

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorooctanoic acid; may also be referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CAS # 335-67-1)

Remarks: The 3M production lot number was 269. The test substance is a white powder. The test sample is FC-26 referred to by the test laboratory as N2803-3. The purity of the sample was not sufficiently characterized, although current information indicates it is a mixture of 96.5 - 100% test substance and 0 - 3.5% C₆, C₇, and C₉ perfluoro homologue compounds.

Remarks field: The sample preparation directions given to the laboratory were to dissolve the test material in a 50:50 water:isopropanol solution. In the protocol amendment, it was stated that the sample was combined with isopropanol in a 50:50 ratio prior to use.

METHOD

Method: U.S. EPA-TSCA Guideline 797.1400

Type: Static acute

GLP: Yes

Year study conducted: 1995

Year study completed: 1996.

Species: *Pimephales promelas*

Supplier: Aquatic Biosystems, Fort Collins, CO

Analytical monitoring: DO, conductivity, pH and temperature were monitored daily.

Exposure period: 96-hours

Statistical methods: LC₅₀ values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test fish age: Not given.

Length and weight of fish in control: Average total length = 33.4 mm, average wet weight = 0.35 g.

Loading: 0.23 g fish/L

Pretreatment: None

Test conditions:

Dilution Water: Deionized water adjusted to a hardness of 40-48 mg/L as CaCO₃/L

Dilution water chemistry: Representative sample given in Table 1 of the final report.

Temperature: $22 \pm 2^{\circ}\text{C}$

Lighting: Cool-white fluorescent bulbs with an intensity of 31 ft-c. Photoperiod of 16-hours light, 8-hours dark with a 15 minute transition period.

Stock and test solutions preparation: Appropriate amounts of test substance were added directly to the dilution water in the test vessels. No insoluble material was noted during the test.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Not noted.

Exposure vessels: 20 L glass aquaria containing approximately 15 L of test solution, water depth approximately 18 cm.

Number of replicates: two

Number of fish-per replicate: ten

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

6.4 – 8.7 mg/L (control exposure)

5.2 – 8.8 mg/L (220 mg/L exposure)

Conductivity range (0 – 48 hours)

120 $\mu\text{mhos/cm}$ (control exposure)

110 $\mu\text{mhos/cm}$ (220 mg/L exposure)

pH range (0 – 96 hours)

7.1 – 7.6 (control exposure)

6.2 – 7.2 (220 mg/L exposure)

Test temperature range (0 – 96 hours)

21.9 – 22.6 $^{\circ}\text{C}$ (control exposure)

21.9 – 22.6 $^{\circ}\text{C}$ (220 mg/L exposure)

Remarks: The pH of the test solutions for the 360, 600 and 1,000 mg/L exposure concentrations were in the range of 3.0-4.3 at test initiation. This low pH would have adversely affected the survival of the fathead minnow.

RESULTS

Nominal concentrations: Blank control, 130, 220, 360, 600, 1,000 mg/L (Tested as 50:50 mixture of test substance and isopropanol). The concentration of test substance in solution was 65, 110, 180, 300 and 500 mg/L.

Element value (as-tested):

24-hour LC_{50} = 280 (220 – 360) mg/L

48-hour LC_{50} = 280 (220 – 360) mg/L

72-hour LC_{50} = 280 (220 – 360) mg/L

96-hour LC_{50} = 280 (220 - 360) mg/L

96-hour NOEC = 220 mg/L

Element value (based on concentration of test substance in solution):

24-hour LC_{50} = 140 (110 - 180) mg/L

48-hour LC_{50} = 140 (110 - 180) mg/L

72-hour LC_{50} = 140 (110 - 180) mg/L

96-hour LC_{50} = 140 (110 - 180) mg/L

96-hour NOEC = 110 mg/L

All element values based on nominal concentrations.

A toxicity test conducted previously with isopropanol at 500 mg/L showed no mortality or sublethal effects.

Statistical Evaluation of Mortality: All of the LC_{50} values were determined by binomial interpolation.

Biological observations after 96-hours: Fish in the control and the 130, and 220 mg/L exposure concentrations appeared normal. Total mortality was observed by 24 hours in the 360, 600 and 1,000 mg/L exposure concentrations.

