

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)

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 797.1300

Test type: Static acute

GLP: Yes

Year study conducted: 1995

Year study completed: 1996.

Species: *Daphnia magna*

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

Exposure period: 48-hours

Statistical methods: LC₅₀ and EC₅₀ 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 daphnid source: Obtained from cultures maintained by T.R. Wilbury Laboratories Inc, Marblehead, MA from an original culture from Aquatic Research Organisms, Hampton, NH.

Test daphnid age at study initiation: < 24-hours

Test conditions

Dilution water: Filtered, deionized water adjusted to a hardness of 160 mg/L as CaCO₃/L

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

Temperature: 19.4 – 20.2°C

Lighting: Cool-white fluorescent bulbs with an intensity of 58 ft-c. Photoperiod of 16-hours light, 8-hours dark with a 15 minute transition period.

Stock and test solutions preparation: A 1,000 mg/L primary stock solution (50:50 mixture of test substance and isopropanol) was prepared in dilution water. After mixing, the primary stock was proportionally diluted with dilution water to prepare the test concentrations. No insoluble material was noted during the test.

Exposure vessels: 300 mL glass beakers containing 250 mL of test solution. The approximate depth of test solution was 9 cm.

Number of replicates: two

Number of daphnids per replicate: ten

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 48 hours):

8.7 – 8.8 mg/L (control exposure)

8.7 – 8.8 mg/L (1,000 mg/L exposure conc.)

Conductivity range (0 – 48 hours)

590 – 610 μ mhos/cm (control exposure)

570 – 590 μ mhos/cm (1,000 mg/L exposure conc.)

pH range (0 – 48 hours)

7.9 – 8.4 (control exposure)

7.5 – 8.0 (1,000 mg/L exposure conc.)

Test temperature range (0 – 48 hours)

19.5 – 20.2°C (control exposure)

19.4 – 20.2°C (1,000 mg/L exposure conc.)

Element basis: mortality and immobilization

RESULTS

Nominal concentrations: Blank control, 130, 200, 360, 600, 1,000 mg/L (Tested as a 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 EC_{50} = 840 (740 – 970) mg/L

24-hour LC_{50} = 1,000 (600 - >1,000) mg/L

48-hour EC_{50} = 720 (660 – 780) mg/L

48-hour LC_{50} = 800 (700 – 920) mg/L

48-hour NOEC = 360 mg/L

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

24-hour EC_{50} = 420 (370 – 490) mg/L

24-hour LC₅₀ = 500 (300 - >500) mg/L
48-hour EC₅₀ = 360 (300 – 500) mg/L
48-hour LC₅₀ = 400 (350 – 460) mg/L
48-hour NOEC = 180 mg/L

All element values based on nominal concentrations.

A toxicity test conducted previously with isopropanol at 390 mg/L showed no mortality or sublethal effects.

Statistical Evaluation: The LC₅₀ and EC₅₀ values and 95% confidence intervals were calculated when possible by probit analysis, the moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Biological observations: Daphnids in the control, the 130, 200, and 360 mg/L treatments appeared healthy and normal throughout the test with no mortality, immobility or overt clinical signs of toxicity.

Cumulative percent mortality:

Nominal As-Tested Conc., mg/L	24-hours	48-hours
Control	0	0
130	0	0
200	0	0
360	0	0
600	10	15
1,000	50	80

Control response: satisfactory

CONCLUSIONS

The as-tested test substance 48-hour LC₅₀ for *Daphnia magna* was determined to be 800 mg/L with a 95% confidence interval of 700 – 920 mg/L. The as-tested test substance 48-hour EC₅₀ for *Daphnia magna* was determined to be 720 mg/L with a 95% confidence interval of 660 – 780 mg/L. The as-tested test substance 48-hour no observed effect concentration (NOEC) was 360 mg/L. The 48-hour LC₅₀ for *Daphnia magna* based on the concentration of test substance in solution was determined to be 400 mg/L with a 95% confidence interval of 350 – 460 mg/L. The 48-hour EC₅₀ for *Daphnia magna* based on the concentration of test substance in solution was determined to be 360 mg/L with a 95%

confidence interval of 300 – 500 mg/L. The 48-hour no observed effect concentration (NOEC) for the test substance in solution was 180 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.

