Study Title

Growth and Reproduction Toxicity Test with N2803-4
and the Freshwater Alga, *Selenastrum capricornutum*

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1050

Authors

Timothy J. Ward
Peter L. Kowalski
Robert L. Boeri

Study Completed

March 7, 1996

Sponsor

3M Company
Environmental Laboratory
935 Bush Avenue
Building 2-3E-09
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 under test conditions was assumed but not verified at T.R. Wilbury Laboratories. Deviations from the study protocol are presented in section XII of this report.

Peter L. Kowalski
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 896-TH, Growth and Reproduction Toxicity Test with N2803-4 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). 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:	8/ 4/95	8/ 4/95	8/ 4/95
In-life:	9/26/95	9/26/95	3/ 7/96
Draft Report:	1/18/96	1/18/96	3/ 7/96
Final Report:	3/ 7/96	3/ 7/96	3/ 7/96

3/7/96
Arthur P. Paradice
Quality Assurance Officer

III. TABLE OF CONTENTS

IV. INDEX OF TABLES AND FIGURES

V. SUMMARY

The toxicity of N2803-4 to the freshwater alga, *Selenastrum capricornutum*, is described in this final report. The test was conducted for 3M Company for 96 hours from September 22 to 26, 1995, at T.R. Wilbury Laboratories, Inc. in Marblehead, Massachusetts. It was conducted according to the protocol developed for T.R. Wilbury Study Number 896-TH. N2803-4 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 ± 2°C. Nominal concentrations of N2803-4 were 0 (control), 62, 130, 250, 500, and 1,000 mg/L. The dilution water was sterile enriched media adjusted to a pH of 7.5.

Exposure of algae to the test substance for 96 hours resulted in a median effective concentration (EC50) of 310 mg/L N2803-4 (95% confidence interval = 280 to 350 mg/L) when calculated using the number of cells/ml, and greater than 1,000 mg/L when calculated using the average specific growth rate. The 96 hour no observed effect concentration (NOEC) is 62 mg/L and the lowest observed effect concentration (LOEC) is 130 mg/L when calculated using the number of cells/ml and the NOEC is 500 mg/L and the LOEC is 1,000 mg/L when calculated using the average specific growth rate.

VI. GENERAL INFORMATION

Material Tested:	N2803-4
Sponsor:	3M Company Environmental Laboratory 935 Bush Avenue Building 2-3E-09 St. Paul, Minnesota 55144
Test Substance Shipped From:	3M Company Environmental Laboratory 935 Bush Avenue Building 2-3E-09 St. Paul, Minnesota 55144
Reported Purity:	100%
Study Initiation Date:	August 16, 1995 (protocol signed)
Experimental Start Date:	September 22, 1995
Experimental Completion Date:	September 26, 1995
Study Completion Date:	March 7, 1996
Archive Location for the 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 N2803-4 (T.R. Wilbury Laboratories Sample Number 522) was delivered to T.R. Wilbury Laboratories on August 14, 1995 in a cardboard box at ambient temperature. It was contained in an 50 ml glass jar. The label attached to the bottle included the following information: "N2803-4, (See MSDS)".

N2803-4 (white powder) was supplied by 3M Company, St Paul, Minnesota. Prior to use the test substance was stored in the dark at room temperature. 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 water used to formulate 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 an 8 day old culture. Identification of the culture organisms was verified using an appropriate taxonomic key.

Table 1. Chemical characterization of a representative sample of water used to formulate test media for the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and N2803-4.