Cumulative percent mortality:

Nominal As-Tested Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
130	0	0	0	0
220	0	0	0	0
360	100	100	100	100
600	100	100	100	100
1,000	100	100	100	100

Lowest concentration causing 100% mortality: 360 mg/L

Mortality of controls: None

CONCLUSIONS

The as-tested test substance 96-hour LC_{50} for fathead minnow was determined to be 280 mg/L with a 95% confidence interval of 220 – 360 mg/L. The as-tested test substance 96-hour no observed effect

concentration (NOEC) was 220 mg/L. The 96-hour LC₅₀ for fathead minnow based on the concentration of test substance in solution was determined to be 140 mg/L with a 95% confidence interval of 110 – 180 mg/L. The 96-hour no observed effect concentration (NOEC) for the test substance in solution was 110 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 3. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized. Additionally, there appears to be a discrepancy between the sample preparation directions given to the laboratory and the procedure conducted by the laboratory to prepare the test solutions. The absence of partial mortality at intermediate doses resulted in the sharp dose-response curve seen in this study. As a result, the LC₅₀ values determined in this study may not accurately reflect the true toxicity of the solution. The low pH values in the high concentrations tested may have had an adverse affect on survival.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company.

OTHER

Last changed: 5/25/00

Study Title

Acute Toxicity of N2803-3 to the
Fathead Minnow, *Pimephales promelas*

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1400

Authors

Timothy J. Ward
Jeanne P. Magazu
Robert L. Boeri

Study Completed

January 24, 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 exception. The stability of the test substance under test conditions 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

Jeanne P. Magazu
Study Director

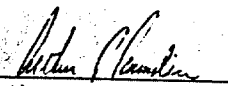
II. QUALITY ASSURANCE STATEMENT

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

Certification:

T.R. Wilbury Laboratories, Inc. study number 891-TH, Acute Toxicity of N2803-3 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/ 4/95	8/ 4/95	8/ 4/95
In-life:	9/ 5/95	9/ 5/95	1/24/96
Draft Report:	9/13/95	9/13/95	1/24/96
Final Report:	1/24/96	1/24/96	1/24/96


Arthur P. Paradise
Quality Assurance Officer

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V. SUMMARY

The acute toxicity of N2803-3 to the fathead minnow, *Pimephales promelas*, is described in this final report. The test was conducted for 3M Company for 96 hours from September 1 to 5, 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 891-TH. N2803-3 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 21.8 to 22.6°C. The dilution water was filtered deionized water collected at Marblehead, Massachusetts and adjusted to a hardness of 40-48 mg/L as CaCO₃. Aeration was initiated at 72 hours to maintain dissolved oxygen concentrations above an acceptable level.

The sample of N2803-3 supplied by the sponsor was combined with isopropanol in a 50:50 ratio prior to use, and this mixture is defined as the test substance as-tested. A toxicity test was also conducted with 500 mg/L isopropanol and no mortality or sublethal effects were observed. Results of the toxicity test are reported twice, once based on nominal concentrations of test substance as-tested and a second time based on concentrations of test substance as-received (N2803-3 without isopropanol). Nominal concentrations of N2803-3 as-tested were 0 (control), 130, 220, 360, 600, and 1,000 mg/L and nominal concentrations of N2803-3 as-received were 0 (control), 65, 110, 180, 300, and 500 mg/L and nominal. 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 0.35 g and an average total length of 33.4 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 280 mg/L N2803-3, with a 95% confidence interval of 220 to 360 mg/L and a 96 hour no observed effect concentration (NOEC) of 220 mg/L, based on test substance as-tested (50% N2803-3 and 50% isopropanol). The 96 hour LC50 is 140 mg/L N2803-3, with a 95% confidence interval of 110 to 180 mg/L and the 96 hour NOEC is 110 mg/L, based on test substance as-received (N2803-3 without isopropanol).

VI. GENERAL INFORMATION

Material Tested: N2803-3

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

Reported Purity: 100%

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

Experimental Start Date: September 1, 1995

Experimental Completion Date: September 5, 1995

Study Completion Date: January 24, 1996

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 N2803-3 (T.R. Wilbury Laboratories Sample Number 530) was delivered to T.R. Wilbury Laboratories on August 29, 1995 in a 5 gallon metal bucket within a cardboard box at ambient temperature. It was contained in an approximately 200 ml glass jar. The label attached to the bottle included the following information: "N2803-3, S. Beach, 2-3E-09, (612) 778-7452, 8/22/95."