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 Daphnid, *Daphnia magna*

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1300

Authors

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Study Completed

March 7, 1996

Sponsor

3M Company
Environmental Laboratory
935 Bush Avenue
Building 2-3E-C9
St. Paul, Minnesota 55144

Testing Facility

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40 Oaks 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

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 892-TH, Acute Toxicity of N2803-3 to the Daphnid, *Daphnia magna*, 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/ 1/95	9/ 1/95	3/ 7/96
	9/ 6/95	9/ 6/95	3/ 7/96
Draft Report:	1/ 2/96	1/ 3/96	3/ 7/96
Final Report:	3/ 7/96	3/ 7/96	3/ 7/96

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

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V. SUMMARY

The acute toxicity of N2803-3 to the daphnid, *Daphnia magna*, is described in this final report. The test was conducted for 3M Company for 48 hours from August 30 to September 1, 1995, at T.R. Wilbury Laboratories, Inc. in Marblehead, Massachusetts. It was conducted by Jeanne Magazu, Peter Kowalski, Jacqueline Navius, Keven Stevens, Peter Navius, Robert Boeri, and Timothy Ward according to the protocol developed for T.R. Wilbury Study Number 892-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 19.4-20.2°C. The dilution water was filtered deionized water collected at Marblehead, Massachusetts and adjusted to a hardness of 160 mg/L as CaCO₃. Aeration was not required 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 (a toxicity test was conducted with 390 mg/L isopropanol and no mortality or sublethal effects were observed). Nominal concentrations of N2803-3 as-received were 0 (control), 65, 110, 180, 300, and 500 mg/L. Daphnids used in the test were less than 24 hours old at the start of the test. They were produced at T.R. Wilbury Laboratories and acclimated under test conditions for their entire life (cultures that produced test specimens were maintained at test conditions for more than 7 days). After 48 hours of exposure the control daphnids had an average wet weight (blotted) of 0.53 mg. All daphnids were in good condition at the beginning of the study.

Exposure of daphnids to N2803-3 resulted in a 24 hour median lethal concentration (LC50) of 500 mg/L N2803-3, a 48 hour LC50 of 400 mg/L N2803-3, a 24 hour median effective concentration (EC50) of 420 mg/L, and a 48 hour EC50 of 360 mg/L N2803-3, based the concentration of N2803-3 in the test substance. The 48 hour no observed effect concentration (NOEC) is 180 mg/L, based on the concentration of N2803-3 in the test substance. The 24 hour LC50 is 1,000 mg/L, the 48 hour LC50 is 800 mg/L, the 24 hour EC50 is 840 mg/L, the 48 hour EC50 is 720 mg/L, and the 48 hour NOEC is 360 mg/L, based on test substance as-tested (50% N2803-3 and 50% isopropanol).

VI. GENERAL INFORMATION

Material Tested: N2803-3

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

Material 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: August 30, 1995

Experimental Completion Date: October 6, 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 JM Company, St Paul, Minnesota. The objective of the study was to determine the 24 and 48 hour median lethal concentrations (LC50s) and median effective concentrations (EC50s) of the test substance to the daphnid, *Daphnia magna*, a freshwater invertebrate, 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 521) was delivered to T.R. Wilbury Laboratories on August 14, 1995 in a cardboard box at ambient temperature. It was contained in an approximately 50 ml glass jar. The label attached to the bottle included the following information: "N2803-3".

N2803-3 (a white solid) was supplied by JM Company, St Paul, Minnesota. Prior to use the 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 but all results are reported on an active ingredient basis (active ingredient is the test substance as-received minus the isopropanol). The test substance was assumed to have a purity of 100% active ingredient and to be stable under storage and testing conditions. Unused N2803-3 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 160-180 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 daphnids, *Daphnia magna*, employed as test organisms were less than 24 hours old at the start of the test. They were from a single source and were identified using an appropriate taxonomic key. Test specimens were produced by adult daphnids that were maintained under test conditions at the T.R. Wilbury facility for more than 7 days.

Table 1. Chemical characterization of a representative sample of dilution water used for the toxicity test with daphnids, *Daphnia magna*, and H2803-3.

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	NL
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.

The original culture was obtained on June 1, 1994 from Aquatic Research Organisms, Hampton, New Hampshire. Measurements made during the 7 days before the start of the test with N2803-3 indicated that the culture temperature ranged from 19.5 to 20.5°C and the dissolved oxygen concentration was always at least 9.1 mg/L and measurements made during the 7 days before the start of the test with isopropanol indicated that the culture temperature ranged from 20.1 to 20.9°C and the dissolved oxygen concentration was always at least 8.3 mg/L. The cultures each produced young prior to day 12 and a subsample of adults produced at least 3 young/daphnid/day for 7 days before the start of the tests.

