

LRN2332

Study Title

Acute Toxicity of L-13492 to the
Fathead Minnow, *Pimephales promelas*

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1400

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Study Completed

July 13, 1995

Sponsor

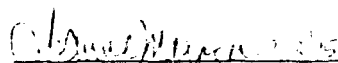
3M Company
Environmental Laboratory
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55144

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.



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 839-TH, Acute Toxicity of L-13492 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). 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:	6/26/95	6/26/95	7/13/95
Draft Report:	7/ 5/95	7/ 5/95	7/13/95
Final Report:	7/13/95	7/13/95	7/13/95


Arthur P. Paradise
Quality Assurance Officer

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V. SUMMARY

The acute toxicity of L-13492 to the fathead minnow, *Pimephales promelas*, 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 839-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 21.9 to 22.3°C. The dilution water was filtered deionized water collected at Marblehead, Massachusetts and adjusted to a hardness of 44 mg/L as CaCO₃. Aeration was initiated at 72 hours to maintain dissolved oxygen concentrations above an acceptable level.

Nominal concentrations of L-13492 were 0 (control), 130, 220, 360, 600, 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 0.28 g and an average total length of 32.8 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 890 mg/L L-13492, with a 95% confidence interval of 600 to >1,000 mg/L. The 96 hour no observed effect concentration (NOEC) is 600 mg/L.

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: July 13, 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 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 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 April 11, 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 L-13492.

Parameter	Unit of Measurement	Detection Limit	Measured Value
pH	pH units	--	7.9
Hardness	mg/L as CaCO ₃	--	44
Alkalinity	mg/L as CaCO ₃	--	38
Acidity	mg/L as CaCO ₃	--	6
Particulate Matter	mg/L	10	ND
Total Organic Carbon	mg/L	1	ND
Total Phosphorus	mg/L	0.03	0.46
Nitrate	mg/L as N	0.05	0.08
Chloride	mg/L	1	14
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. Hardness, alkalinity, acidity, and particulate matter were measured in dilution water at the beginning of the toxicity test. Total organic carbon was measured in dilution water used for this toxicity test. 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.

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 22.1 to 22.9°C and the dissolved oxygen concentration was always at least 8.0 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 32.8 mm and an average wet weight of 0.28 g (loading during the definitive toxicity test was 0.19 g/L).

TOXICITY TESTING:

A static screening test was conducted from June 14 to 18, 1995 with five concentrations of test substance and a dilution water control. Nominal concentrations of L-13492 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 20% survival at 1,000 mg/L.

The definitive toxicity test was performed from June 26 to 30, 1995, according to T.R. Wilbury Protocol 839-TH (Acute Toxicity of L-13492 To The Fathead Minnow, *Pimephales promelas*). 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 $22 \pm 2^\circ\text{C}$ with five concentrations of test substance and a dilution water control. Nominal concentrations of the test substance were: 0 mg/L (control), 130, 220, 360, 600, 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 28 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 L-13492 and fathead minnows, *Pimephales promelas*.

Species:	<i>Pimephales promelas</i>
Age/Size of Test Organisms:	Juveniles/≈0.28 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:	21.9-22.3°C
Minimum Dissolved Oxygen:	4.2 mg/L
Photoperiod:	16 hours light and 8 hours dark with a 15 minute transition period.
Light Intensity:	28 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 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:	≤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 4.2 to 8.9 mg/L (mean = 7.3 mg/L), the temperature ranged from 21.9 to 22.3°C (mean = 22.1°C), conductivity ranged from 170 to 210 μ mhos/cm (mean = 190 μ mhos/cm), and pH ranged from 7.2 to 7.9. 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 L-13492 are presented in Table 4. The 96 hour LC50 for fathead minnows exposed to L-13492 is 890 mg/L, with a 95% confidence interval of 600 to >1,000. The slope of the 96 hour dose response curve could not be determined with these data. The 96 hour no observed effect concentration (NOEC) is 600 mg/L.

Table 3. Survival and sublethal effect data from the toxicity test with fathead minnows, *Pimephales promelas*, and L-13492.

Nominal Concentration of L-13492 (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
130	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
220	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
360	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
600	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
1,000	1	10	3	3	3	3	0	1	2	3	3
	2	10	7	5	3	3	0	2	1	3	3

Note: Affected fathead minnows were lethargic at 48 and 72 hours and they were lethargic and exhibiting a loss of equilibrium at 24 and 96 hours.