Parameter[1]	Unit of Measurement	Detection Limit	Measured Value
Metals			
Aluminum	mg/L	0.1	ND[2]
Arsenic	mg/L	0.01	ND
Boron	mg/L	0.5	ND
Cadmium	mg/L	0.0002	ND
Chromium	mg/L	0.01	ND
Cobalt	mg/L	0.03	ND
Copper	mg/L	0.005	ND
Iron	mg/L	0.03	0.03
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
Nitrate	mg/L as N	0.05	ND
Chloride	mg/L	1	
Fluoride	mg/L	0.1	ND
Total Organic Carbon	mg/L	1	ND
Total Phosphorus	mg/L	0.03	ND
Organochlorine Pesticides	µg/L	0.5	ND
Toxaphene	µg/L	2	ND
Organophosphorus Pesticides	µg/L	0.5	ND
Dimethoate	µg/L	2.0	ND
TEPP	µg/L	2.0	ND
Monocrotophos	µg/L	2.0	ND
PCBs	µg/L	0.5	ND

Notes:
1. Parameters were measured in deionized water (used to formulate dilution water) that was collected during August, 1995 and analyzed by Pace, Inc. as part of routine water quality testing.
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 September 7 to 11, 1995. At the conclusion of the test the number of cells/ml equalled the following percent of the control: 0.10, 1.0, 10, and 100 mg/L = >100% and 1,000 mg/L = 8%.

The definitive toxicity test was performed from September 22 to 26, 1995, according to T.R. Wilbury Protocol 896-TH (Growth and Reproduction Toxicity Test with N2803-4 and the Freshwater Alga, *Selenastrum capricornutum*). The protocol, which was signed by the study director on August 16, 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 ± 2°C with five concentrations of test substance and a dilution water control. Nominal concentrations of the test substance were: 0 mg/L (control), 62, 130, 250, 500, and 1,000 mg/L. A 1,000 mg/L stock solution was formulated on September 22, 1995 by adding 0.5 g N2803-4 to sterile enriched media and adjusting the final media volume to 500 ml. Appropriate amounts of this stock solution were added directly to dilution water to formulate test media.

Algae were distributed among three replicates of each treatment at the rate of approximately 10,000 cells/ml. The test was performed in 250 ml glass Erlenmeyer flasks that contained 50 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 400 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 1,000 mg/L vessel was transferred to a vessel containing fresh media without N2803-4 and incubated for 72 hours under test conditions).

Table 2. Test system summary for the toxicity test with N2803-4 and the freshwater alga, *Selenastrum capricornutum*.

Species:	*Selenastrum capricornutum*
Culture Acclimation Period:	>14 days
Test Duration:	96 hours
Test Vessels:	250 ml glass flasks
Volume of Test Media:	50 ml
Number of Replicate Test Vessels:	3
Inoculum:	≈10,000 cells per ml
Oscillation Rate:	100 rpm
Incubator Temperature Range:	23.5-23.7°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 binomial/interpolation or probit methods (Stephan, 1983) were used for calculating EC10, EC50, and EC90 values. All calculations were performed using the number of cells/ml and the average specific growth rates and nominal concentrations of N2803-4. The no observed effect concentration (NOEC) was determined using a parametric one-way analysis of variance (ANOVA) or a non-parametric Kruskal and Wallis' test and the number of cells/ml and the 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 vessels grew well, resulting in an average of 1,407,000 cells/ml after 96 hours (Table 3). Water quality throughout the test was within acceptable limits (Appendix A). The range of incubator temperatures was 23.5 to 23.7°C (Table A.1). The pH of test media (Table A.2) was slightly affected by the test substance only at 1,000 mg/L at the beginning of the test.

Biological data generated by the acute toxicity test with N2803-4 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 2.5 based on cells/ml at 96 hours). The 72 and 96 hour EC50 values for algae exposed to N2803-4 are presented in Table 5. The 96 hour EC50 (and associated 95 percent confidence limits) is 310 mg/L (280 and 350 mg/L) when calculated using the number of cells per ml, and greater than 1,000 mg/L when calculated using the average specific growth rate. 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 62 mg/L and the lowest observed effect concentration (LOEC) is 130 mg/L N2803-4, when calculated using the number cells/ml, and the NOEC is 500 mg/L and the LOEC is 1,000 mg/L, when calculated using the average specific growth rate.

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 1,000 mg/L vessel was transferred to a vessel containing fresh media without N2803-4 and incubated for 72 hours under test conditions. Algae increased from <10,000 cells/mL to 642,000 cells/mL, indicating that the effect of N2803-4 at this concentration was algistatic rather than algicidal.