During acclimation daphnids were not treated for disease, they were free of apparent sickness, injuries, and abnormalities at the beginning of the test and there was no mortality during the 48 hours preceding the start of the test. The culture was supplied with a yeast/trout chow mix (Lot# DC02) and the freshwater alga, *Selenastrum capricornutum*, daily during acclimation. Daphnids were not fed during the test. Control daphnids, blotted and weighed at the conclusion of the toxicity test with N2803-3, averaged 0.53 mg and the loading rate during the definitive toxicity test was approximately 10.4 mg/L and 40 daphnids/L.

TOXICITY TESTING:

A static screening test was conducted from August 23 to 25, 1995 with five concentrations of test substance and a dilution water control. Nominal concentrations of N2803-3 were 0.050, 0.50, 5.0, 50, and 500 mg/L. After 48 hours of exposure there was at least 95% survival in the control and at 0.050, 0.50, 5.0, and 50 mg/L and 15% survival at 500 mg/L (surviving daphnids exposed to 500 mg/L were immobilized).

The definitive toxicity test was performed from August 30 to September 1, 1995, according to T.R. Wilbury Protocol 892-TH (Acute Toxicity of N2803-3 To The Daphnid, *Daphnia magna*). 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 20 ± 1°C with five concentrations of test substance and a dilution water control. Nominal concentrations of the test substance were: 0 mg/L (control), 130, 200, 360, 600, and 1,000 mg/L (concentrations of N2803-3 were 65, 110, 300 and 500 mg/L). A 1,000 mg/L (500 mg/L N2803-3) stock solution was formulated by combining 2.0 g of test substance (50:50 mixture of N2803-3 and isopropanol) and dilution water in a Class A volumetric flask and adjusting the final stock solution volume to 2,000 ml. The stock solution was mixed and then appropriate amounts of this stock solution were added to dilution water in test vessels to formulate test media with the use of a solvent.

Table 2. Test system summary for the toxicity test with N2803-3 and daphnids, *Daphnia magna*.

Species:	<i>Daphnia magna</i>
Age/Size of Test Organisms:	<24 hours old
Test Duration:	48 hours
Test Vessels:	300 ml glass beakers
Test Media Volume:	250 ml
Depth of Test Media:	9 cm
Number of Replicate Test Vessels:	2
Organisms per Replicate:	10
Temperature Range:	19.4-20.2°C
Minimum Dissolved Oxygen:	8.7 mg/L
Photoperiod:	16 hours light and 8 hours dark.
Light Intensity:	58 footcandles
Water Quality Measurements:	Dissolved oxygen, pH, temperature, and conductivity daily in each test vessel. Hardness, total organic carbon, and alkalinity once in the control. Residual chlorine daily. Dilution water analysis is summarized in Table 1.
End Points:	24 and 48 hour LC50 and EC50 (based on immobilization), 48 hour no observed effect level.
Acceptability Criteria:	≤10% control mortality and acceptable temperature range.

Twenty daphnids were indiscriminately and equally distributed among two replicates of each treatment. The test was performed in 300 ml glass beakers that contained 250 ml of test solution (water depth was approximately 9 cm). Test vessels were randomly arranged in an incubator during the 48 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 58 footcandles. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels and daphnids were not fed during the test.

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 and 48 hours. 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 a beaker of water incubated among the test vessels was recorded continuously during the test.

A static test was conducted from October 4 to 6, 1995 with 390 mg/L isopropanol (0.125 ml isopropanol brought up to 250 ml with dilution water) and a dilution water control. The test was established with 2 replicates of each concentration and 10 daphnids 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 13 to 25 footcandles.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate LC50s and EC50s. All calculations were performed by using nominal concentrations of both the test substance (as tested) and N2803-3 (as received) and the number of live or unaffected daphnids. The no observed effect concentration is the highest concentration of test substance that did not cause any toxicant-related mortality or sublethal effects.

