboratories Incorporated
May 8, 2000

Sample No.: 123511
Date Collected: 4/12/00
Time Collected: 9 45 a.m. and 3:00 p.m.
Description: EPA Moderately Hard Dilution Water

Analysis	Result	MDL	Date/Time Analyzed	Analyst
Total Aluminum (EPA 202.2)	0.005 mg/L	0.003 mg/L	5/02/00, 12:00 p.m.	HT
Dissolved Aluminum (EPA 202.2)	ND	0.003 mg/L	5/02/00, 12:00 p.m.	HT
Total Arsenic (EPA 200.9)	ND	0.001 mg/L	4/25/00, 2:00 p.m.	HT
Dissolved Arsenic (EPA 200.9)	ND	0.001 mg/L	4/25/00, 2:00 p.m.	HT
Total Cadmium (EPA 200.9)	ND	0.0001 mg/L	4/20/00, 9:30 a.m.	BN
Dissolved Cadmium (EPA 200.9)	ND	0.0001 mg/L	4/20/00, 9:30 a.m.	BN
Total Chromium III (EPA 200.9-SM 3500 Cr,D)	ND	0.001 mg/L	4/20/00, 4:00 p.m.	HT
Dissolved Chromium III (EPA 200.9-SM 3500 Cr,D)	ND	0.001 mg/L	4/20/00, 4:00 p.m.	HT
Total Hexavalent Chromium (SM 3500 Cr,D)	ND	0.005 mg/L	4/13/00, 1:30 p.m.	SAH
Dissolved Hexavalent Chromium (SM 3500 Cr,D)	ND	0.005 mg/L	4/13/00, 1:30 p.m.	SAH
Total Cobalt (EPA 200.9)	ND	0.001 mg/L	4/26/00, 3:00 p.m.	HT
Dissolved Cobalt (EPA 200.9)	ND	0.001 mg/L	4/26/00, 3:00 p.m.	HT
Total Copper (EPA 200.9)	ND	0.001 mg/L	4/18/00, 9:00 p.m.	HT/BN
Dissolved Copper (EPA 200.9)	ND	0.001 mg/L	4/18/00, 9:00 p.m.	HT/BN
Total Iron (EPA 236.2)	0.006 mg/L	0.001 mg/L	5/03/00, 12:00 p.m.	HT
Dissolved Iron (EPA 236.2)	0.006 mg/L	0.001 mg/L	5/03/00, 12:00 p.m.	HT
Total Lead (EPA 200.9)	ND	0.001 mg/L	5/01/00, 2:00 p.m.	HT
Dissolved Lead (EPA 200.9)	ND	0.001 mg/L	5/01/00, 2:00 p.m.	HT
Total Mercury (EPA 245.1)	ND	0.0002 mg/L	4/21/00, 9:00 a.m.	BN
Dissolved Mercury (EPA 245.1)	ND	0.0002 mg/L	4/21/00, 9:00 a.m.	BN
Total Nickel (EPA 200.9)	ND	0.001 mg/L	4/18/00, 7:30 a.m.	BN
Dissolved Nickel (EPA 200.9)	ND	0.001 mg/L	4/18/00, 7:30 a.m.	BN
Total Selenium (EPA 200.9)	ND	0.002 mg/L	4/17/00, 11:45 a.m.	BN
Dissolved Selenium (EPA 200.9)	ND	0.002 mg/L	4/17/00, 11:45 a.m.	BN
Total Silver (EPA 200.9)	ND	0.0002 mg/L	4/18/00, 5:00 p.m.	HT
Dissolved Silver (EPA 200.9)	ND	0.0002 mg/L	4/18/00, 5:00 p.m.	HT
Total Zinc (EPA 200.7)	ND	0.005 mg/L	4/22/00, 11:30 a.m.	BN
Dissolved Zinc (EPA 200.7)	ND	0.005 mg/L	4/22/00, 11:30 a.m.	BN
Priority Pollutant Organics:				
Acid Compounds (EPA 625):			4/28/00, 7:56 a.m.	JSL
2-Chlorophenol	ND	10 µg/L		
2,4-Dichlorophenol	ND	10 µg/L		
2,4-Dimethylphenol	ND	10 µg/L		
4,6-Dinitro-o-cresol	ND	10 µg/L		
2,4-Dinitrophenol	ND	50 µg/L		
2-Nitrophenol	ND	10 µg/L		
4-Nitrophenol	ND	50 µg/L		
p-Chloro-m-cresol	ND	20 µg/L		
Pentachlorophenol	ND	50 µg/L		
Phenol	ND	10 µg/L		
2,4,6-Trichlorophenol	ND	10 µg/L		

