

Study Title

Acute Toxicity of N2803-2 to the
Fathead Minnow, *Pimephales promelas*

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1400

Authors

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Study Completed

November 16, 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 exception. The stability of the test substance was assumed but not verified at T.R. Wilbury Laboratories. Deviations from the study protocol and protocol amendments are presented in Section XII of this report.

Peter L. Kowalski 111645
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 888-TH, Acute Toxicity of N2803-2 to the Fathead Minnow, *Pimephales promelas*, 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:	9/12/95	9/12/95	11/16/95
Draft Report:	9/22/95	9/22/95	11/16/95
Final Report:	11/16/95	11/16/95	11/16/95


Arthur P. Paradice
Quality Assurance Officer

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V. SUMMARY

The acute toxicity of N2803-2 to the fathead minnow, *Pimephales promelas*, is described in this final report. The test was conducted for 3M Company for 96 hours from September 8 to 12, 1995, at T.R. Wilbury Laboratories, Inc. in Marblehead, Massachusetts. It was conducted by Peter L. 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 888-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 21.6 to 22.0°C. The dilution water was filtered deionized water collected at Marblehead, Massachusetts and adjusted to a hardness of 40 mg/L as CaCO₃. Aeration was initiated at 72 hours to maintain dissolved oxygen concentrations above an acceptable level.

Nominal concentrations of N2803-2 were 0 (control), 160, 250, 400, 630, and 1,000 mg/L. Fathead minnows used in the test were procured from a commercial supplier and acclimated to test conditions at T.R. Wilbury Laboratories. After 96 hours of exposure the control fish had an average wet weight (blotted dry) of 0.36 g and an average total length of 34.9 mm. All fish were in good condition at the beginning of the study.

Exposure of fathead minnows to the test substance resulted in a 96 hour median lethal concentration (LC50) of 960 mg/L N2803-2, with a 95% confidence interval of 830 to >1,000 mg/L. The 96 hour no observed effect concentration (NOEC) is 630 mg/L.

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
367 Grove Street
Building 53-5S-02
St. Paul, Minnesota 55101

Reported Purity: 100%

Study Initiation Date: August 16, 1995 (protocol signed)

Experimental Start Date: September 8, 1995

Experimental Completion Date: September 12, 1995

Study Completion Date: November 16, 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, St Paul, Minnesota. The objective of the study was to determine the 24, 48, 72, and 96 hour median lethal concentrations (LC50s) of the test substance to the fathead minnow, *Pimephales promelas*, a freshwater fish, under static conditions. The test protocol was based on U.S. EPA methods (U.S. 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 529) was delivered to T.R. Wilbury Laboratories on August 29, 1995 in a 5 gallon metal can within a cardboard box at ambient temperature. The test substance was contained in an approximately 150 ml plastic jar. The attached label included the following information: "N2803-2, S. Beach, 2-3E-09, (612) 778-7452, 8/22/95".

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 deionized water collected at T.R. Wilbury Laboratories in Marblehead, Massachusetts. Water was adjusted to a hardness of 40-48 mg/L as CaCO₃ and stored in 500-gallon polyethylene tanks where it was aerated and continuously passed through a particle filter, ultraviolet sterilizer, and activated carbon. Results of chemical analysis of a representative sample of water are presented in Table 1.

TEST ORGANISM:

Juvenile fathead minnows, *Pimephales promelas*, employed as test organisms were from a single source and were procured from Aquatic Biosystems, Fort Collins, Colorado on August 10, 1995. The species of the fish was verified using an appropriate taxonomic key.

Table 1. Chemical characterization of a representative sample of dilution water used for the toxicity test with fathead minnows, *Pimephales promelas*, and N2803-2.

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	ND
Fluoride	mg/L	0.1	ND
Total Organic Carbon	mg/L	1	ND
Total Phosphorus	mg/L	0.03	ND
Organochlorine Pesticides			
Toxaphene	µg/L	0.5	ND
	µg/L	2	ND
Organophosphorus Pesticides			
Dimethoate	µg/L	0.5	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.

Prior to testing the fish were maintained under flow through conditions in a 270 liter fiberglass tank or a 40 liter glass aquarium. During acclimation fish were not treated for disease, they were free of apparent sickness, injuries, and abnormalities at the beginning of the test, and mortality during the final 48 hours of acclimation was less than 3%.