II. RESULTS

No insoluble material was noted during the test. Biological and water quality data generated by the acute toxicity test are presented in Table 3 and Appendix A, respectively. During the test with N2803-3 the measured concentrations of dissolved oxygen ranged from 8.7 to 8.8 mg/L, the temperature ranged from 19.4 to 20.7°C, conductivity ranged from 570 to 610 μ mhos/cm, and pH ranged from 8.3 to 8.5. During the test with isopropanol the measured concentrations of dissolved oxygen ranged from 8.1 to 8.5 mg/L, the temperature ranged from 20.4 to 20.9°C, conductivity ranged from 530 to 540 μ mhos/cm, and pH ranged from 8.3 to 8.5. One hundred percent survival occurred in the control exposures and these daphnids did not exhibit any sublethal effects. During the toxicity test with 390 mg/L isopropanol no mortality or sublethal effects were observed and the 48 hour EC50 and LC50 values are greater than 390 mg/L (Table 4).

The 24 and 48 hour LC50s and EC50s for daphnids exposed to N2803-3 are presented in Table 5. Exposure of daphnids to N2803-3 resulted in a 24 hour median lethal concentration (LC50) of 500 mg/L N2803-3, a 48 hour LC50 of 400 mg/L N2803-3, a 24 hour median effective concentration (EC50) of 420 mg/L, and a 48 hour EC50 of 360 mg/L N2803-3, based on the N2803-3 concentration in the test substance. The 48 hour no observed effect concentration (NOEC) is 180 mg/L, based on the N2803-3 concentration in the test substance. The 24 hour LC50 is 1,000 mg/L, the 48 hour LC50 is 800 mg/L, the 24 hour EC50 is 840 mg/L, the 48 hour EC50 is 720 mg/L, and the 48 hour NOEC is 360 mg/L, based on test substance as-tested (50% N2803-3 and 50% isopropanol). The slope of the 48 hour dose response curve (based on mortality and immobilization) is 10.5.

Table 3. Survival and sublethal effect data from the toxicity test with daphnids, *Daphnia magna*, and N2803-3.

Nominal Concentration of N2803-3 (mg/L)	Rep.	Number alive			Number affected		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
65	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
110	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
180	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
300	1	10	9	8	0	0	0
	2	10	9	9	0	0	0
500	1	10	5	2	0	3	2
	2	10	5	2	0	2	2

Note: Affected daphnids were immobilized.

Table 4. Survival and sublethal effect data from the toxicity test with daphnids, *Daphnia magna*, and isopropanol.

Nominal Concentration of Isopropanol (mg/L)	Rep.	Number alive			Number affected		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
390	1	10	10	10	0	0	0
	2	10	10	10	0	0	0

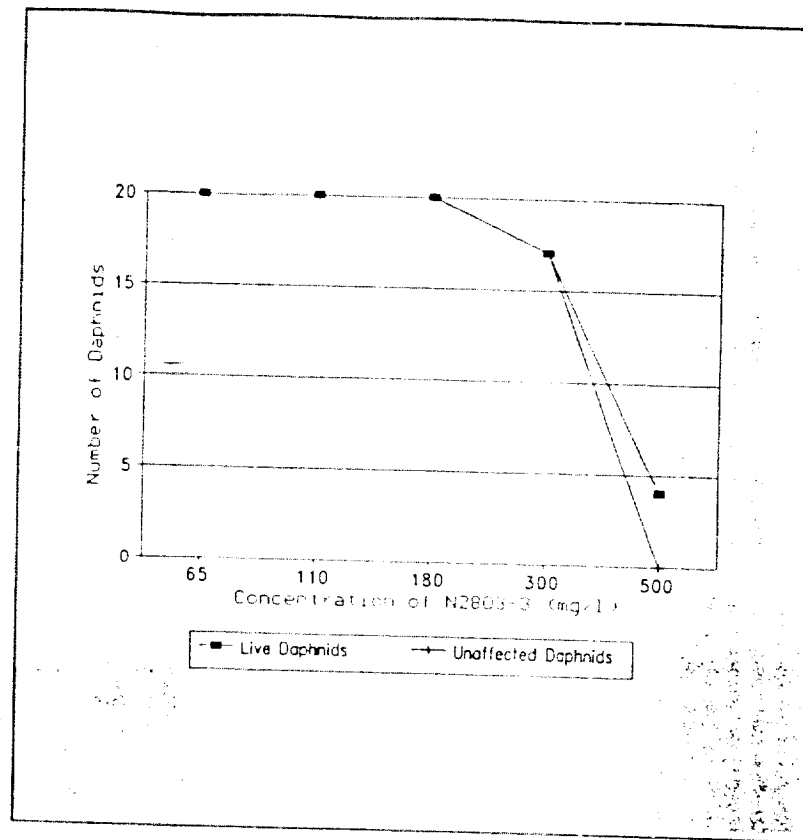


Figure 1. Survival and sublethal effects of daphnids, *Daphnia magna*, exposed to N2803-3 for 48 hours.

