

LR N 2332

Study Title

Growth and Reproduction Toxicity Test with L-13492
and the Freshwater Alga, *Selenastrum capricornutum*

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1050

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Study Completed

August 3, 1995

Sponsor

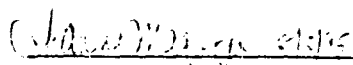
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Environmental Laboratory
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St. Paul, Minnesota 55144

Testing Facility

T.R. Wilbury Laboratories, Inc.
40 Doaks Lane
Marblehead, Massachusetts 01945

I. GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to EPA Good Laboratory Practice Regulations (40 CFR 792), with the following exceptions. The stability of the test substance was assumed but not verified at T.R. Wilbury Laboratories and the date of one of the 3 micropipette calibrations can not be verified from the raw data (the year was apparently entered incorrectly). Deviations from the study protocol are discussed in Section XII of this report.



Jeanne P. Magazu
Study Director

II. QUALITY ASSURANCE STATEMENT

Submitted by: T.R. Wilbury Laboratories, Inc.
40 Doaks Lane
Marblehead, Massachusetts

Certification:

T.R. Wilbury Laboratories, Inc. study number 841-TH, Growth and Reproduction Toxicity Test with L-13492 and the Freshwater Alga, *Selenastrum capricornutum*, was audited by the Quality Assurance Unit for compliance with the protocol, standard operating procedures, and applicable Good Laboratory Practices (U.S. EPA, 1993). The original raw data and a copy of the final report will be archived at T.R. Wilbury Laboratories, Inc. for at least 10 years.

Quality assurance audits were performed and the findings reported to T.R. Wilbury Laboratories management and the study director on the following dates:

Aspect Audited	Audit Date	Reported to Study Director	Reported to Management
Protocol:	6/ 8/95	6/ 9/95	6/9/95
In-life:	6/30/95	6/30/95	8/3/95
Draft Report:	7/11/95	7/11/95	8/3/95
Final Report:	8/ 3/95	8/ 3/95	8/3/95


Arthur P. Paradice
Quality Assurance Officer

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V. SUMMARY

The toxicity of L-13492 to the freshwater alga, *Selenastrum capricornutum*, is described in this final report. The test was conducted for 3M Company for 96 hours from June 26 to 30, 1995, at T.R. Wilbury Laboratories, Inc. in Marblehead, Massachusetts. It was conducted by Jeanne Magazu, Jacqueline Nevius, Peter Kowalski, Keven Stevens, Peter Nevius, Robert Boeri, and Timothy Ward according to the protocol developed for T.R. Wilbury Study Number 841-TH. L-13492 was supplied by the sponsor.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a temperature of $24 \pm 2^\circ\text{C}$. Nominal concentrations of L-13492 were 0 (control), 1.0, 2.0, 4.0, 8.0, and 16 mg/L. The dilution water was sterile enriched media adjusted to a pH of 7.5. Cell counts were made daily with a hemocytometer.

Exposure of algae to the test substance for 96 hours resulted in a median effective concentration (EC50) of 4.9 mg/L L-13492, with 95% confidence limits of 3.5 and 6.7 mg/L. The 96 hour no observed effect concentration (NOEC) is 1.0 mg/L and the lowest observed effect concentration (LOEC) is 2.0 mg/L.

A toxicity test was performed simultaneously with isopropyl alcohol, a component that represents 27% of L-13492. Algal growth was not affected at 4.4 mg/L (the concentration of isopropyl alcohol present in the 16 mg/L L-13492 solutions) or at 1,000 mg/L, indicating that the concentration of isopropyl alcohol in L-13492 can not, by itself, account for the toxicity of L-13492 to algae.

VI. GENERAL INFORMATION

Material Tested: L-13492

Sponsor: 3M Company
Environmental Laboratory
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55144

Test Substance 3M Company
Shipped From: Environmental Laboratory
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55144

Reported Purity: 100%

Study Initiation Date: June 14, 1995 (protocol signed)

Experimental Start Date: June 26, 1995

Experimental Completion Date: June 30, 1995

Study Completion Date: August 3, 1995

Archive Location for
the Biological Raw Data
and a Copy of the Final
Report: T.R. Wilbury Laboratories, Inc.
Marblehead, Massachusetts

VII. INTRODUCTION

This study was sponsored by 3M Company. The objective of the study was to determine the 24, 48, 72, and 96 hour effective concentrations (EC10s, EC50s, EC90s) and no observed effect concentration (NOEC) of the test substance to the freshwater alga, *Selenastrum capricornutum*, under static conditions. The test protocol was based on U.S. EPA methods (EPA, 1993). The report contains sections that describe the methods and materials employed in the study, and results of the investigation. The report also contains an appendix that presents water quality data collected during the test.