Table 3. Cell growth data from the acute toxicity test with N2803-4 and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of N2803-4 (mg/L)	Rep.	Number of Cells/ml x 10^3 (hour) 0	24	48	72	96
0 (control)	1	10	16	142	466	1,544
	2	10	18	154	584	1,320
	3	10	12	180	592	1,356
	mean	10	15	159	547	1,407
	% control	--	--	--	--	--
62	1	10	17	162	602	1,446
	2	10	15	184	576	1,272
	3	10	15	168	586	1,616
	mean	10	16	171	588	1,445
	% control	100	107	108	107	103
130	1	10	16	160	556	1,080
	2	10	19	144	598	1,184
	3	10	10	136	584	1,124
	mean	10	15	147	579	1,129
	% control	100	100	92	106	80
250	1	10	14	138	590	762
	2	10	15	174	552	752
	3	10	11	140	528	744
	mean	10	13	151	557	753
	% control	100	87	95	102	54
500	1	10	11	92	274	354
	2	10	<10	104	234	484
	3	10	13	110	300	492
	mean	10	11	102	286	443
	% control	100	73	64	52	31
1,000	1	10	<10	90	106	200
	2	10	<10	62	134	216
	3	10	10	72	124	128
	mean	10	<10	75	121	181
	% control	100	<67	47	22	13

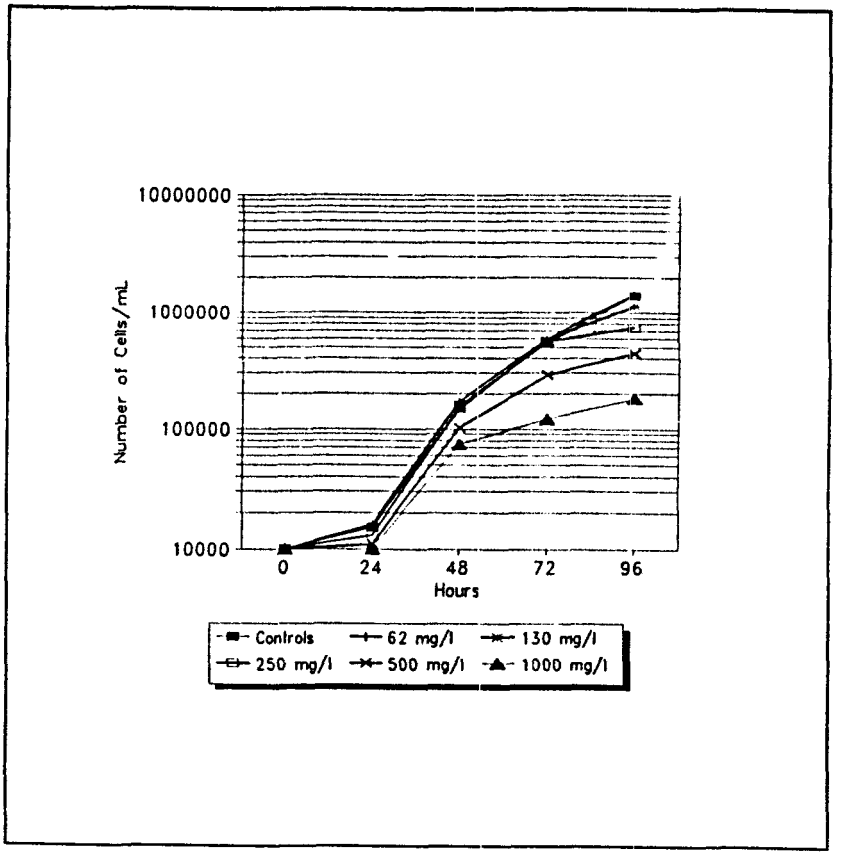

Figure 1. Growth of *Selenastrum capricornutum* exposed to N2803-4 for 96 hours.

Table 4. Average specific growth rate and percent change from the control from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and N2803-4.

