Study Title

Growth and Reproduction Toxicity Test with N2803-2
and the Freshwater Alga, *Selenastrum capricornutum*

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1050

Authors

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Study Completed

November 28, 1995

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 was assumed but not verified at T.R. Wilbury Laboratories. Deviations from the study protocol are discussed in Section XII of this report.

Peter L. Kowalski 11-3-95
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 890-TH, Growth and Reproduction Toxicity Test with N2803-2 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/ 3/95	8/ 4/95	8/ 4/95
In-life:	9/22/95	9/22/95	11/28/95
Draft Report:	10/25/95	10/25/95	11/28/95
Final Report:	11/28/95	11/28/95	11/28/95

Arthur P. Paradice 11/28/95
Arthur P. Paradice
Quality Assurance Officer

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V. SUMMARY

The toxicity of N2803-2 to the freshwater alga, *Selenastrum capricornutum*, is described in this final report. The test was conducted for 3M Company for 96 hours from September 18 to 22, 1995, at T.R. Wilbury Laboratories, Inc. in Marblehead, Massachusetts. It was conducted by Peter Kowalski, Jeanne Magazu, Jacqueline Nevius, Keven Stevens, Peter Nevius, Robert Boeri, and Timothy Ward according to the protocol developed for T.R. Wilbury Study Number 890-TH. N2803-2 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 N2803-2 were 0 (control), 0.99, 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 2.9 mg/L N2803-2, (95% confidence limits = 1.0 to 7.7 mg/L) when the value was calculated using the number of algal cells/ml, and 8.4 mg/L (95% confidence interval = 5.9 to 14 mg/L) when the value was calculated using the average specific growth rate. The 96 hour no observed effect concentration (NOEC) is 0.99 mg/L and the lowest observed effect concentration (LOEC) is 2.0 mg/L when calculated using the number of cells/ml and the NOEC is 2.0 mg/L and the LOEC is 4.0 mg/L when calculated using the growth rate.

VI. GENERAL INFORMATION

Material Tested:	N2803-2
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 18, 1995
Experimental Completion Date:	September 22, 1995
Study Completion Date:	November 28, 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 N2803-2 (T.R. Wilbury Laboratories Sample Number 520) was delivered to T.R. Wilbury Laboratories on August 14, 1995 in a cardboard box at ambient temperature. It was contained in a 50 ml glass jar. The label attached to the jar included the following information: "N2803-2, See MSDS".

N2803-2 (a 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*.

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. Metals, nitrate, chloride, fluoride, total organic carbon, total phosphorus, organochlorine pesticides, organophosphorus pesticides, and PCBs 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 August 30 to September 3, 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 = >98%, 10 mg/L, 100 mg/L, and 1,000 mg/L = <1%.

The definitive toxicity test was performed from September 18 to 22, 1995, according to T.R. Wilbury Protocol 890-TH (Growth and Reproduction Toxicity Test with N2803-2 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), 0.99, 2.0, 4.0, 8.0, and 16 mg/L. A 160 mg/L stock solution was formulated on September 18, 1995 by adding 0.080 g N2803-2 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 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 380 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 N2803-2 and incubated for 144 hours under test conditions).