VIII. METHODS AND MATERIALS

TEST SUBSTANCE:

The sample of L-13492 (T.R. Wilbury Laboratories Sample Number 502) was delivered to T.R. Wilbury Laboratories on June 14, 1995 in a cardboard box at ambient temperature. It was contained in a 125 ml plastic bottle. The label attached to the bottle included the following information: "L-13492, Hazardous Components/Special Precautions: Isopropyl Alcohol, Originator: S.J. Martin + S.A. Beach, 53-4N-02 + 2-3E-09, 8-5774, 8-7065, 5/4/95, 6/7/95, N2332-1".

L-13492 (a thin, clear liquid) was supplied by 3M Company, St Paul, Minnesota. Prior to use the test substance was stored in the dark at room temperature. Test substance stock solutions were formulated in dilution water without the use of a solvent. The test substance was assumed to have a purity of 100% active ingredient and to be stable under storage and testing conditions. Unused test substance will be returned to the sponsor.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was sterile enriched media (U.S. EPA, 1978; T.R. Wilbury Standard Operating Procedure number 6) adjusted to a target pH of 7.5 with 0.1 N HCl prior to use. Characterization of a representative sample of media is presented in Table 1.

TEST ORGANISM:

Algae used for the test (*Selenastrum capricornutum*, UTEX 1648) was from a culture originally procured from the Culture Collection of Algae at the University of Texas at Austin and delivered to T.R. Wilbury Laboratories on April 11, 1995. The culture was transferred to sterile enriched media identical to media used for this test and maintained at test conditions for at least 14 days before the definitive test. The subsample of algae used to inoculate media at the start of the definitive test came from a 7 day old culture. Identification of the culture organisms was verified using an appropriate taxonomic key.

Table 1. Chemical characterization of a representative sample of test media and water used to formulate test media for the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and L-13492.

Parameter	Unit of Measurement	Detection Limit	Measured Value
pH	pH units	--	7.5
Total Phosphorus	mg/L	0.03	0.46
Nitrate	mg/L as N	0.05	0.08
Chloride	mg/L	1	14
Total Organic Carbon	mg/L	1	ND
Metals			
Arsenic	mg/L	0.01	ND
Cadmium	mg/L	0.0002	0.0002
Copper	mg/L	0.005	ND
Fluoride	mg/L	0.1	ND
Boron	mg/L	0.5	ND
Aluminum	mg/L	0.1	ND
Chromium	mg/L	0.01	ND
Cobalt	mg/L	0.03	ND
Iron	mg/L	0.03	0.08
Lead	mg/L	0.005	ND
Mercury	mg/L	0.0003	ND
Nickel	mg/L	0.03	ND
Silver	mg/L	0.02	ND
Zinc	mg/L	0.02	ND
Organochlorine			
Pesticides	µg/L	0.5	ND
plus Toxaphene	µg/L	2	ND
Organophosphorus			
Pesticides	µg/L	0.52	ND
PCBs	µg/L	0.5	ND

- Notes: 1. The pH was measured in a control test vessel at the start of the test. Total organic carbon, total phosphorus, nitrate, chloride, fluoride, metals, pesticide, and PCB data were collected during February, 1995, as part of routine biannual water quality testing (these analysis were performed by Pace, Inc., Hampton, New Hampshire).
2. ND = not detected at or above the detection limit.

TOXICITY TEST:

Results of a range finding test were used to select the concentrations for the definitive test. The range finding test was performed from June 15 to 19, 1995. At the conclusion of the test the number of cells/ml equalled the following percent of the control: 0.10, and 1.0 mg/L = >89%, 10 mg/L = 4%, 100 mg/L = 2%, and 1,000 mg/L = <1%.

The definitive toxicity test was performed from June 26 to 30, 1995, according to T.R. Wilbury Protocol 841-TH (Growth and Reproduction Toxicity Test with L-13492 and the Freshwater Alga, *Selenastrum capricornutum*). The protocol, which was signed by the study director on June 14, 1995, is based on procedures of the U.S. Environmental Protection Agency (1993). A summary of the test system is presented in Table 2.

