

TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

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 797.1050

Test: Static acute

GLP: Yes

Year completed: Study completed in 1995. Report completed in 1996.

Species: *Selenastrum capricornutum*

Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at T.R. Wilbury, Inc., Marblehead, MA.

Element basis: Reported two ways: number of cells/mL and growth rate.

Exposure period: 96-hours

Year study conducted: 1995

Year study completed: 1996

Analytical monitoring: Nominal concentrations. pH and temperature.

Test organisms laboratory culture: Algae cultures were growing in U.S. EPA-recommended sterile enriched medium for at least 14 days prior to test initiation.

Test Conditions:

Algal medium: prepared to U.S. EPA recommended concentrations by spiking deionized water with nutrient stocks. The pH of the synthetic algal medium at test initiation was 7.4. Algal medium used for culturing and as diluent.

Stock and test solutions preparation: A 1,000 mg/L primary stock solution was prepared in sterile enriched media. Appropriate amounts of this stock solution were added directly to dilution water to formulate the test media.

Exposure vessels: 250 mL glass Erlenmeyer flasks containing 50 mL of test solution.

Agitation: Shaken continuously at 100 rpm

Number of replicates: three

Initial algal cell loading: 1.0×10^4 cells/mL

Number of concentrations: Five plus a negative control

Water chemistry:

pH range: (0 – 96 hours)

7.4 – 10.3 (control exposure)

2.9 – 3.0 (1,000 mg/L exposure)

Test temperature range: (0 – 96 hours)

23.5 – 24.0°C

Light levels: (0 – 96 hours)

~380 ft-c from continuous cool-white fluorescent lighting

Remarks: The pH of the test solutions for the 250, 500 and 1,000 mg/L exposure concentrations were in the range of 2.9-4.0 at test initiation. This low pH would have adversely affected the survival and subsequent growth of the algae.

RESULTS

Nominal concentrations: Blank control, 63, 125, 250, 500, 1,000 mg/L (Tested as 50:50 mixture of test substance and isopropanol). The concentration of test substance in solution was 32, 63, 130, 250 and 500 mg/L.

Element value (as-tested):

72-hour EC_{50} (cell density) = 180 (125-250) mg/L

72-hour E_rC_{50} (growth rate) = 180 (125-250) mg/L

96-hour EC_{50} (cell density) = 180 (125-250) mg/L

96-hour E_rC_{50} (growth rate) = 180 (125-250) mg/L

96-hour NOEC: 125 mg/L

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

72-hour EC_{50} (cell density) = 90 (63-130) mg/L

72-hour E_rC_{50} (growth rate) = 90 (63-130) mg/L

96-hour EC_{50} (cell density) = 90 (63-130) mg/L

96-hour E_rC_{50} (growth rate) = 90 (63-130) mg/L

96-hour NOEC: 63 mg/L

All element values based on nominal concentrations.

A toxicity test conducted previously with isopropanol at 1,000 mg/L showed no effect on algal growth.

Statistical methods: Cell densities, growth rates and percent inhibition values used to estimate the EC₁₀, EC₅₀, and EC₉₀ values and 95% confidence limits were calculated using the computer software of C.E. Stephan. The no observed effect concentration (NOEC) was calculated using one-way analysis of variance (ANOVA).

Control response: Satisfactory

Biological observations after 96-hours:

Nominal Concentration (as-tested), mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Growth Rate
Control	1,671,000	-	-
63	1,732,000	-4	-2
125	1,560,000	7	0
250	<10,000	100	100
500	<10,000	100	100
1,000	<10,000	100	100

Observations: Algal cell counts in each test vessel were determined by means of direct microscope counts with a hemocytometer. After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the flasks in the control or any test treatment group. In addition, there were no noticeable changes in cell size, color or morphology when compared to the control.

Reversibility of Growth Inhibition: Effect of the test substance was determined to be algistatic based on the results of the post-definitive test exposure.

CONCLUSIONS

The test sample 96-hour EC₅₀ and 95% confidence interval for *Selenastrum capricornutum* was determined using two calculation methods. The as-tested test substance 96-hour EC₅₀ for *Selenastrum capricornutum* was determined to be 180 mg/L with a 95% confidence interval of 125 – 260 mg/L, when calculated using either the cell density or

growth rate. The as-tested test substance 96-hour no observed effect concentration (NOEC) was 125 mg/L. The 96-hour EC₅₀ for *Selenastrum capricornutum* based on the concentration of test substance in solution was determined to be 90 mg/L with a 95% confidence interval of 63 – 130 mg/L using either the cell density or growth rate. The 96-hour no observed effect concentration (NOEC) for the test substance in solution was 63 mg/L. No signs of aggregation, flocculation, or adherence were noted in any of the test solutions. This test substance was determined to be algistatic.