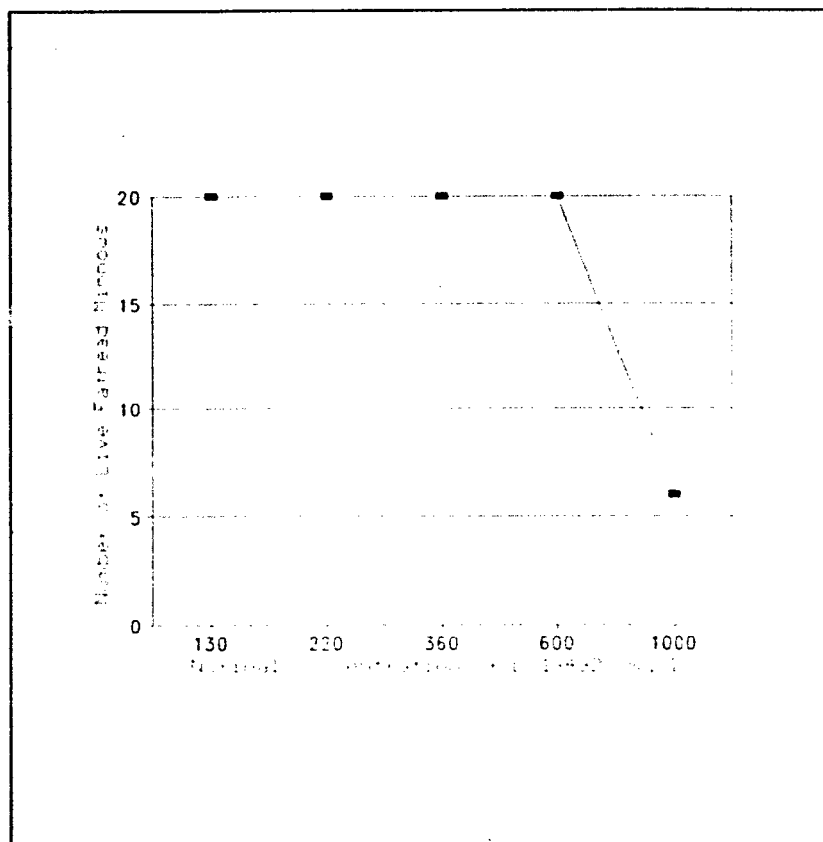


Figure 1. Survival of fathead minnows, *Pimephales promelas*, exposed to L-13492 for 96 hours.

Table 4. Median lethal concentrations (LC50s) from the toxicity test with fathead minnows, *Pimephales promelas*, and L-13492.

Exposure period	LC50	95 percent confidence limit	Calculation method
24 hours	1,000 mg/L	600 to >1,000 mg/L	Binomial
48 hours	940 mg/L	600 to >1,000 mg/L	Binomial
72 hours	890 mg/L	600 to >1,000 mg/L	Binomial
96 hours	890 mg/L	600 to >1,000 mg/L	Binomial

X. REFERENCES

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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XII. PROTOCOL AMENDMENTS AND DEVIATIONS

PROTOCOL AMENDMENTS:

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

PROTOCOL DEVIATIONS:

Deviations were made from the protocol. Dissolved oxygen concentrations were <60% saturation at 72 hr.

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 L-13492.

Nominal Concentration of L-13492 (mg/L)	Rep.	Dissolved Oxygen (mg/L)						Conductivity (µmhos/cm)				
		0	24	48	72 hr		96	0	24	48	72	96
		hr	hr	hr	b	a	hr	hr	hr	hr	hr	
0 (control)	1	8.8	7.5	6.9	6.1	8.2	8.5	170	170	170	180	180
	2	8.8	7.9	7.4	7.1	8.2	8.4	170	170	170	170	180
130	1	8.8	7.9	6.9	4.9	6.4	7.4	180	180	170	180	180
	2	8.8	7.7	6.8	6.1	7.0	7.7	180	180	170	180	180
220	1	8.8	7.4	6.8	6.0	7.7	8.3	180	180	170	180	180
	2	8.8	7.4	6.4	5.7	7.4	8.3	180	180	180	180	180
360	1	8.8	7.3	6.2	5.1	6.3	7.1	180	180	180	190	190
	2	8.8	7.6	6.4	5.9	6.9	7.7	180	180	180	190	190
600	1	8.8	6.8	5.9	4.2	6.2	7.5	190	190	190	190	190
	2	8.8	7.5	6.1	5.3	7.1	7.6	190	190	190	190	190
1,000	1	8.9	8.1	7.8	7.1	7.7	7.3	200	210	200	210	210
	2	8.9	7.8	6.6	5.1	6.4	6.5	200	210	200	210	210

Note: Aeration was initiated in each test vessel following the 72 hour measurements. The "b" measurements were made before aeration and the "a" measurements were made 5 hours after the initiation of aeration.

Table A.1. Continued.

Nominal Concentration of L-13492 (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	7.9	7.5	7.4	7.2	7.8	22.1	22.1	22.2	22.1	22.2
	2	7.9	7.5	7.4	7.2	7.8	22.1	22.1	22.1	22.1	22.2
130	1	7.9	7.6	7.4	7.2	7.6	22.0	22.2	22.1	22.2	22.2
	2	7.9	7.6	7.4	7.2	7.6	21.9	22.1	22.1	22.1	22.2
220	1	7.9	7.5	7.4	7.3	7.7	22.0	22.1	22.1	22.1	22.2
	2	7.8	7.5	7.4	7.2	7.6	22.0	22.1	22.2	22.1	22.2
360	1	7.9	7.5	7.4	7.3	7.6	22.0	22.1	22.2	22.1	22.3
	2	7.9	7.5	7.4	7.3	7.6	22.0	22.1	22.2	22.1	22.2
600	1	7.8	7.5	7.4	7.3	7.6	22.0	22.1	22.2	22.1	22.2
	2	7.8	7.5	7.4	7.3	7.6	22.0	22.1	22.2	22.1	22.2
1,000	1	7.8	7.6	7.5	7.3	7.7	22.0	22.1	22.1	22.1	22.1
	2	7.8	7.6	7.5	7.3	7.6	21.9	22.1	22.1	22.2	22.2

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 2
ARCHIVED FINAL REPORT

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

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