The test was conducted at a target temperature of $24 \pm 2^\circ\text{C}$ with five concentrations of test substance and a dilution water control. Nominal concentrations of the test substance were: 0 mg/L (control), 1.0, 2.0, 4.0, 8.0, and 16 mg/L. A 16 mg/L stock solution was formulated on June 26, 1995 by adding 0.016 g L-13492 to sterile enriched media and adjusting the final media volume to 1,000 ml. Appropriate amounts of this stock solution were added directly to dilution water to formulate test media. A test was performed simultaneously with isopropyl alcohol (Mallinckrodt, Lot 3035), a component that represents 27% of L-13492. This test was conducted at 4.4 mg/L (the concentration of isopropyl alcohol in vessels containing 16 mg/L L-13492) and at 1,000 mg/L. A 1,000 mg/L stock solution was formulated on June 26, 1995 by adding 0.5 g isopropyl alcohol to sterile enriched media and adjusting the final media volume to 500 ml.

Algae were distributed among three replicates of each treatment at the rate of 10,000 cells/ml. The test was performed in 250 ml glass Erlenmeyer flasks that contained 100 ml of test solution. Test vessels were randomly arranged on a rotary shaker adjusted to 100 rpm in an incubator during the test (a random numbers table was used to select the location of each vessel). A 24 hour light and 0 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of approximately 350-360 footcandles.

The number of algal cells/ml in each test vessel and the occurrence of relative size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells was determined visually by means of direct microscopic examination with a hemocytometer. Cell counts were made and recorded daily during the 96 hour test. The determination of whether toxic effects were algistatic or algicidal was performed at the conclusion of the toxicity test (0.5 ml of media from each 16 mg/L vessel was transferred to a vessel containing fresh media without L-13492 and incubated for 192 hours under test conditions).

Table 2. Test system summary for the toxicity test with L-13492 and the freshwater alga, *Selenastrum capricornutum*.

Species:	<i>Selenastrum capricornutum</i>
Culture Acclimation Period:	>14 days
Test Duration:	96 hours
Test Vessels:	250 ml glass flasks
Volume of Test Media:	100 ml
Number of Replicate Test Vessels:	3
Inoculum:	10,000 cells per ml
Oscillation Rate:	100 rpm
Incubator Temperature Range:	23.4-23.7°C
Photoperiod:	24 hours light and 0 hours dark.
Light Intensity:	~400 footcandles
Water Quality Measurements:	pH in each test vessel at the beginning and end of the test. Incubator temperature daily. Dilution water analysis is summarized in Table 1.
End Points:	24, 48, 72, and 96 hour EC10, EC50, and EC90; no observed effect concentration.
Acceptability Criteria:	Logarithmic growth at 96 hours and acceptable temperature range.

Temperature of the incubator was measured and recorded daily (thermometer number 2968), and pH (Beckman model pHI 12 meter; instrument number 124) was determined in each test vessel at the beginning and end of the test. The temperature in a representative vessel of water incubated with the test vessels was continuously recorded.

STATISTICAL METHODS:

The average specific growth rate was calculated as the natural log of the number of cells/ml at 24, 48, 72, and 96 hours minus the natural log of the number of cells/ml at 0 hours divided by the exposure period. The percent change from the control was calculated by subtracting the treatment average specific growth rate from the control average specific growth rate, dividing the difference by the average specific growth rate in the control, and multiplying that value by 100.

Results of the toxicity test were interpreted by standard statistical techniques. The statistical method used for calculating 24, 48, 72, and 96 hour EC values (and confidence intervals) was based on a nonlinear regression estimation procedure (Bruce and Versteeg, 1992). All calculations were performed using the average specific growth rates and nominal concentrations of L-13492. The no observed effect concentration (NOEC) was determined using a parametric one-way analysis of variance (ANOVA) and average specific growth rate in each test vessel at the end of the test.

IX. RESULTS

No insoluble material was noted in any test vessel during the test. The algal population in the control test vessels grew well, resulting in an average of 1,249,000 cells/ml after 96 hours (Table 3). Water quality throughout the test was within acceptable limits (Appendix A). The range of incubator temperatures was 23.4 to 23.7°C (Table A.1) and the pH of test media was not affected by the test substance at the beginning of the test (Table A.2).