OLVER
LABORATORIES

Client Sheet No
Client
Date

0004151
Olver Laboratories Incorporated
May 8, 2000

Sample No.: 123511
Date Collected: 4/12/00
Time Collected: 9 45 a m and 3 00 p m
Description: EPA Moderately Hard Dilution Water

<u>Analysis</u>	<u>Result</u>	<u>MDL</u>	<u>Date/Time Analyzed</u>	<u>Analyst</u>
Priority Pollutant Organics (continued)				
Base/Neutral Compounds (EPA 625)			4/28/00, 7 56 a m	JSL
Acenaphthene	ND	10 µg/L		
Acenaphthylene	ND	10 µg/L		
Anthracene	ND	10 µg/L		
Benidine 4	ND	10 µg/L		
Benzo(a)anthracene	ND	10 µg/L		
Benzo(a)pyrene	ND	10 µg/L		
3,4-Benzofluoranthene	ND	10 µg/L		
Benzo(g,h,i)perylene	ND	10 µg/L		
Benzo(k)fluoranthene	ND	10 µg/L		
Bis(2-chloroethoxy)methane	ND	10 µg/L		
Bis(2-chloroethyl)ether	ND	10 µg/L		
Bis(2-chloroisopropyl)ether	ND	10 µg/L		
Bis(2-ethylhexyl)phthalate	ND	10 µg/L		
4-Bromophenyl Phenyl Ether	ND	10 µg/L		
Butyl Benzyl Phthalate	ND	10 µg/L		
2-Chloronaphthalene	ND	10 µg/L		
4-Chlorophenyl Phenyl Ether	ND	10 µg/L		
Chrysene	ND	10 µg/L		
Dibenzo(a,h)anthracene	ND	10 µg/L		
1,2-Dichlorobenzene	ND	10 µg/L		
1,3-Dichlorobenzene	ND	10 µg/L		
1,4-Dichlorobenzene	ND	10 µg/L		
3,3'-Dichlorobenzidine	ND	20 µg/L		
Diethyl Phthalate	ND	10 µg/L		
Dimethyl Phthalate	ND	10 µg/L		
Di-n-Butyl Phthalate	ND	10 µg/L		
2,4-Dinitrotoluene	ND	10 µg/L		
2,6-Dinitrotoluene	ND	10 µg/L		
Di-n-Octyl Phthalate	ND	10 µg/L		
1,2-Diphenylhydrazine	ND	10 µg/L		
Fluoranthene	ND	10 µg/L		
Fluorene	ND	10 µg/L		
Hexachlorobenzene	ND	10 µg/L		

OLVER
LABORATORIES

Client Sheet No 0004151
Client Olver Laboratories Incorporated
Date May 8, 2000

Sample No.: 123511
Date Collected: 4/12/00
Time Collected: 9:45 a.m. and 3:00 p.m.
Description: EPA Moderately Hard Dilution Water

<u>Analysis</u>	<u>Result</u>	<u>MDL</u>	<u>Date/Time Analyzed</u>	<u>Analyst</u>
Base/Neutral Compounds (EPA 625) (continued).			4/28/00, 7 56 a.m.	JSL
Hexachlorobutadiene	ND	10 µg/L		
Hexachlorocyclopentadiene	ND	10 µg/L		
Hexachloroethane	ND	10 µg/L		
Indeno(1,2,3-cd)pyrene	ND	10 µg/L		
Isophorone	ND	10 µg/L		
Naphthalene	ND	10 µg/L		
Nitrobenzene	ND	10 µg/L		
N-Nitroso-dimethylamine	ND	10 µg/L		
N-Nitroso-di-n-propylamine	ND	10 µg/L		
N-Nitroso-diphenylamine	ND	10 µg/L		
Phenanthrene	ND	10 µg/L		
Pyrene	ND	10 µg/L		
1,2,4-Trichlorobenzene	ND	10 µg/L		

OLVER
LABORATORIES

Client Sheet No
Client
Date

0004151
Olver Laboratories Incorporated
May 8, 2000

Sample No.: 123511
Date Collected: 4/12/00
Time Collected: 9:45 a m and 3 00 p.m.
Description: EPA Moderately Hard Dilution Water