Table 2. Test system summary for the toxicity test with N2803-2 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:	23.4-23.7°C
Photoperiod:	24 hours light and 0 hours dark.
Light Intensity:	≈380 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 pHT 4 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 probit method was used for calculating 24, 48, 72, and 96 hour EC values (and confidence intervals). The probit method was use despite the occasional cautionary note because EC50 values computed by this method were similar to values computed with other methods (Stephan, 1983). The calculations were performed using both the number of cells/ml and the average specific growth rates. The no observed effect concentration (NOEC) was determined using a parametric one-way analysis of variance (ANOVA) 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 test vessels grew well, resulting in an average of 3,227,000 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 N2803-2 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 4.1). The 24, 48, 72, and 96 hour EC10, EC50, and EC90 values for algae exposed to N2803-2 are presented in Table 5. Exposure of algae to the test substance for 96 hours resulted in a median effective concentration (EC50) of 2.9 mg/L N2803-2, (95% confidence limits = 1.0 to 7.7 mg/L) when the value was calculated using the number of algal cells/ml, and 8.4 mg/L (95% confidence interval = 5.9 to 14 mg/L) when the value was calculated using the average specific growth rate. The 96 hour no observed effect concentration (NOEC) is 0.99 mg/L and the lowest observed effect concentration (LOEC) is 2.0 mg/L when calculated using the number of cells/ml and the NOEC is 2.0 mg/L and the LOEC is 4.0 mg/L when calculated using the 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 16 mg/L vessel was transferred to a vessel containing fresh media without N2803-2 and incubated for 144 hours under test conditions. The number of cells/ml equalled 190,000 cells/mL, indicating that the effect of N2803-2 at this concentration was algistatic rather than algicidal.

Table 3. Cell growth data from the acute toxicity test with N2803-2 and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of N2803-2 (mg/L)	rep.	Number of Cells/ml x 10^3 (hour) 0	24	48	72	96
0 (control)	1	10	116	480	2,040	3,180
	2	10	88	388	1,760	3,620
	3	10	94	364	1,680	2,880
	mean	10	99	411	1,827	3,227
	% control	--	--	--	--	--
0.99	1	10	84	286	1,950	3,480
	2	10	98	368	1,840	3,600
	3	10	112	450	2,010	3,820
	mean	10	98	368	1,937	3,633
	% control	100	99	90	106	113
2.0	1	10	92	376	1,120	2,940
	2	10	96	370	1,010	2,540
	3	10	84	366	850	1,840
	mean	10	91	371	993	2,440
	% control	100	92	90	54	76
4.0	1	10	112	240	496	492
	2	10	86	252	432	714
	3	10	96	236	512	536
	mean	10	98	243	480	581
	% control	100	99	59	26	18
8.0	1	10	80	176	262	168
	2	10	84	240	254	180
	3	10	76	188	246	158
	mean	10	80	201	254	169
	% control	100	81	49	14	5
16	1	10	48	114	82	58
	2	10	44	102	80	56
	3	10	36	118	92	70
	mean	10	43	111	85	61
	% control	100	43	27	5	2