During the 14 day period before the start of the test the acclimation temperature range was 21.0 to 22.3°C and the dissolved oxygen concentration was always at least 8.6 mg/L. During acclimation fish were fed a dry commercial food (Tetra Min® Staple Food, Lot # TM05) daily except during the 48 hours immediately preceding the test. Fish were not fed during the test. At the conclusion of the test the control fish had an average total length of 34.9 mm and an average wet weight of 0.36 g (loading during the definitive toxicity test was 0.24 g/L).

TOXICITY TESTING:

A static screening test was conducted from August 18 to 22, 1995 with five concentrations of test substance and a dilution water control. Nominal concentrations of N2803-2 were 0.10, 1.0, 10, 100, and 1,000 mg/L. After 96 hours of exposure there was 100% survival in the control and at 0.10, 1.0, 10, and 100 mg/L, and 0% survival at 1,000 mg/L.

The definitive toxicity test was performed from September 8 to 12, 1995, according to T.R. Wilbury Protocol 888-TH (Acute Toxicity of N2803-2 To The Fathead Minnow, *Pimephales promelas*). 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 22 ± 2°C with five concentrations of test substance and a dilution water control. Nominal concentrations of the test substance were: 0 mg/L (control), 160, 250, 400, 630, and 1,000 mg/L. Appropriate amounts of test substance were added directly to dilution water in test vessels to formulate test media without the use of a solvent.

Twenty fathead minnows were indiscriminately and equally distributed among two replicates of each treatment. The test was performed in 20 liter glass aquaria that contained 15 liters of test solution (water depth was approximately 18 cm). Test vessels were randomly arranged in a water bath during the 96 hour test (a random numbers table was used to select the location of each vessel) and they were loosely covered. A 16 hour light and 8 hour dark photoperiod with a 15 minute transition period was automatically maintained with cool-white fluorescent lights that provided a light intensity of 25 footcandles. Aeration was initiated after the 72 hour dissolved oxygen concentration measurements to maintain dissolved oxygen concentrations above acceptable levels.

Table 2. Test system summary for the toxicity test with N2803-2 and fathead minnows, *Pimephales promelas*.

Species:	<i>Pimephales promelas</i>
Age/Size of Test Organisms:	Juveniles/ $\approx$ 0.36 grams, wet weight
Test Duration:	96 hours
Test Vessels:	20 liter glass aquaria
Depth of Test Media:	18 cm
Number of Replicate Test Vessels:	2
Organisms per Replicate:	10
Temperature Range:	21.6-22.0°C
Minimum Dissolved oxygen:	4.6 mg/L (prior to aeration)
Photoperiod:	16 hours light and 8 hours dark with a 15 minute transition period.
Light Intensity:	25 footcandles
Water Quality Measurements:	Dissolved oxygen, pH, temperature, and conductivity daily in each test vessel. Temperature measured at least every hour in 1 test vessel. Hardness, alkalinity, acidity, and particulate matter and TOC in the dilution water. Dilution water analysis is summarized in Table 1.
End Points:	24, 48, 72, and 96 hour LC50; 96 hour NOEC.
Acceptability Criteria:	$\leq$ 10% control mortality and acceptable temperature range.

The number of surviving organisms and the occurrence of sublethal effects (immobilization, loss of equilibrium, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24, 48, 72, and 96 hours (mortality and sublethal effects were monitored twice each day). Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument numbers 1), pH (Beckman model PHI 12 meter; instrument number 124), conductivity (Cole Parmer meter, instrument number 94), and temperature (Beckman model PHI 12 meter; instrument number 124, probe PHT-1) were measured and recorded daily in each test chamber that contained live animals. The temperature in one test vessel was recorded at least hourly during the test.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate the median lethal concentrations (LC50s). All calculations were performed by the author using nominal concentrations of test substance and the number of live fathead minnows. The no observed effect concentration is the highest concentration of test substance that did not cause any toxicant-related mortality or sublethal effects.