N2803-3 (a white solid) was supplied by 3M Company, St Paul, Minnesota. Prior to use the sample of N2803-3 was stored in the dark at room temperature. The sample of N2803-3 supplied by the sponsor was combined with isopropanol in a 50:50 ratio prior to use. This 50:50 mixture was then considered to be 100% test substance during the performance of the toxicity test. All results are reported both on the basis of test substance as-tested (50% N2803-3 and 50% isopropanol) and test substance as-received (N2803-3 without isopropanol). The N2803-3 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 N2803-3.

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	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.

Prior to testing the fish were maintained under flow through conditions in a 270 liter fiberglass tank. 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 8.0 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 33.4 mm and an average wet weight of 0.35 g (loading during the definitive toxicity test was 0.23 g/L).

TOXICITY TESTING:

A static screening test was conducted from August 18 to 22, 1995 with five concentrations of test substance as-received and a dilution water control. Nominal concentrations of N2803-3 were 0.05, 0.50, 5.0, 50, and 500 mg/L. After 96 hours of exposure there was 100% survival in the control, 90% survival at 0.05 mg/L, 100% survival at 0.50, 5.0, and 50 mg/L, and 0% survival at 500 mg/L.

The definitive toxicity test was performed from September 1 to 5, 1995, according to T.R. Wilbury Protocol 891-TH (Acute Toxicity of N2803-3 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 $22 \pm 2^\circ\text{C}$ with five concentrations of test substance and a dilution water control. Nominal concentrations of the test substance as-tested 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.

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 31 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 N2803-3 and fathead minnows, *Pimephales promelas*.

Species:	<i>Pimephales promelas</i>
Age/Size of Test Organisms:	Juveniles/-0.35 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:	21.9-22.6°C
Minimum Dissolved Oxygen:	5.2 mg/L
Photoperiod:	16 hours light and 8 hours dark with a 15 minute transition period.
Light Intensity:	31 footcandles
Water Quality Measurements:	Dissolved oxygen, pH, temperature, and conductivity daily in each test vessel. Hardness and alkalinity were measured at the start and the end of test in dilution water. Acidity, TOC and particulate matter were measured in dilution water. Temperature measured at least every hour in 1 test vessel. 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 number 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-4) 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.

A static test was conducted from September 29 to October 3, 1995 with 500 mg/L isopropanol (7.5 g isopropanol added to 15 liters of dilution water) and a dilution water control. The test was established with 2 replicates of each concentration and 10 fish per replicate, and it was conducted in a manner similar to the test with N2803-3. Test vessels were randomly arranged in a water bath and the light intensity was 28 footcandles.

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 using nominal concentrations of test substance as tested 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 4 and Appendix A, respectively. The measured concentrations of dissolved oxygen ranged from 5.2 to 9.0 mg/L, the temperature ranged from 21.8 to 22.6°C, conductivity ranged from 110 to 370 μ mhos/cm, and pH ranged from 3.0 to 7.6 (conductivity and pH were affected by the test substance). One hundred percent survival occurred in the control exposure and these fathead minnows did not exhibit any sublethal effects. During the toxicity test with 500 mg/L isopropanol no mortality or sublethal effects were observed and the 96 hour LC50 value is greater than 500 mg/L.

The 24, 48, 72, and 96 hour LC50s for fathead minnows exposed to the test substance are presented in Table 5. The 96 hour LC50 for fathead minnows exposed to the test substance is 280 mg/L, with a 95% confidence interval of 220 to 360 mg/L and the 96 hour NOEC is 220 mg/L, based on test substance as-tested (50% N2803-3 and 50% isopropanol). The 96 hour LC50 is 140 mg/L, with a 95% confidence interval of 110 to 180 mg/L and the 96 hour NOEC is 110 mg/L, based on test substance as-received (N2803-3 without isopropanol). The slope of the 96 hour dose response curve could not be determined with these data.