<u>Analysis</u>	<u>Result</u>	<u>MDL</u>	<u>Date/Time Analyzed</u>	<u>Analyst</u>
Volatile Compounds (EPA 624)			4/13/00, 4 06 p m	BP
Acrolein	ND	10 µg/L		
Acrylonitrile	ND	10 µg/L		
Benzene	ND	5 µg/L		
Bromodichloromethane	ND	5 µg/L		
Bromoform	ND	5 µg/L		
Bromomethane	ND	10 µg/L		
Carbon Tetrachloride	ND	10 µg/L		
Chlorobenzene	ND	5 µg/L		
Chloroethane	ND	10 µg/L		
2-Chloroethyl Vinyl Ether	ND	5 µg/L		
Chloroform	ND	5 µg/L		
Chloromethane	ND	10 µg/L		
Dibromochloromethane	ND	5 µg/L		
1,1-Dichloroethane	ND	10 µg/L		
1,2-Dichloroethane	ND	5 µg/L		
1,1-Dichloroethene	ND	10 µg/L		
trans-1,2-Dichloroethene	ND	10 µg/L		
1,2-Dichloropropane	ND	5 µg/L		
cis-1,3-Dichloropropene	ND	5 µg/L		
trans-1,3-Dichloropropene	ND	5 µg/L		
Ethylbenzene	ND	5 µg/L		
Methylene Chloride	ND	5 µg/L		
sym-Tetrachloroethane	ND	5 µg/L		
Tetrachloroethene	ND	5 µg/L		
Toluene	ND	5 µg/L		
1,1,1-Trichloroethane	ND	10 µg/L		
1,1,2-Trichloroethane	ND	5 µg/L		
Trichloroethene	ND	10 µg/L		
Trichlorofluoromethane	ND	10 µg/L		
Vinyl Chloride	ND	10 µg/L		

OLVER
LABORATORIES

Client Sheet No
Client
Date

0004151
Olver Laboratories Incorporated
May 8, 2000

Sample No.: 123511
Date Collected: 4/12/00
Time Collected: 9:45 a.m and 3 00 p m.
Description: EPA Moderately Hard Dilution Water

<u>Analysis</u>	<u>Result</u>	<u>MDL</u>	<u>Date/Time Analyzed</u>	<u>Analyst</u>
Pesticides (EPA 608)			4/21/00, 9 26 p m	SD
Aldnn	ND	1 µg/L		
alpha-BHC	ND	1 µg/L		
beta-BHC	ND	1 µg/L		
delta-BHC	ND	1 µg/L		
gamma-BHC	ND	1 µg/L		
Chlordane	ND	1 µg/L		
4,4'-DDD	ND	1 µg/L		
4,4'-DDE	ND	1 µg/L		
4,4'-DDT	ND	1 µg/L		
Dieldnn	ND	1 µg/L		
Endosulfan I	ND	1 µg/L		
Endosulfan II	ND	1 µg/L		
Endosulfan Sulfate	ND	1 µg/L		
Endnn	ND	1 µg/L		
Endnn Aldehyde	ND	1 µg/L		
Heptachlor	ND	1 µg/L		
Heptachlor Epoxide	ND	1 µg/L		
Toxaphene	ND	1 µg/L		
PCBs (EPA 608)			4/21/00, 9 26 p m	SD
PCB-1016	ND	50 µg/L		
PCB-1221	ND	50 µg/L		
PCB-1232	ND	50 µg/L		
PCB-1242	ND	50 µg/L		
PCB-1248	ND	50 µg/L		
PCB-1254	ND	50 µg/L		
PCB-1260	ND	50 µg/L		



CHAIN OF CUSTODY RECORD

Consulting Engineers and Environmental Laboratories
1116 South Main Street • Blacksburg, Virginia • 24060
Telephone: (540) 552-6974 • FAX: (540) 552-1715

Client: Olver Laboratories, Incorporated
Company Contact: Bioassay Laboratory
Address: 1116 South Main Street, Blacksburg, VA 24060
Telephone: (540) 552-6974

Purchase Order No. _____

* Client should complete all information requested to the right and in the unshaded areas below.
DO NOT WRITE IN THE SHADED AREAS
* Samples will be discarded or returned to the client two weeks after the analytical report has been mailed, unless prior arrangements have been made