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. Initial low pH values in the higher concentrations may have had an adverse effect on the survival and growth of the algae.

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

Growth and Reproduction Toxicity Test with N2803-3
and the Freshwater Alga, *Selenastrum capricornutum*

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1050

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

T.R. Wilbury Study Number 893-TH

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I. GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to EPA Good Laboratory Practice Regulations (40 CFR 792), with the following exceptions. The stability of the test substance under test conditions was assumed but not verified at T.R. Wilbury Laboratories. Deviations from the study protocol are discussed in Section XII of this report.

Jeanne P. Magazu
Jeanne P. Magazu
Study Director

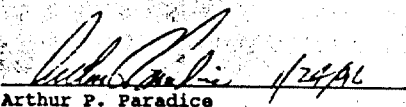
II. QUALITY ASSURANCE STATEMENT

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

Certification:

T.R. Wilbury Laboratories, Inc. study number 893-TH, Growth and Reproduction Toxicity Test with N2803-3 and the Freshwater Alga, *Selenastrum capricornutum*, was audited by the Quality Assurance Unit for compliance with the protocol, standard operating procedures, and applicable Good Laboratory Practices (U.S. EPA, 1993). Quality assurance audits were performed and the findings reported to T.R. Wilbury Laboratories management and the study director on the following dates:

Aspect Audited	Audit Date	Reported to Study Director	Reported to Management
Protocol:	8/ 4/95	8/ 4/95	8/ 4/95
In-life:	9/ 8/95	9/ 8/95	1/24/96
Draft Report:	9/29/95	9/29/95	1/24/96
Final Report:	1/24/96	1/24/96	1/24/96


Arthur P. Paradice
Quality Assurance Officer

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V. SUMMARY

The toxicity of N2803-3 to the freshwater alga, *Selenastrum capricornutum*, is described in this final report. The test was conducted for 3M Company for 96 hours from August 8 to 12, 1995, at T.R. Wilbury Laboratories, Inc. in Marblehead, Massachusetts. It was conducted by Jeanne Magazu, Peter Kowalski, Jacqueline Nevius, Kaven Stevens, Peter Nevius, Robert Boeri, and Timothy Ward according to the protocol developed for T.R. Wilbury Study Number 893-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 $24 \pm 2^\circ\text{C}$.

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 previously conducted with 1,000 mg/L isopropanol (Ward et.al., 1995) and algal growth was not affected at 1,000 mg/L, indicating that the concentration of isopropyl alcohol in the sample of N2803-3 can not, by itself, account for the toxicity of N2803-3 to algae. 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), 63, 125, 250, 500, and 1,000 mg/L and nominal concentrations of N2803-3 as-received were 0 (control), 32, 63, 130, 250, and 500 mg/L. The dilution water was sterile enriched media.

Exposure of algae to the test substance for 96 hours resulted in a median effective concentration (EC50) of 180 mg/L N2803-3, with 95% confidence limits of 125 and 260 mg/L, when calculated using either the number of cells per ml or the average specific growth rate, based on test substance as-tested (50% N2803-3 and 50% isopropanol). The 96 hour no observed effect concentration (NOEC) is 125 mg/L and the lowest observed effect concentration (LOEC) is 250 mg/L, based on test substance as-tested. The 96 hour EC50 is 90 mg/L N2803-3 (95% confidence limits = 63 and 130 mg/L, when calculated using either the number of cells per ml or the average specific growth rate and the 96 hour NOEC is 63 mg/L and the LOEC is 130 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
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55144

Reported Purity: 100%

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

Experimental Start Date: September 8, 1995

Experimental Completion Date: September 12, 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. The objective of the study was to determine the 24, 48, 72, and 96 hour effective concentrations (EC10s, EC50s, EC90s) and no observed effect concentration (NOEC) of the test substance to the freshwater alga, *Selenastrum capricornutum*, under static conditions. The test protocol was based on U.S. EPA methods (EPA, 1993). The report contains sections that describe the methods and materials employed in the study, and results of the investigation. The report also contains an appendix that presents water quality data collected during the test.

VIII. METHODS AND MATERIALS

TEST SUBSTANCE:

The sample of N2803-3 (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 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 sterile enriched media (U.S. EPA, 1978; T.R. Wilbury Standard Operating Procedure number 6) adjusted to a target pH of 7.5 with 0.1 N HCl prior to use. Characterization of a representative sample of media is presented in Table 1.