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

Nominal Concentration of N-2803-3 (mg/L)		Rep.	Number Alive					Number Affected				
as-Tested	as-Received		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 65		1	10	10	10	10	10	0	0	0	0	0
		2	10	10	10	10	10	0	0	0	0	0
220 110		1	10	10	10	10	10	0	0	0	0	0
		2	10	10	10	10	10	0	0	0	0	0
360 180		1	10	0	0	0	0	0	--	--	--	--
		2	10	0	0	0	0	0	--	--	--	--
600 300		1	10	0	0	0	0	6	--	--	--	--
		2	10	0	0	0	0	7	--	--	--	--
1,000 500		1	10	0	0	0	0	0	--	--	--	--
		2	10	0	0	0	0	0	--	--	--	--

- Notes: 1. All of the fish exposed to 1,000 mg/L test substance as-tested died immediately and 7 of the 20 fish exposed to 600 mg/L test substance as-tested died immediately.
2. Affected fish were swimming in short, irregular bursts and then floated motionless to the bottom of the test vessels.

Table 4. Survival and sublethal effect data from the toxicity test with fathead minnows, *Pimephales promelas*, and isopropanol.

Nominal Concentration of Isopropanol (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
500	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0

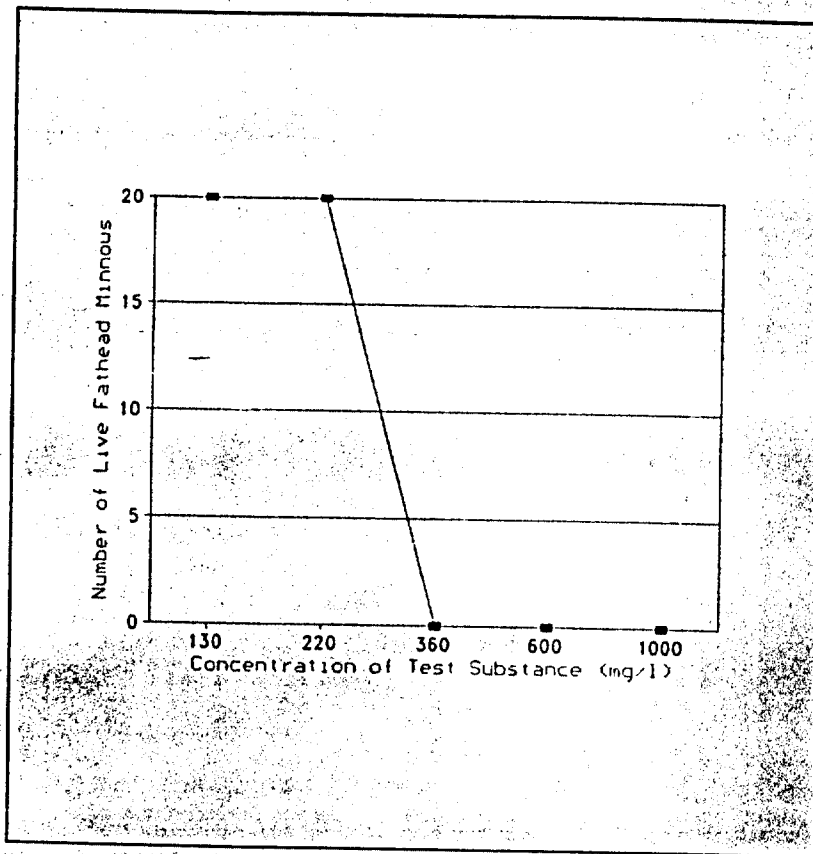


Figure 1. Survival of fathead minnows, *Pimephales promelas*, exposed to W2803-3 for 96 hours.

Table 5. Median lethal concentrations (LC50s) from the toxicity test with fathead minnows, *Pimephales promelas*, and W2803-3.

Exposure period	LC50	95 percent confidence limit	Calculation method
Calculated based on Test Substance as-Tested (50% W2803-3 and 50% Isopropanol)			
24 hours	280 mg/L	220 to 360 mg/L	Binomial
48 hours	280 mg/L	220 to 360 mg/L	Binomial
72 hours	280 mg/L	220 to 360 mg/L	Binomial
96 hours	280 mg/L	220 to 360 mg/L	Binomial
Calculated based on Test Substance as-Received (W2803-3 Without Isopropanol)			
24 hours	140 mg/L	110 to 180 mg/L	Binomial
48 hours	140 mg/L	110 to 180 mg/L	Binomial
72 hours	140 mg/L	110 to 180 mg/L	Binomial
96 hours	140 mg/L	110 to 180 mg/L	Binomial

Note: The 24, 48, 72, and 96 hour LC50 values from the toxicity test with isopropanol are greater than 500 mg/L.