Biological data generated by the acute toxicity test with L-13492 are presented in Tables 3 and 4. The growth curve for algae exposed to the test substance for 96 hours is presented in Figure 1 (the slope of the dose-response curve is 3.3). The 48, 72, and 96 hour EC10, EC50, and EC90 values for algae exposed to L-13492 are presented in Table 6. The 96 hour EC50 (and associated 95 percent confidence limits) is 4.9 mg/L (3.5 and 6.7 mg/L). No effects (size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells) were noted. The 96 hour no observed effect concentration (NOEC) is 1.0 mg/L and the lowest observed effect concentration (LOEC) is 2.0 mg/L L-13492.

The determination of whether toxic effects were algistatic or algicidal was performed at the conclusion of the toxicity test. A 0.5 ml aliquot of media from each 16 mg/L vessel was transferred to a vessel containing fresh media without L-13492 and incubated for 192 hours under test conditions. Algae increased from 400 cells/mL to 580,000 cells/mL, indicating that the effect of L-13492 at this concentration was algistatic rather than algicidal.

Biological data generated by the exposure of algae to isopropyl alcohol are presented in Table 4. Algal growth was not affected at 4.4 mg/L or 1,000 mg/L, indicating that the concentration of isopropyl alcohol in L-13492 can not, by itself, account for the toxicity of L-13492 to algae.

Table 3. Cell growth data from the acute toxicity test with L-13492 and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of L-13492 (mg/L)	rep.	Number of Cells/ml $\times 10^3$ (hour)				
		0	24	48	72	96
0 (control)	1	10	22	134	538	1,132
	2	10	20	132	568	1,260
	3	10	23	114	614	1,356
	mean	10	22	127	573	1,249
	% control	--	--	--	--	--
1.0	1	10	20	142	606	1,378
	2	10	18	112	592	1,156
	3	10	28	118	610	1,400
	mean	10	22	124	603	1,311
	% control	100	100	98	105	105
2.0	1	10	11	140	178	270
	2	10	18	102	198	252
	3	10	19	136	202	304
	mean	10	16	126	193	275
	% control	100	73	99	34	22
4.0	1	10	26	86	100	106
	2	10	19	78	160	102
	3	10	14	80	104	132
	mean	10	20	81	121	113
	% control	100	91	64	21	9
8.0	1	10	18	44	66	98
	2	10	16	52	72	80
	3	10	21	40	48	74
	mean	10	18	45	62	84
	% control	100	82	35	11	7
16	1	10	12	34	40	24
	2	10	11	38	70	34
	3	10	18	34	38	22
	mean	10	14	35	49	27
	% control	100	64	28	9	2

Table 4. Cell growth data from the acute toxicity test with isopropyl alcohol and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of L-13492 (mg/L)	rep.	Number of Cells/ml x 10 ³ (hour)				
		0	24	48	72	96
0 (control)	1	10	22	134	538	1,132
	2	10	20	132	568	1,260
	3	10	23	114	614	1,356
	mean	10	22	127	573	1,249
	% control	--	--	--	--	--
4.4	1	10	14	112	584	1,236
	2	10	32	128	554	1,270
	3	10	16	144	584	1,064
	mean	10	21	128	574	1,190
	% control	100	95	101	100	95
1,000	1	10	31	136	608	1,370
	2	10	26	144	580	1,338
	3	10	24	106	512	1,334
	mean	10	27	129	567	1,347
	% control	100	123	102	99	108