Sample Location (i.e., influent, outfall 001, MW1, etc.)	Date Collected	Time Collected	Sample Type	Container Type	Preservation	Composite (S)	Grab (S)	# of Containers	Analyses Requested
EPA Moderately Hard Water	4/12/00	3:00 pm	Lab Dilution Water	PE	None		✓	1	Alkalinity, Conductivity, Hardness, pH
EPA Moderately Hard Water		9:45 am	Lab Dilution Water	PE	NaOH		✓	1	Total Cyanide
EPA Moderately Hard Water		9:45 am	Lab Dilution Water	PE	H ₂ SO ₄ / Na ₂ S ₂ O ₈		✓	1	Ammonia
EPA Moderately Hard Water		9:45 am	Lab Dilution Water	GL	H ₂ SO ₄		✓	1	Total Phenols
EPA Moderately Hard Water		9:45 am	Lab Dilution Water	PE	HNO ₃		✓	1	Total Al, As, Cd, Cr, Co, Cu, Fe, Pb, Ni, Se, Ag (GFAA), Total Zn (ICP), Hardness
EPA Moderately Hard Water		9:45 am	Lab Dilution Water	GL	HNO ₃		✓	1	Total Mercury
EPA Moderately Hard Water		3:00 pm	Lab Dilution Water	PE	None		✓	1	Hexavalent Chromium, Diss. Hexavalent Chromium
EPA Moderately Hard Water		9:45 am	Lab Dilution Water	PE	None		✓	1	Diss. Al, As, Cd, Cr, Co, Cu, Fe, Pb, Ni, Se, Ag (GFAA), Diss. Zn (ICP)

Special Instructions

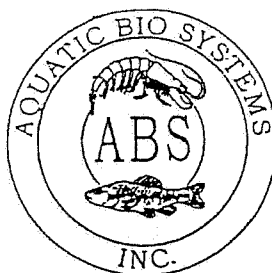
Temperature upon receipt in lab = 4.2°C
Method of Shipment: 500
4/12/00 UPS Oliver Personnel
Comments: _____

Supplier (S.L.) Signature: [Signature]
Relinquished By (Signature): [Signature] Date: 4/12/00 Time: 4:45
Relinquished By (Signature): [Signature] Date: _____ Time: _____
Relinquished By (Signature): _____ Date: _____ Time: _____

APPENDIX 11

CHEMICAL ANALYSES OF
BRINE SHRIMP AND YCT FOOD MIXTURES

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

SALMON STARTER #1
38-4763-91-50

PROCESS DATE:
07/26/00

GUARANTEED ANALYSIS

Crude Protein, Min 55.0%
Crude Fat, Min. 15.0%
Crude Fiber, Max. 2.0%

Toxic Metals and Pesticide Analysis (Note: This analysis is on Lot 3/16/00)
Performed by Energy Labs, Billings, MT

Total Metals		
	Detection Limit ug/g	Result ug/g
Aluminum	5	20
Arsenic	5	<5
Cadmium	1	<1
Chromium	5	<5
Cobalt	5	<5
Copper	5	9
Iron	5	347
Lead	5	<5
Mercury	1	<1
Nickel	5	<5
Silver	5	<5
Zinc	5	103

EPA Methods 8081: Organochlorine Pesticides & 8082: PCB's Analysis Report			
Compound*	Result ug/g	Compound*	RESU LT ug/g
Aldrin	<0.10	Endrin ketone	<0.20
alpha-BHC	<0.10	Heptachlor	<0.10
beta-BHC	<0.10	Heptachlor epoxide	<0.10
delta-BHC	<0.10	Methoxychlor	<1.0
gamma-BHC (Lindane)	<0.10	Chlordane (technical)	<1.0
alpha-Chlordane	<0.10	Toxaphene	<10
gamma-Chlordane	<0.10	Aroclor-1016	<2.0
4,4' - DDD	<0.20	Aroclor-1221	<4.0
4,4' - DDE	<0.20	Aroclor-1232	<2.0
4,4' - DDT	<0.20	Aroclor-1242	<2.0
Dieldrin	<0.20	Aroclor-1248	<2.0
Endosulfan I	<0.10	Aroclor-1254	<2.0
Endosulfan II	<0.20	Aroclor-1260	<2.0
Endosulfan sulfate	<0.20	Aroclor-1262	<2.0
Endrin	<0.20	Aroclor-1268	<2.0
Endrin aldehyde	<0.20		

*Compound analyzed for but not detected.