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 that the test substance would be comprised of 50% N2803-3 and 50% isopropanol, to note the nominal concentrations for the definitive test, and to note that an additional toxicity test would be conducted with isopropanol.

PROTOCOL DEVIATIONS:

Dissolved oxygen concentrations were <60% saturation at 72 hr prior to aeration. Test vessels were aerated prior to sponsor approval. The slope of the dose response curve was not provided. In the isopropanol test, not all fish were between 0.1 and 1.0 g. These deviations did not affect the outcome of the study 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 M2803-3.

Nominal Concentration of M2803-3 as-Tested (mg/L)	Rep.	Dissolved Oxygen (mg/L)					Conductivity (μ mhos/cm)				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	8.6	7.5	6.5	6.5	7.4	120	120	110	110	120
	2	8.7	7.4	6.5	6.4	7.5	120	120	110	120	120
130	1	8.7	7.5	6.5	5.8	7.5	110	110	110	120	120
	2	8.9	7.5	6.5	5.5	7.5	110	110	110	120	120
220	1	8.7	7.4	6.5	5.2	7.2	110	110	110	120	110
	2	8.8	7.5	6.5	5.3	7.2	110	110	110	120	110
360	1	8.7	8.0	--	--	--	120	120	--	--	--
	2	8.7	8.0	--	--	--	120	120	--	--	--
600	1	8.9	8.0	--	--	--	180	200	--	--	--
	2	8.9	8.1	--	--	--	200	200	--	--	--
1,000	1	9.0	--	--	--	--	350	--	--	--	--
	2	9.0	--	--	--	--	370	--	--	--	--

Table A.1. Continued.

Nominal Concentration of N2803-3 as-Tested (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	7.6	7.3	7.2	7.1	7.4	22.1	22.6	21.9	22.0	21.9
	2	7.6	7.3	7.2	7.1	7.4	22.1	22.6	21.9	22.0	21.9
130	1	6.8	7.2	7.2	7.1	7.4	21.9	22.6	21.8	22.0	21.9
	2	6.7	7.2	7.2	7.1	7.4	21.9	22.6	21.8	22.0	21.9
220	1	6.3	6.9	6.9	6.9	7.2	22.4	22.5	21.9	22.0	21.9
	2	6.2	6.8	6.9	6.9	7.2	22.0	22.6	21.9	22.0	21.9
360	1	4.2	4.5	--	--	--	22.1	22.5	--	--	--
	2	4.3	4.6	--	--	--	22.1	22.5	--	--	--
600	1	3.4	3.5	--	--	--	22.1	22.6	--	--	--
	2	3.4	3.4	--	--	--	22.1	22.5	--	--	--
1,000	1	3.0	--	--	--	--	22.5	--	--	--	--
	2	3.0	--	--	--	--	22.5	--	--	--	--

Table A.2. Dissolved oxygen concentration, conductivity, pH, and temperature measured during the toxicity test with fathead minnows, *Pimephales promelas*, and isopropanol.

Nominal Concentration of Isopropanol (mg/L)	Rep.	Dissolved Oxygen (mg/L)					Conductivity (μ mhos/cm)				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	8.6	7.9	7.6	7.1	6.2	140	160	160	160	160
	2	8.6	8.0	7.6	7.4	6.8	140	160	160	160	160
500	1	8.6	8.1	7.8	6.2	5.5	140	160	160	160	160
	2	8.6	8.0	7.6	6.3	5.6	140	160	160	160	160

Table A.2. Continued.

Nominal Concentration of isopropanol (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	7.8	7.8	7.7	7.8	7.7	21.4	21.9	21.9	21.5	22.1
	2	7.8	7.8	7.7	7.8	7.7	21.4	21.9	21.9	21.5	22.1
500	1	7.8	7.8	7.8	7.8	7.6	21.4	21.9	21.9	21.5	22.1
	2	7.8	7.8	7.8	7.8	7.6	21.4	21.9	21.9	21.5	22.1