Nominal Concentration of N2803-4 (mg/L)	Average Specific Growth Rate				Percent Change From Control			
	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr
0 (control)	0.017	0.058	0.056	0.052	--	--	--	--
62	0.020	0.059	0.057	0.052	-18	-2	-2	0
130	0.017	0.056	0.056	0.049	0	3	0	6
250	0.011	0.057	0.056	0.045	35	2	0	13
500	0.004	0.048	0.047	0.039	76	17	16	25
1,000	0.000	0.042	0.035	0.030	100	28	37	42

Table 5. Effective concentrations (EC10s, EC50s, EC90s) from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and N2803-4.

Time	EC Value (mg/L)	95 Percent Confidence Limits (mg/L)
Calculated Using the Number of Cells/ml		
72 hours	EC10 = 310	110 - 440
	EC50 = 520	250 - 1,000
	EC90 = >1,000	800 - >1,000
96 hours	EC10 = 97	77 - 120
	EC50 = 310	280 - 350
	EC90 = 1,000	830 - >1,000
Calculated Using the Average Specific Growth Rate		
72 hours	EC10 = 470	380 - 550
	EC50 = >1,000	>1,000
	EC90 = >1,000	>1,000
96 hours	EC10 = 220	160 - 280
	EC50 = >1,000	950 - >1,000
	EC90 = >1,000	>1,000

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.

Stephan, C.E. 1983. Computer Program for Calculation of LC50 Values. Personal communication.

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.

XI. SIGNATURE PAGE

Peter L. Kowalski 3.7.96
Peter L. Kowalski
Study Director
Aquatic Toxicologist

Jeanne P. Magazu 3/7/96
Jeanne P. Magazu
Aquatic Toxicologist

Jacqueline M. Nevius 3/7/96
Jacqueline M. Nevius
Biologist

Keven M. Stevens 3/7/96
Keven M. Stevens
Biologist

Robert L. Boeri 3-7-96
Robert L. Boeri
Coauthor

Timothy J. Ward 3/7/96
Timothy J. Ward
Author

XII. PROTOCOL AMENDMENTS AND DEVIATIONS

PROTOCOL AMENDMENTS:

The protocol was amended to specify the nominal concentrations for the definitive study. No other amendments were made to the protocol.

PROTOCOL DEVIATIONS:

Two nominal concentrations were slightly different than the ones listed in the protocol. This deviation did not affect the outcome of this test and no other deviations were made from the protocol.

Appendix A: WATER QUALITY DATA FROM THE TOXICITY TEST

Table A.1. Temperature measured during the toxicity test with N2803-4 and the freshwater alga, *Selenastrum capricornutum*.

Hour of Exposure	Temperature of Incubator (°C)
0	23.5
24	23.6
48	23.6
72	23.7
96	23.5

Table A.2. pH values measured during the toxicity test with N2803-4 and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of N2803-4 (mg/L)	Replicate	pH	
		Initial	Final
0 (control)	1	7.5	8.2
	2	7.5	9.6
	3	7.5	9.6
65	1	7.5	8.9
	2	7.5	9.0
	3	7.5	8.6
130	1	7.5	6.4
	2	7.5	6.5
	3	7.5	6.5
250	1	7.5	5.8
	2	7.5	5.4
	3	7.5	5.4
500	1	7.5	5.1
	2	7.5	5.0
	3	7.5	5.0
1,000	1	7.4	5.4
	2	7.3	5.4
	3	7.3	5.5

LAB REQUEST NO. N2803
REQUESTOR NAME: SAB/GOEBEL/PICKETT
DEPARTMENT: 3048
PROJECT NO: BI703004UQ
DATE RECEIVED: 08/01/1995
DESC: FC-143 AND RELATED PRODUCTS ECOTOX WORK

CONTRACT LAB(S): TRWIL
EXP COMP DATE: 10/04/1995
DATE COMPLETED: 03/27/1996
PROJECT LEAD:
PHONE NO:
3M FAX NO: 651-778-6176

Samples received from S.J. Martin.

Samples 1-5 sent to TRWIL on 8/11/95. An additional 4L of sample 5 sent on 8/22/95.