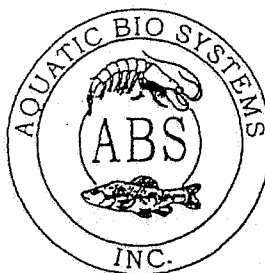
INGREDIENTS

Fish Meal, Soy Flour, Corn Gluten Meal, Blood Meal, Fish Oil, Liquid Fish Solubles, Torula Dried Yeast, Brewers Yeast, Soy Lecithin, Lignin Sulfonate, L-Ascorbyl-2-Polyphosphate (Vitamin C), Choline Chloride, Ethoxyquin (preservative), Canthaxanthin (pigmenting agent), Vitamin B12 Supplement, Niacin, Calcium Pantothenate, Riboflavin, Pyridoxine Hydrochloride, Vitamin A Acetate, D-Activated Animal Sterol (source of Vitamin D3), Thiamine Mononitrate, Folic Acid, Biotin, dl-Alpha Tocopherol Acetate (source of Vitamin E), Menadione (Vitamin K), Zinc Sulfate, Copper Sulfate, Manganous Sulfate, Ethylenediamine Dihydroiodide, Cobalt Carbonate, Ferrous Sulfate, Calcium Carbonate

MANUFACTURED BY: ZEIGLER BROS., INC. P.O. BOX 95, GARDNERS, PA 17324

Aquatic BioSystems, Inc • Quality Research Organisms

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524



Toll Free: 800/331-5916
Tel: 970/484-5091 Fax: 970/484-2514

YTC TOTAL SOLIDS MEASUREMENT
(Method from EPA/505/8-89-002a)

YTC Process Date:
Average Total Solids

12/4/00: Best if used by 04/30/01
1760 mg/l

Ingredient Lot Numbers

Pines International Wheat Grass WG98S00090; Zeigler Salmon Starter #1: 38-4763-9150, Fleischmanns Yeast G-3

EPA Required Toxic Metals and Pesticide Analyses*

Analyzed Metals	Report Limits	Results (mg/L)
Aluminum	0.1	<0.1
Arsenic	0.005	<0.005
Cadmium	0.001	<0.001
Chromium	0.01	<0.01
Copper	0.01	0.01
Iron	0.03	0.18
Lead	0.01	<0.01
Mercury	0.001	<0.001
Nickel	0.01	<0.01
Silver	0.005	<0.005
Zinc	0.01	0.42

Compounds	Results (ug/L)	Qualifier
Aldrin	<2.0	U
alpha-BHC	<2.0	U
beta-BHC	<2.0	U
delta-BHC	<2.0	U
gamma-BHC (Lindane)	<2.0	U
alpha-Chlordane	<2.0	U
gamma-Chlordane	<2.0	U
4,4' - DDD	<4.0	U
4,4' - DDE	<4.0	U
4,4' - DDT	<4.0	U
Dieldrin	<4.0	U
Endosulfan I	<2.0	U
Endosulfan II	<4.0	U
Endosulfan sulfate	<4.0	U
Endrin	<4.0	U
Endrin aldehyde	<4.0	U
Endrin ketone	<4.0	U
Heptachlor	<2.0	U
Heptachlor epoxide	<2.0	U
Methoxychlor	<20	U
Chlordane (technical)	<20	U
Toxaphene	<200	U
Aroclor-1016	<40	U
Aroclor-1221	<80	U
Aroclor-1232	<40	U
Aroclor-1242	<40	U
Aroclor-1248	<40	U
Aroclor-1254	<40	U
Aroclor-1260	<40	U
Aroclor-1262	<40	U
Aroclor-1268	<40	U

U - Indicates compound was analyzed for but not detected

*Testing performed by Energy Labs, Billings, Montana

Aquatic BioSystems, Inc • Quality Research Organisms

Company Sanitized. Does not contain TSCA CBI



Consulting Engineers • Environmental Laboratories
1116 South Main Street Blacksburg, Virginia 24060

Client Sheet No.: 0005275 Job No.: 60001
Date: July 11, 2000 Date Received: 5/25/00
Client: Olver Laboratories Incorporated
Source: Blacksburg, Virginia
Shipping Information: Delivered to Olver Laboratories Incorporated by Olver Personnel

Sample No.: 124686
Time Collected: 3:45 p.m.
Date Collected: 5/25/00
Description: Tetramin Flakes (Lot No. 121599)