IX. RESULTS

Insoluble material was not noted during the test. Biological and water quality data generated by the acute toxicity test are presented in Table 3 and Appendix A, respectively. The measured concentrations of dissolved oxygen ranged from 4.6 to 8.8 mg/L (aeration was initiated after the 72 hour dissolved oxygen determinations), the temperature ranged from 21.6 to 22.0°C, conductivity ranged from 140 to 180 μ mhos/cm, and pH ranged from 7.4 to 7.8. One hundred percent survival occurred in the control exposure and these fathead minnows did not exhibit any sublethal effects.

The 24, 48, 72, and 96 hour LC50s for fathead minnows exposed to N2803-2 are presented in Table 4. The 96 hour LC50 for fathead minnows exposed to N2803-2 is 960 mg/L, with a 95% confidence interval of 830 to >1,000 mg/L. The slope of the 96 hour dose response curve is 7.3. The 96 hour no observed effect concentration (NOEC) is 630 mg/L.

Table 3. Survival and sublethal effect data from the toxicity test with fathead minnows, *Pimephales promelas*, and N2803-2.

Nominal Concentration of N2803-2 (mg/L)	Rep.	Number alive					Number affected				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
160	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
250	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
400	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
630	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	8	0	0	0	0	0
1,000	1	10	6	4	3	3	0	0	0	0	0
	2	10	8	7	6	6	0	0	0	0	0

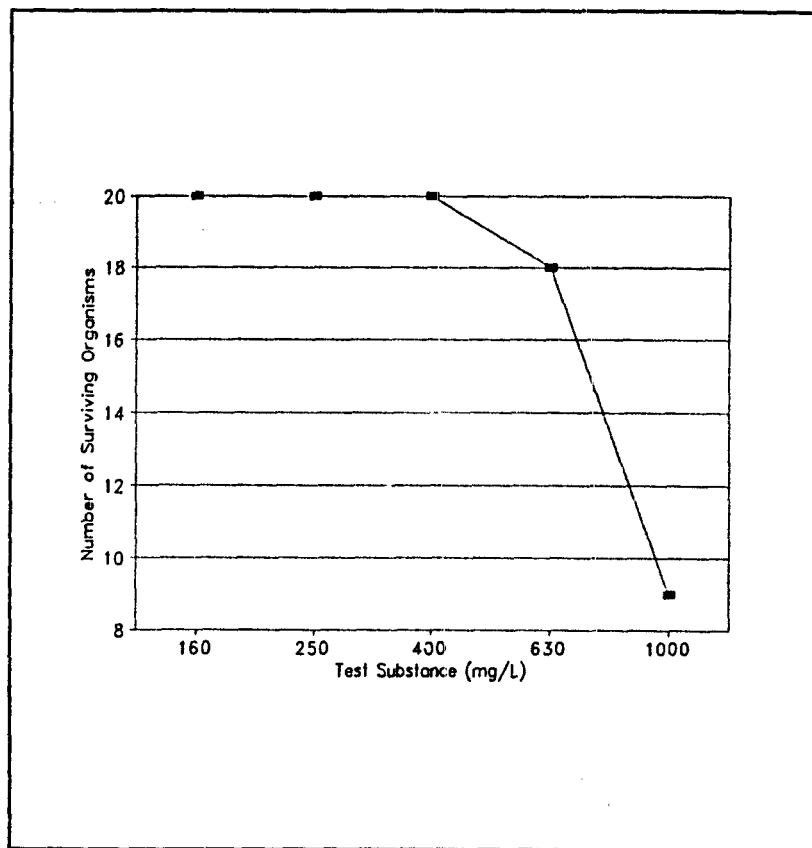


Figure 1. Survival of fathead minnows, *Pimephales promelas*, exposed to N2803-2 for 96 hours.

Table 4. Median lethal concentrations (LC50s) from the toxicity test with fathead minnows, *Pimephales promelas*, and N2803-2.

Exposure period	LC50	95 percent confidence limit	Calculation method
24 hours	>1,000 mg/L	--	--
48 hours	>1,000 mg/L	--	--
72 hours	970 mg/L	630 to >1,000 mg/L	Binomial
96 hours	960 mg/L	830 to >1,000 mg/L	Probit

X. REFERENCES

ASTM. 1986. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E-729-80. In Annual Book of Standards.

Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.

U.S. EPA. 1993. Toxic Substances Control Act Test Guidelines; Final Rules. Section 797.1400.

U.S. EPA. 1993. 40 CFR Part 792. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards. Code of Federal Regulations, Title 40, July 1, 1993.

XI. SIGNATURE PAGE

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Author

XII. PROTOCOL AMENDMENTS AND DEVIATIONS

PROTOCOL AMENDMENTS:

The protocol was amended to specify the nominal concentrations of test substance for the definitive test and the source of the test organisms.

PROTOCOL DEVIATIONS:

The dissolved oxygen concentrations were not always maintained above 60% saturation (aeration was initiated as soon as dissolved oxygen concentrations below 60% saturation were observed). 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. Dissolved oxygen concentration, conductivity, pH, and temperature measured during the toxicity test with fathead minnows, *Pimephales promelas*, and N2803-2.

Nominal Concentration of N2803-2 (mg/L)	Rep.	Dissolved Oxygen (mg/L)						Conductivity (µmhos/cm)				
		0 hr	24 hr	48 hr	72 hr		96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
					b	a						
0 (control)	1	8.4	7.1	6.6	6.3	7.1	8.6	150	140	150	150	150
	2	8.4	7.2	6.7	6.3	7.2	8.6	150	150	150	150	150
160	1	8.4	7.0	6.6	6.2	7.0	8.4	150	140	150	150	150
	2	8.4	6.9	6.6	6.3	7.2	8.7	150	140	150	150	150
250	1	8.4	6.9	6.5	4.9	6.8	8.8	150	150	150	150	150
	2	8.4	6.9	6.4	5.8	7.0	8.5	150	150	150	150	150
400	1	8.4	6.7	6.2	5.2	6.6	8.3	150	150	150	150	160
	2	8.4	6.8	6.2	5.3	6.9	8.6	150	150	150	150	160
630	1	8.4	6.6	5.8	4.6	6.8	8.6	160	150	150	150	160
	2	8.4	6.6	5.8	4.7	7.1	7.8	160	160	160	150	170
1,000	1	8.4	6.8	5.6	4.8	6.7	8.0	170	170	170	170	180
	2	8.4	6.7	5.5	4.6	6.9	8.4	170	170	170	170	180

Note: All test vessels were aerated following the 72 hour dissolved oxygen measurements. Measurements made at 72 hours before aeration are noted above in the column headed "b" and the measurements made after aeration are noted in the column headed "a".

Table A.1. Continued.

Nominal Concentration of N2803-2 (mg/L)	Rep.	pH					Temperature (°C)				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	7.5	7.4	7.6	7.4	7.6	21.9	21.9	21.8	21.6	21.6
	2	7.5	7.4	7.6	7.4	7.7	21.9	21.9	21.9	21.6	21.9
160	1	7.5	7.4	7.6	7.4	7.7	21.9	21.9	21.8	21.6	21.9
	2	7.5	7.5	7.6	7.5	7.7	21.9	21.9	21.8	21.7	21.8
250	1	7.5	7.5	7.6	7.5	7.8	21.9	21.9	21.8	21.7	21.8
	2	7.5	7.5	7.6	7.5	7.7	21.9	21.9	21.8	21.7	21.7
400	1	7.5	7.5	7.6	7.5	7.7	21.9	21.9	21.8	21.6	21.9
	2	7.5	7.4	7.6	7.5	7.8	21.9	21.8	21.8	21.7	21.9
630	1	7.5	7.5	7.6	7.5	7.7	22.0	21.9	21.8	21.7	21.9
	2	7.5	7.5	7.6	7.5	7.8	21.9	21.9	21.8	21.6	21.9
1,000	1	7.5	7.5	7.6	7.5	7.7	21.8	21.8	21.8	21.6	21.6
	2	7.5	7.5	7.6	7.5	7.7	21.9	21.9	21.8	21.6	21.8

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 Exposure
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)

04/01/2001

ENVIRONMENTAL LABORATORY
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2

LAB REQUEST NO. N2803

CONTINUED

SAMPLE	DATE	CODE	DESCRIPTION	RESULT	MDL or (95% C.I.)
1	08/11/95	TB NH40H	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