Table 5. Median lethal concentrations (LC50s) and median effective concentrations (EC50s) from the toxicity test with daphnids, *Daphnia magna*, and N2803-3.

Exposure period	LC50/EC50	95 percent confidence limit	Calculation method
Calculated based on Test Substance as-Tested			
LC50			
24 hours	1,000 mg/L	600 to >1,000 mg/L	Binomial/Nonlinear Interpolation
48 hours	800 mg/L	700 to 920 mg/L	Probit
EC50			
24 hours	840 mg/L	740 to 970 mg/L	Probit
48 hours	720 mg/L	660 to 780 mg/L	Probit
Calculated based on the N2803-3 concentration in the Test Substance			
LC50			
24 hours	500 mg/L	300 to >500 mg/L	Binomial/Nonlinear Interpolation
48 hours	400 mg/L	350 to 460 mg/L	Probit
EC50			
24 hours	420 mg/L	370 to 490 mg/L	Probit
48 hours	360 mg/L	300 to 500 mg/L	Binomial/Nonlinear Interpolation

Note: The 24 and 48 hour EC50 and LC50 values from the toxicity test with isopropanol are greater than 390 mg/L.

I. 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 797.1300.

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, to indicate that the test substance was combined in a 50:50 ratio with isopropanol, and to note that a toxicity test would also be conducted with a single concentration of isopropanol.

PROTOCOL DEVIATIONS:

The nominal isopropanol concentration was 390 mg/L rather than 500 mg/L. 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 daphnids, *Daphnia magna*, and N2803-3.

Nominal Concentration of N2803-3 (mg/L)	Rep.	Dissolved Oxygen (mg/L)			Conductivity (μ mhos/cm)		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	8.7	8.8	8.7			
	2	8.7	8.8	8.7	590	600	610
65	1	8.7	8.8	8.7	590	600	610
	2	8.7	8.8	8.7	590	600	610
110	1	8.7	8.8	8.7	590	600	610
	2	8.7	8.8	8.7	590	600	610
180	1	8.7	8.8	8.7	590	600	600
	2	8.7	8.8	8.7	590	600	600
300	1	8.7	8.8	8.7	580	590	600
	2	8.7	8.8	8.7	580	590	600
500	1	8.7	8.8	8.7	570	590	590
	2	8.7	8.8	8.7	570	590	590

Table A.1. Continued.

Nominal Concentration of N2803-3 (mg/L)	Rep.	pH			Temperature (°C)		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	8.0	7.9	8.4	20.2	19.6	19.5
	2	8.0	7.9	8.4	20.2	19.6	19.5
65	1	7.8	7.9	8.4	20.2	19.5	19.5
	2	7.8	7.9	8.4	20.2	19.6	19.5
110	1	7.7	7.8	8.4	20.2	19.6	19.6
	2	7.7	7.8	8.4	20.2	19.5	19.6
180	1	7.6	7.7	8.3	20.1	19.6	19.5
	2	7.6	7.7	8.3	20.1	19.5	19.4
300	1	7.6	7.7	8.2	20.1	19.6	19.4
	2	7.6	7.7	8.2	20.1	19.6	19.4
500	1	7.5	7.6	8.0	20.2	19.6	19.4
	2	7.5	7.6	7.9	20.2	19.5	19.4

Table A.2. Dissolved oxygen concentration, conductivity, pH, and temperature measured during the toxicity test with daphnids, *Daphnia magna*, and isopropanol.

Nominal Concentration of isopropanol (mg/L)	Rep.	Dissolved Oxygen (mg/L)			Conductivity (μ mhos/cm)		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	8.1	8.5	8.4	540	530	530
	2	8.1	8.5	8.4	540	530	530
390	1	8.1	8.5	8.4	540	530	530
	2	8.1	8.5	8.4	540	530	530

Table A.2. Continued.

Nominal Concentration of Isopropanol (mg/L)	Rep.	pH			Temperature (°C)		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	8.5	8.4	8.3	20.4	20.9	20.6
	2	8.5	8.4	8.3	20.4	20.8	20.6
390	1	8.5	8.4	8.3	20.4	20.8	20.6
	2	8.5	8.4	8.3	20.4	20.9	20.6