Analysis	Result	MDL	Date/Time Analyzed	Analyst
Total Aluminum (EPA 202.2)	350 mg/kg as received	31.2 mg/kg as received*	6/27/00; 8:00 a.m.	AMT
Total Arsenic (EPA 206.2)	ND **	0.15 mg/kg as received	7/01/00; 10:00 a.m.	BN
Total Cadmium (EPA 213.2)	0.0125 mg/kg as received	0.005 mg/kg as received	6/19/00; 11:30 a.m.	AMT
Total Chromium (EPA 218.2)	1.05 mg/kg as received	0.15 mg/kg as received	7/10/00; 1:30 p.m.	BN
Total Cobalt (EPA 219.2)	0.425 mg/kg as received	0.05 mg/kg as received	6/29/00; 1:00 p.m.	HT
Total Copper (EPA 220.2)	13.3 mg/kg as received	0.5 mg/kg as received*	6/14/00; 3:00 p.m.	HT
Total Iron (EPA 200.7)	905 mg/kg as received	0.35 mg/kg as received	6/22/00; 2:00 p.m.	HT
Total Lead (EPA 239.2)	0.275 mg/kg as received	0.05 mg/kg as received*	6/23/00; 2:00 p.m.	HT
Total Mercury (EPA 245.1)	ND	0.25 mg/kg as received	7/05/00; 2:00 p.m.	HT
Total Nickel (EPA 200.9)	1.3 mg/kg as received	0.05 mg/kg as received	7/05/00; 2:00 p.m.	HT/BN
Total Silver (EPA 272.2)	0.055 mg/kg as received**	0.01 mg/kg as received	6/27/00; 3:00 p.m.	HT
Total Zinc (EPA 200.7)	82.3 mg/kg as received	0.5 mg/kg as received	6/27/00; 8:00 a.m.	AMT

MDL = Minimum Detection Limit

ND = Not Detectable

* Sample required dilution; MDL was raised accordingly.

** Due to low spike recovery, the Method of Standard Additions was used to analyze this sample.

All tests according to Standard Methods for the Examination of Water and Wastewater, 18th Edition, Test Methods for Evaluating Solid Waste (Physical/Chemical), 2nd and 3rd Editions, and Methods for the Chemical Analysis of Water and Wastes, EPA.

By: Brit Ritchey
Brit Ritchey
Laboratory Manager

Unless otherwise indicated, this report sets forth the results of our analysis of samples delivered to our laboratory and shall not be construed to be a representation by Olver Laboratories Incorporated as to the source or method of procuring such samples. All reports are submitted as the confidential property of clients and authorization for publication of any statements contained in our reports is reserved pending our written approval.

OLVER
LABORATORIES

Client Sheet No
Client
Date

0005275
Olver Laboratories Incorporated
July 11, 2000

Sample No.: 124686
Time Collected: 3:45 p.m.
Date Collected: 5/25/00
Description: Tetramin Flakes (Lot No. 121599)

<u>Analysis</u>	<u>Result</u>	<u>MDL</u>	<u>Date/Time Analyzed</u>	<u>Analyst</u>
Pesticides (EPA 608):			6/06/00; 5:56 a.m.	ASM/PMW
Aldrin	ND	5 µg/kg as received		
alpha-BHC	ND	5 µg/kg as received		
beta-BHC	ND	5 µg/kg as received		
delta-BHC	ND	5 µg/kg as received		
gamma-BHC	ND	5 µg/kg as received		
Chlordane	ND	50 µg/kg as received		
4,4'-DDD	ND	5 µg/kg as received		
4,4'-DDE	ND	5 µg/kg as received		
4,4'-DDT	ND	5 µg/kg as received		
Dieldrin	ND	5 µg/kg as received		
Endosulfan I	ND	5 µg/kg as received		
Endosulfan II	ND	5 µg/kg as received		
Endosulfan Sulfate	ND	5 µg/kg as received		
Endrin	ND	5 µg/kg as received		
Endrin Aldehyde	ND	5 µg/kg as received		
Heptachlor	ND	5 µg/kg as received		
Heptachlor Epoxide	ND	5 µg/kg as received		
Toxaphene	ND	50 µg/kg as received		
PCBs (EPA 608):			6/06/00, 5:56 a.m.	ASM/PMW
PCB-1016	ND	50 µg/kg as received		
PCB-1221	ND	50 µg/kg as received		
PCB-1232	ND	50 µg/kg as received		
PCB-1242	ND	50 µg/kg as received		
PCB-1248	ND	50 µg/kg as received		
PCB-1254	ND	50 µg/kg as received		
PCB-1260	ND	50 µg/kg as received		

OLVER LABORATORIES

Client Sheet No
Client
Date

0005275
Olver Laboratories Incorporated
July 11, 2000

Sample No.: 124687
Time Collected: 3:45 p.m.
Date Collected: 5/25/00
Description: TYC (Lot No. 51900)