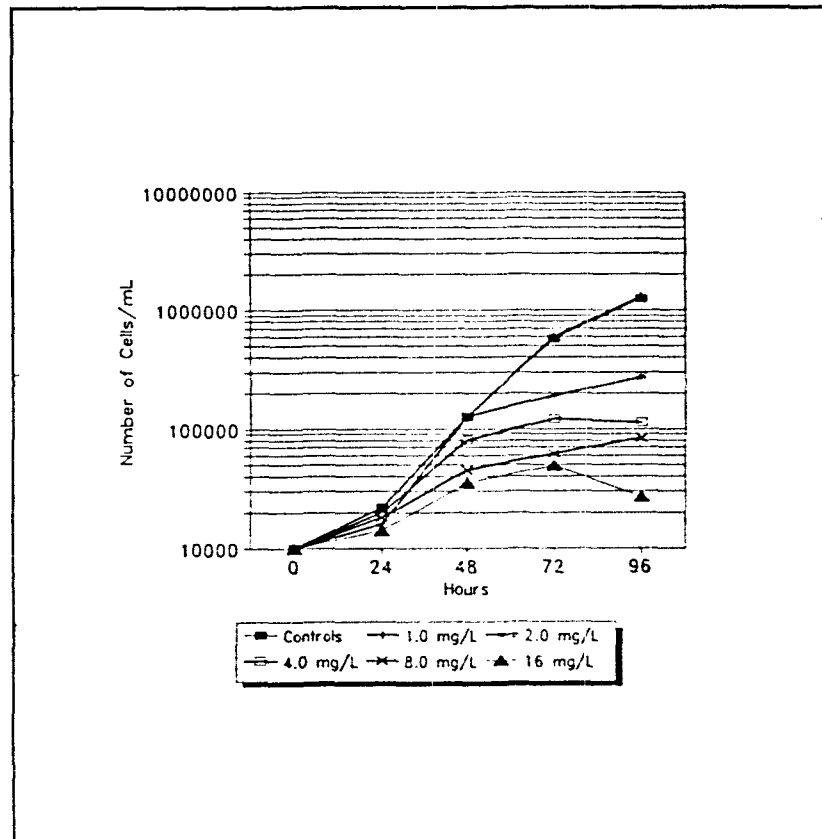


Figure 1. Growth of *Selenastrum capricornutum* exposed to L-13492 for 96 hours.

Table 5. Average specific growth rate and percent change from the control from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and L-13492.

Nominal Concentration of L-13492 (mg/L)	Average Specific Growth Rate				Percent Change From Control			
	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr
0 (control)	0.032	0.053	0.056	0.050	--	--	--	--
1.0	0.032	0.052	0.057	0.051	0	2	-2	0
2.0	0.018	0.053	0.041	0.035	44	0	27	30
4.0	0.027	0.044	0.035	0.025	16	17	37	50
8.0	0.025	0.031	0.025	0.022	22	42	55	56
16	0.012	0.026	0.022	0.010	63	51	61	80

Table 6. Effective concentrations (EC10s, EC50s, EC90s) from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and L-13492.

Time	EC Value (mg/L)	95 Percent Confidence Limits (mg/L)
24 hours	EC10 = <1.0 EC50 = 15 EC90 = >16	<1.0 9.8 - >16 7.7 - >16
48 hours	EC10 = <1.0 EC50 = 14 EC90 = >16	<1.0 8.2 - >16 >16
72 hours	EC10 = <1.0 EC50 = 7.1 EC90 = >16	<1.0 4.7 - 11 >16
96 hours	EC10 = <1.0 EC50 = 4.9 EC90 = >16	<1.0 3.5 - 6.7 >16

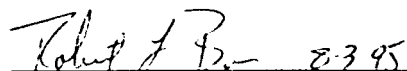
X. REFERENCES

- Bruce, R.D., and Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. Environ. Toxicol. and Chem. Vol.11. No. 10, pp. 1,485-1,494.
- U.S. EPA. 1978. The *Selenastrum capricornutum* Printz Algal Assay Bottle Test. EPA-600/9-78-018. Environmental Research Laboratory, Corvallis, Oregon.
- U.S. EPA. 1993. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Section 797.1050.
- U.S. EPA. 1993. 40 CFR Part 792. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Final Rule.

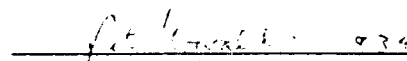
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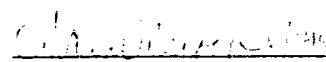
Jeanne P. Magazu
Study Director
Aquatic Toxicologist



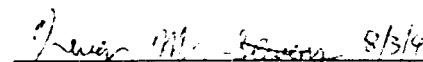
Robert L. Boeri
Coauthor



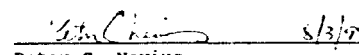
Peter L. Kowalski
Aquatic Toxicologist



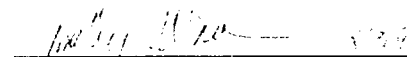
for: Jacqueline M. Nevius
Aquatic Toxicologist



Keven M. Stevens
Biologist



Peter C. Nevius
Biologist



Timothy J. Ward
Author

XII. PROTOCOL AMENDMENTS AND DEVIATIONS

PROTOCOL AMENDMENTS:

The protocol was amended to specify the nominal concentrations for the definitive test and to note that a test would also be conducted with 4.4 and 1,000 mg/L isopropyl alcohol.