TEST ORGANISM:

Algae used for the test (*Selenastrum capricornutum*, UTEX 1648) was from a culture originally procured from the Culture Collection of Algae at the University of Texas at Austin and delivered to T.R. Wilbury Laboratories on April 11, 1995. The culture was transferred to sterile enriched media identical to media used for this test and maintained at test conditions for at least 14 days before the definitive test. The subsample of algae used to inoculate media at the start of the definitive test came from a 10 day old culture. Identification of the culture organisms was verified using an appropriate taxonomic key.

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Table 1. Chemical characterization of a representative sample of test media and water used to formulate test media for the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and N2803-3.

Parameter ¹	Unit of Measurement	Detection Limit	Measured Value
pH	pH units	--	7.4
Total Phosphorus	mg/L	0.03	0.46
Nitrate	mg/L as N	0.05	0.08
Chloride	mg/L	1	14
Total Organic Carbon	mg/L	1	ND ²
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. Total organic carbon, 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.

TOXICITY TEST:

Results of a range finding test were used to select the concentrations for the definitive test. The range finding test was performed from August 25 to 29, 1995. At the conclusion of the test the number of cells/ml equalled the following percent of the control: 0.050, 0.50, 5.0, and 50 mg/L = >100% and 500 mg/L = <1%, based on test substance as-received.

The definitive toxicity test was performed from September 8 to 12, 1995, according to T.R. Wilbury Protocol 893-TH (Growth and Reproduction Toxicity Test with N2803-3 and the Freshwater Alga, *Selenastrum capricornutum*). The protocol, which was signed by the study director on August 16, 1995, is based on procedures of the U.S. Environmental Protection Agency (1993). A summary of the test system is presented in Table 2.

The test was conducted at a target temperature of $24 \pm 2^\circ\text{C}$ with five concentrations of test substance and a dilution water control. Nominal concentrations of the test substance as-tested were: 0 mg/L (control), 63, 125, 250, 500, and 1,000 mg/L. A 1,000 mg/L stock solution of test substance as-tested was formulated on September 8, 1995 by adding 1.0 g N2803-3 as-tested to sterile enriched media and adjusting the final media volume to 1,000 ml. Appropriate amounts of this stock solution were added directly to dilution water to formulate test media.

A test was performed from June 26 to 30, 1995 with isopropyl alcohol (Mallinckrodt, Lot 3035), a component that represents 50% of test substance (Ward, et.al., 1995). This test was conducted at 4.4 mg/L and at 1,000 mg/L. A 1,000 mg/L stock solution was formulated on June 26, 1995 by adding 0.5 g isopropyl alcohol to sterile enriched media and adjusting the final media volume to 500 ml.

Algae were distributed among three replicates of each treatment at the rate of 10,000 cells/ml. The test was performed in 250 ml glass Erlenmeyer flasks that contained 100 ml of test solution. Test vessels were randomly arranged on a rotary shaker adjusted to 100 rpm in an incubator during the test (a random numbers table was used to select the location of each vessel). A 24 hour light and 0 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of approximately 380 footcandles.

The number of algal cells/ml in each test vessel and the occurrence of relative size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells was determined visually by means of direct microscopic examination with a hemocytometer. Cell counts were made and recorded daily during the 96 hour test.

Table 2. Test system summary for the toxicity test with N2803-3 and the freshwater alga, *Selenastrum capricornutum*.

Species:	<i>Selenastrum capricornutum</i>
Culture Acclimation Period:	>14 days
Test Duration:	96 hours
Test Vessels:	250 ml glass flasks
Volume of Test Media:	100 ml
Number of Replicate Test Vessels:	3
Inoculum:	10,000 cells per ml
Oscillation Rate:	100 rpm
Incubator Temperature Range:	23.5-24.0°C
Photoperiod:	24 hours light and 0 hours dark.
Light Intensity:	~380 footcandles
Water Quality Measurements:	pH in each test vessel at the beginning and end of the test. Incubator temperature daily. Dilution water analysis is summarized in Table 1.
End Points:	24, 48, 72, and 96 hour EC10, EC50, and EC90; no observed effect concentration.
Acceptability Criteria:	Logarithmic growth at 96 hours and acceptable temperature range.

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The determination of whether toxic effects were algistatic or algicidal was performed at the conclusion of the toxicity test (0.5 ml of media from each 250 mg/L vessel was transferred to a vessel containing fresh media without N2803-3 and incubated for 216 hours under test conditions).

Temperature of the incubator was measured and recorded daily (thermometer number 2968), and pH (Beckman model pH 12 meter; instrument number 124) was determined in each test vessel at the beginning and end of the test. The temperature in a representative vessel of water incubated with the test vessels was continuously recorded.

STATISTICAL METHODS:

The average specific growth rate was calculated as the natural log of the number of cells/ml at 24, 48, 72, and 96 hours minus the natural log of the number of cells/ml at 0 hours divided by the exposure period. The percent change from the control was calculated by subtracting the treatment average specific growth rate from the control average specific growth rate, dividing the difference by the average specific growth rate in the control, and multiplying that value by 100.