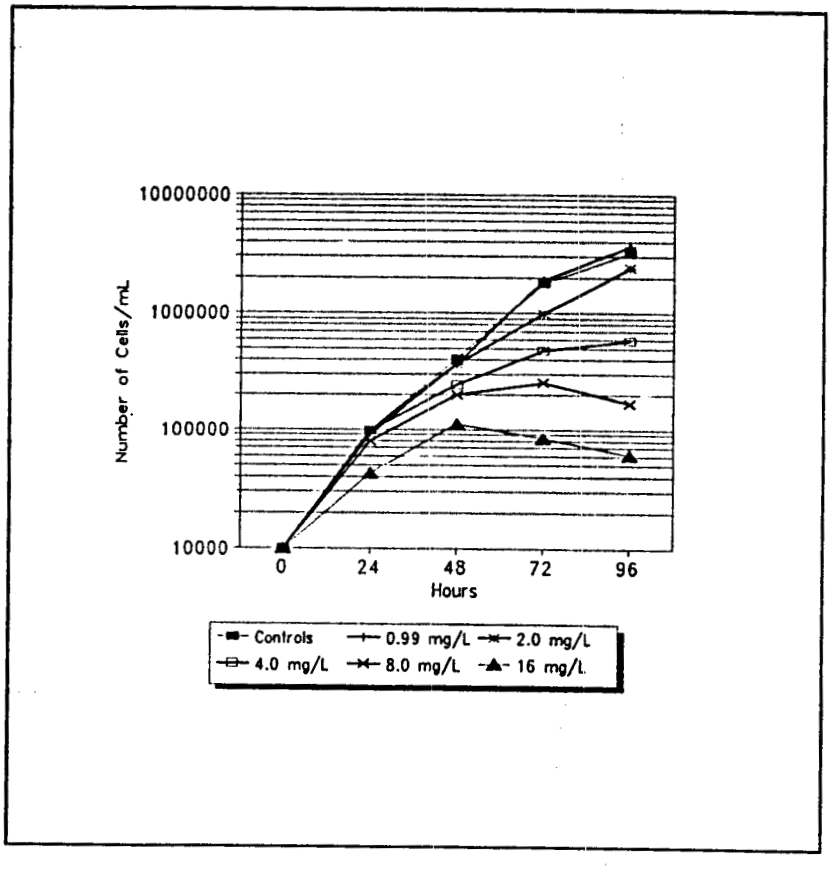

Figure 1. Growth of *Selenastrum capricornutum* exposed to N2803-2 for 96 hours.

Table 4. Average specific growth rate and percent change from the control from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and N2803-2.

Nominal Concentration of N2803-2 (mg/L)	Average Specific Growth Rate				Percent Change From Control			
	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr
0 (control)	0.096	0.077	0.072	0.060	--	--	--	--
0.99	0.095	0.075	0.073	0.061	1	3	-1	-2
2.0	0.092	0.075	0.064	0.057	4	3	11	5
4.0	0.095	0.066	0.054	0.042	1	14	25	30
8.0	0.087	0.063	0.045	0.029	9	18	38	52
16	0.061	0.050	0.030	0.019	36	35	58	68

Table 5. Effective concentrations (EC10s, EC50s, EC90s) from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and N2803-2.

Time	EC Value (mg/L)	95 Percent Confidence Limits (mg/L)
Calculated Using Cells/ml		
24 hours	EC10 = 4.1	0 - 9.0
	EC50 = >16	7.7 - >16
	EC90 = >16	>16
48 hours	EC10 = 1.2	<0.99 - 1.6
	EC50 = 7.1	6.0 - 8.6
	EC90 = >16	>16
72 hours	EC10 = <0.99	<0.99 - 1.9
	EC50 = 2.8	1.1 - 5.8
	EC90 = 8.4	4.5 - >16
96 hours	EC10 = 1.4	<0.99 - 2.4
	EC50 = 2.9	1.0 - 7.7
	EC90 = 6.0	3.5 - >16
Calculated Using Growth Rate		
24 hours	EC50 = >16	--
48 hours	EC50 = >16	--
72 hours	EC10 = 2.2	1.6 - 2.8
	EC50 = 11	9.4 - 15
	EC90 = >16	>16
96 hours	EC10 = 2.3	<0.99 - 3.6
	EC50 = 8.4	5.9 - 14
	EC90 = >16	>16

X. REFERENCES

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

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XII. PROTOCOL AMENDMENTS AND DEVIATIONS

PROTOCOL AMENDMENTS:

The protocol was amended to specify the nominal concentrations for the definitive test.

PROTOCOL DEVIATIONS:

Concentration #1 is 0.99 mg/L rather than the 1 mg/L specified in Protocol Amendment #1. This deviation did not affect the outcome of the test and no other deviations were made from the protocol.

Appendix A: WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Temperature measured during the toxicity test with N2803-2 and the freshwater alga, *Selenastrum capricornutum*.

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

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

Nominal Concentration (mg/L)	Replicate	pH	
		Initial	Final
Test with N2803-2			
0 (control)	1	7.5	10.7
	2	7.5	10.8
	3	7.5	10.8
0.99	1	7.5	10.4
	2	7.5	10.7
	3	7.5	10.5
2.0	1	7.5	9.3
	2	7.5	9.1
	3	7.5	9.0
4.0	1	7.5	8.7
	2	7.5	8.7
	3	7.5	8.7
8.0	1	7.5	8.6
	2	7.5	8.5
	3	7.5	8.5
16	1	7.5	8.4
	2	7.5	8.4
	3	7.5	8.4

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