Analysis	Result	MDL	Date/Time Analyzed	Analyst
Total Aluminum (EPA 202.2)	0.118 mg/L	0.025 mg/L*	6/27/00; 8:00 a.m.	AMT
Total Arsenic (EPA 206.2)	0.22 mg/L**	0.15 mg/L*	7/01/00; 10:00 a.m.	BN
Total Cadmium (EPA 213.2)	ND	0.0001 mg/L	6/19/00; 11:30 a.m.	AMT
Total Chromium (EPA 200.9)	0.004 mg/L	0.001 mg/L	7/07/00; 11:30 a.m.	BN
Total Cobalt (EPA 219.2)	ND	0.001 mg/L	6/29/00; 1:00 p.m.	HT
Total Copper (EPA 220.2)	0.054 mg/L	0.001 mg/L	6/14/00; 3:00 p.m.	HT
Total Iron (EPA 200.7)	0.372 mg/L	0.007 mg/L	6/22/00; 2:00 p.m.	HT
Total Lead (EPA 239.2)	0.012 mg/L	0.1 mg/L*	6/23/00; 2:00 p.m.	HT
Total Mercury (EPA 245.1)	ND	0.25 mg/L	6/26/00; 10:00 a.m.	AMT
Total Nickel (EPA 200.9)	0.004 mg/L	0.001 mg/L	7/05/00; 2:00 p.m.	HT/BN
Total Silver (EPA 272.2)	ND **	0.0002 mg/L	6/27/00; 3:00 p.m.	HT
Total Zinc (EPA 200.7)	0.521 mg/L	0.005 mg/L	6/27/00; 8:00 a.m.	AMT
Pesticides (EPA 608):			6/06/00; 6:24 a.m.	ASMPMW
Aldrin	ND	5 µg/kg as received		
alpha-BHC	ND	5 µg/kg as received		
beta-BHC	ND	5 µg/kg as received		
delta-BHC	ND	5 µg/kg as received		
gamma-BHC	ND	5 µg/kg as received		
Chlordane	ND	50 µg/kg as received		
4,4'-DDD	ND	5 µg/kg as received		
4,4'-DDE	ND	5 µg/kg as received		
4,4'-DDT	ND	5 µg/kg as received		
Dieldrin	ND	5 µg/kg as received		
Endosulfan I	ND	5 µg/kg as received		
Endosulfan II	ND	5 µg/kg as received		
Endosulfan Sulfate	ND	5 µg/kg as received		
Endrin	ND	5 µg/kg as received		
Endrin Aldehyde	ND	5 µg/kg as received		
Heptachlor	ND	5 µg/kg as received		
Heptachlor Epoxide	ND	5 µg/kg as received		
Toxaphene	ND	50 µg/kg as received		
PCBs (EPA 608):			6/06/00; 6:24 a.m.	ASMPMW
PCB-1016	ND	50 µg/kg as received		
PCB-1221	ND	50 µg/kg as received		
PCB-1232	ND	50 µg/kg as received		
PCB-1242	ND	50 µg/kg as received		
PCB-1248	ND	50 µg/kg as received		
PCB-1254	ND	50 µg/kg as received		
PCB-1260	ND	50 µg/kg as received		

* Sample required dilution; MDL was raised accordingly.

** Due to low spike recovery, the Method of Standard Additions was used to analyze this sample

OLVER LABORATORIES

Client Sheet No
Client
Date

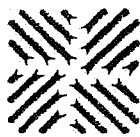
0005275
Olver Laboratories Incorporated
July 11, 2000

Sample No.: 124688
Time Collected: 5:00 p.m.
Date Collected: 5/25/00
Description: Brine Shrimp Nauplii (Lot No. 4400)