Sample 1 = Tetrabutyl ammonium hydroxide, 40% in water.
Sample 2 = L-13492 (45% solids, 27% water, 27% IPA)
Sample 3 = FC-26, 100% solids. Dissolve in 50:50 water:IPA solution.
Sample 4 = FC-143, 100% solids.
Sample 5 = L-13856, 15% solids.
Sample 6 = L-13856, diluted by a factor of 15, tested as an effluent.

Sample #1 (Tetrabutylammonium hydroxide) - a new bottle was ordered from Aldrich because TR Wilbury needed more sample. This was lot 05922BN, red label, cat. # 17,878,0.
sab 9/11/95

NOTE: All data for sample 3 (FC-26) is reported in terms of FC-26, and not as a 50/50 mixture with isopropanol. sab 11/21/95

NOTE: For sample #5, only a range-finding test was run for toxicity to algae. The requestor canceled the request for testing so no definitive test was run. The results for the range finder are as follows:

Test Conc. mg/l	Percent Difference in Avg. Specific Growth Rate 96-hrs Expsure
0.1	2
1.0	18
10	4
100	32
1000	100
EC50 (95% C.I.)	160 (100 - 1000) mg/L.

sab 2/16/96

For N2803-6, the testing was canceled before a definitive study with fish could be completed. Results reflect testing with a single concentration of 13% v/v, which showed no mortality after 96-hours.
sab 3/14/96

SAMPLE	DATE	CODE	DESCRIPTION	RESULT	MDL or (95% C.I.)
1	08/11/95	TB NH4OH	Lot 10317KQ		
TRWIL 48-HR-EC50			*DAPHNIA ACUTE TEST	27 MG/L	(23 - 32)

LAB REQUEST NO. N2803 CONTINUED

SAMPLE	DATE	CODE	DESCRIPTION	RESULT	MDL or (95% C.I.)
1	08/11/95	TB NH4OH	Lot 10317KQ		
TRWIL	96-HR-LC50	*FISH ACUTE TEST - 96 HOUR		720 MG/L	(600 - 1000)
TRWIL	IC50-ALGAE	*ALGAE ASSAY - IC50		3.7 MG/L	(1.8 - 7.7)
2		L-13492	Lot 2		
TRWIL	48-HR-EC50	*DAPHNIA ACUTE TEST		62 MG/L	(36-100)
TRWIL	96-HR-LC50	*FISH ACUTE TEST - 96 HOUR		960 MG/L	(830->1000)
TRWIL	IC50-ALGAE	*ALGAE ASSAY - IC50		8.4 MG/L	(5.9 - 14)
3		FC-26	Lot 135		
TRWIL	48-HR-EC50	*DAPHNIA ACUTE TEST		360 MG/L	(300 - 500)
TRWIL	96-HR-LC50	*FISH ACUTE TEST - 96 HOUR		140 MG/L	(110 - 180)
TRWIL	IC50-ALGAE	*ALGAE ASSAY - IC50		90 MG/L	(63 - 130)
4		FC-143	Lot 427		
TRWIL	48-HR-EC50	*DAPHNIA ACUTE TEST		720 MG/L	(600 - 1000)
TRWIL	96-HR-LC50	*FISH ACUTE TEST - 96 HOUR		740 MG/L	(660 - 830)
TRWIL	IC50-ALGAE	*ALGAE ASSAY - IC50		>1000 MG/L	(-)
5	08/16/95	L-13856	As-is		
TRWIL	48-HR-EC50	*DAPHNIA ACUTE TEST		190 MG/L	(130-600)
TRWIL	96-HR-LC50	*FISH ACUTE TEST - 96 HOUR		>1000 MG/L	(-)
TRWIL	IC50-ALGAE	*ALGAE ASSAY - IC50		SEE COMMENT ABOVE	
6		L-13856	1:15 diluted		
TRWIL	48-HR-EC50	*DAPHNIA ACUTE TEST		1.2 % V/V	(1.0 - 1.7)
TRWIL	96-HR-LC50	*FISH ACUTE TEST - 96 HOUR		>13 % V/V	(-)
TRWIL	IC50-ALGAE	*ALGAE ASSAY - IC50		TESTING CANCELED	

* = CONTRACT LAB DATA