PROTOCOL DEVIATIONS:

No deviations were made from the protocol.

Appendix A: WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Temperature measured during the toxicity test with L-13492 and the freshwater alga, *Selenastrum capricornutum*.

Hour of Exposure	Temperature of Incubator (°C)
0	23.6
24	23.6
48	23.4
72	23.4
96	23.7

Table A.2. pH values measured during the toxicity test with L-13492 and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration (mg/L)	Replicate	pH	
		Initial	Final
Test with L-13492			
0 (control)	1	7.5	9.8
	2	7.5	10.4
	3	7.5	10.0
1.0	1	7.5	9.8
	2	7.5	9.9
	3	7.5	9.8
2.0	1	7.5	8.6
	2	7.5	8.3
	3	7.5	8.2
4.0	1	7.4	8.0
	2	7.4	8.0
	3	7.4	7.9
8.0	1	7.4	7.7
	2	7.4	7.7
	3	7.4	7.7
16	1	7.4	7.6
	2	7.4	7.6
	3	7.4	7.6
Test with Isopropyl Alcohol			
4.4	1	7.4	10.0
	2	7.4	10.1
	3	7.4	10.5
1,000	1	7.4	10.4
	2	7.4	9.9
	3	7.4	9.9

LAB REQUEST NO. N2332
 REQUESTOR NAME: SAB/S.J.MARTIN
 DEPARTMENT: 3048
 PROJECT NO: BI703004UQ
 DATE RECEIVED: 06/07/1995
 DESC: L-13492 ECOTOXICITY EVALUATION

CONTRACT LAB(S): TRWIL
 EXP COMP DATE: 08/30/1995
 DATE COMPLETED: 09/02/1995
 PROJECT LEAD:
 PHONE NO:
 3M FAX NO: 651-778-6176

Tests conducted following 40CFR 797; methods 1050, 1300, 1400.

Sample desc.: L-13492, 45% solids, from S. Martin.

SAB 6/7/95

Sample is a clear liquid. Lot confirmed as Lot #2 as per S.J. Martin, of 3M SCD Lab on 7/18/95. SAB

Results from 48-hr Daphnia magna acute toxicity test:

Test Conc., mg/L	Percent Immobilization and/or Mortality	
	24-hrs Exposure	48-hrs Exposure
0 (control)	0	0
13	0	0
22	5	5
36	0	55
60	0	100
100	14 70	100

sab 7/31/95

Results from 96-hr Fathead minnow acute static toxicity test:

Test Conc. mg/L	Percent Mortality			
	24-hrs exp	48-hrs exp	72-hrs exp	96-hrs exp
0 (control)	0	0	0	0
130	0	0	0	0
220	0	0	0	0
360	0	0	0	0
600	0	0	0	0
1000	50	60	70	70

sab 7/31/95

Results from 96-hr algae growth inhibition test:

Test conc. mg/L	Percent change from Control			
	24-hrs exp.	48-hrs exp.	72-hrs exp.	96-hrs exp.
1.0	0	2	-2	0
2.0	44	17	27	30
4.0	16	17	38	50
8.0	22	42	55	56
16	63	51	61	80
EC50	15	14	7.1	4.9
95% C.I.	(9.8 - >16)	(8.2 - >16)	(4.7 - 11)	(3.5 - 6.7)

sab 9/1/95

SAMPLE	DATE	CODE	DESCRIPTION	RESULT	MDL or (95% C.I.)
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* = CONTRACT LAB DATA

04/01/2001

ENVIRONMENTAL LABORATORY
ARCHIVED FINAL REPORT

2

LAB REQUEST NO. N2332

CONTINUED

SAMPLE	DATE	CODE	DESCRIPTION	RESULT	MDL or (95% C.I.)
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1	05/04/95	L-13492	Lot 2		
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TRWIL 48-HR-EC50	*DAPHNIA ACUTE TEST	34 MG/L	(30 - 39)
TRWIL 96-HR-LC50	*FISH ACUTE TEST - 96 HOUR	890 MG/L	(600 - >1000)
TRWIL IC50-ALGAE	*ALGAE ASSAY - IC50	4.9 MG/L	(3.5 - 6.7)

* = CONTRACT LAB DATA