Analysis	Result	MDL	Date/Time Analyzed	Analyst
Total Aluminum (EPA 202.2)	0.007 mg/L	0.005 mg/L	6/27/00; 8:00 a.m.	AMT
Total Arsenic (EPA 206.2)	0.08 mg/L **	0.15 mg/L *	7/01/00; 10:00 a.m.	BN
Total Cadmium (EPA 213.2)	ND	0.0001 mg/L	6/19/00; 11:30 a.m.	AMT
Total Chromium (EPA 200.9)	0.001 mg/L	0.001 mg/L	7/07/00; 11:30 a.m.	BN
Total Cobalt (EPA 219.2)	ND	0.001 mg/L	6/29/00; 1:00 p.m.	HT
Total Copper (EPA 220.2)	0.015 mg/L	0.001 mg/L	6/14/00; 3:00 p.m.	HT
Total Iron (EPA 200.7)	1.04 mg/L	0.007 mg/L	6/22/00; 2:00 p.m.	HT
Total Lead (EPA 239.2)	ND	0.1 mg/L *	6/23/00; 2:00 p.m.	HT
Total Mercury (EPA 245.1)	ND	0.25 mg/L	6/26/00; 10:00 a.m.	AMT
Total Nickel (EPA 200.9)	0.003 mg/L	0.001 mg/L	7/05/00; 2:00 p.m.	HT/BN
Total Silver (EPA 272.2)	ND **	0.0002 mg/L	6/27/00; 3:00 p.m.	HT
Total Zinc (EPA 200.7)	0.069 mg/L	0.005 mg/L	6/27/00; 8:00 a.m.	AMT
Pesticides (EPA 608):			6/06/00; 6:53 a.m.	ASMPMW
Aldrin	ND	5 µg/kg as received		
alpha-BHC	ND	5 µg/kg as received		
beta-BHC	ND	5 µg/kg as received		
delta-BHC	ND	5 µg/kg as received		
gamma-BHC	ND	5 µg/kg as received		
Chlordane	ND	50 µg/kg as received		
4,4'-DDD	ND	5 µg/kg as received		
4,4'-DDE	ND	5 µg/kg as received		
4,4'-DDT	ND	5 µg/kg as received		
Dieldrin	ND	5 µg/kg as received		
Endosulfan I	ND	5 µg/kg as received		
Endosulfan II	ND	5 µg/kg as received		
Endosulfan Sulfate	ND	5 µg/kg as received		
Endrin	ND	5 µg/kg as received		
Endrin Aldehyde	ND	5 µg/kg as received		
Heptachlor	ND	5 µg/kg as received		
Heptachlor Epoxide	ND	5 µg/kg as received		
Toxaphene	ND	50 µg/kg as received		
PCBs (EPA 608):			6/06/00; 6:53 a.m.	ASMPMW
PCB-1016	ND	50 µg/kg as received		
PCB-1221	ND	50 µg/kg as received		
PCB-1232	ND	50 µg/kg as received		
PCB-1242	ND	50 µg/kg as received		
PCB-1248	ND	50 µg/kg as received		
PCB-1254	ND	50 µg/kg as received		
PCB-1260	ND	50 µg/kg as received		

* Sample required dilution; MDL was raised accordingly.

** Due to low spike recovery, the Method of Standard Additions was used to analyze this sample.



CHAIN OF CUSTODY RECORD

Consulting Engineers and Environmental Laboratories
1116 South Main Street • Blacksburg, Virginia • 24060
Telephone: (540) 552-6974 • FAX: (540) 552-1715

Client: Oliver Laboratories Inc.

Company Contact: Bioassay Laboratory - (5m)

Address: _____

Telephone: _____

Purchase Order No. _____

* Client should complete all information requested to the right and in the unshaded areas below
DO NOT WRITE IN THE SHADDED AREAS
* Samples will be discarded or returned to the client two weeks after the analytical report has been
mailed, unless prior arrangements have been made

CS 0005275 Job # 60201 Oliver Sample ID #	Sample Location (i.e., influent, outfall 001, MW1, etc.)	Date Collected	Time Collected	Sample Type	Container Type	Preservation	Composite (S)	Grab (S)	# of Containers	Analyses Requested
124686-001	Tetramin Flakes - Lot 121599	5/25/00	3:45 pm	Food Source	Plastic Bag	None		✓	1	Total Metals: Hg, Ag Al, As, Cr, Cd, Cu, Co, Fe, Pb, Ni, Zn Pesticides/PCBs (method 608)
124687-001	TYC Food Mixture - Lot 51900	5/25/00	3:45 pm		AG	NA		✓	1	Total Metals: Hg, Ag Al, As, Cr, Cd, Cu, Co, Fe, Pb, Ni, Zn Pesticides/PCBs (method 608)
124688-001	Brine shrimp Nauplii - Lot 4400	5/25/00	5:00 pm		AG	NA		✓	1	Total Metals: Hg, Ag Al, As, Cr, Cd, Cu, Co, Fe, Pb, Ni, Zn Pesticides/PCBs (method 608)
Special Instructions (See attachment for previous data)										
Received By: (Signature) <u>William A. Zettl</u> Date 5/25/00 Received By: (Signature) <u>William A. Zettl</u> Date 5/25/00 Received By: (Signature) Date										
Received By: (Signature) Date Received By: (Signature) Date Received By: (Signature) Date										