Results of the toxicity test were interpreted by standard statistical techniques. The binomial/interpolation method (Stephan, 1984) was used for calculating 72 and 96 hour values. All calculations were performed using the number of cells/ml and the average specific growth rates and nominal concentrations of the test substance. The no observed effect concentration (NOEC) was determined using a parametric one-way analysis of variance (ANOVA) and the number of cells/ml and the average specific growth rate in each test vessel at the end of the test.

IX. RESULTS

No insoluble material was noted in any test vessel during the test. The algal population in the control test vessels grew well, resulting in an average of 1,671,000 cells/ml after 96 hours (Table 3). Water quality throughout the test was within acceptable limits (Appendix A). The range of incubator temperatures was 23.5 to 24.0°C (Table A.1). The pH of test media (Table A.2) was affected by the test substance at the beginning of the test. Test substance concentrations above 125 mg/L reduced pH values to 4.0 and lower.

Biological data generated by the acute toxicity test with N2803-3 are presented in Tables 3 and 4. The growth curve for algae exposed to the test substance for 96 hours is presented in Figure 1 (the slope of the dose-response curve could not be calculated from this data set). The 72 and 96 hour EC50 values for algae exposed to N2803-3 are presented in Table 6.

The 96 hour EC50 (and associated 95 percent confidence limits) is 180 mg/L (125 and 260 mg/L), based on test substance as-tested (50% N2803-3 and 50% isopropanol) and the 96 hour EC50 (and associated 95 percent confidence limits) is 90 mg/L (63 and 130 mg/L), based on test substance as-received (N2803-3 without isopropanol). No effects (size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells) were noted. The 96 hour no observed effect concentration (NOEC) is 125 mg/L and the lowest observed effect concentration (LOEC) is 250 mg/L N2803-3, based on test substance as-tested and the 96 hour NOEC is 63 mg/L and the lowest observed effect concentration (LOEC) is 130 mg/L N2803-3, based on test substance as-received.

The determination of whether toxic effects were algistatic or algicidal was performed at the conclusion of the toxicity test. A 0.5 ml aliquot of media from each 250 mg/L (as-tested) vessel was transferred to a vessel containing fresh media without N2803-3 and incubated for 216 hours under test conditions. Algae increased from <10,000 cells/mL to 4,320,000 cells/mL, indicating that the effect of N2803-3 at this concentration was algistatic rather than algicidal.

Biological data generated by a previous exposure of algae to isopropyl alcohol (Ward, et.al., 1995) are presented in Table 4. Algal growth was not affected at 1,000 mg/L, indicating that the concentration of isopropyl alcohol in N2803-3 can not, by itself, account for the toxicity of N2803-3 to algae.

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Table 3. Cell growth data from the acute toxicity test with M2803-3 and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of M2803-3 (mg/L)			Number of Cells/ml x 10 ³ (hour)				
as-Tested	as-Received	Rep.	0	24	48	72	96
0 (control)		1	10	22	168	360	1,800
		2	10	20	146	402	1,612
		3	10	22	154	510	1,600
		mean	10	21	156	424	1,671
		% control	--	--	--	--	--
63	32	1	10	28	134	532	1,720
		2	10	26	152	520	1,810
		3	10	18	160	410	1,666
		mean	10	24	149	487	1,732
		% control	100	114	96	115	101
125	63	1	10	10	92	540	1,620
		2	10	32	96	480	1,660
		3	10	22	140	520	1,400
		mean	10	21	109	513	1,560
		% control	100	100	70	121	93
250	130	1	10	<10	<10	<10	<10
		2	10	<10	<10	<10	<10
		3	10	<10	<10	<10	<10
		mean	10	<10	<10	<10	<10
		% control	100	<48	<6	<2	<1
500	250	1	10	<10	<10	<10	<10
		2	10	<10	<10	<10	<10
		3	10	<10	<10	<10	<10
		mean	10	<10	<10	<10	<10
		% control	100	<48	<6	<2	<1
1,000	500	1	10	<10	<10	<10	<10
		2	10	<10	<10	<10	<10
		3	10	<10	<10	<10	<10
		mean	10	<10	<10	<10	<10
		% control	100	<48	<10	<2	<1

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Table 4. Historic cell growth data from an acute toxicity test with isopropyl alcohol and the freshwater alga, *Selenastrum capricornutum* (from Ward, et.al., 1995).

Nominal Concentration of Isopropyl Alcohol (mg/L)	rep.	Number of Cells/ml x 10 ³ (hour)				
		0	24	48	72	96
0 (control)	1	10	22	134	538	1,132
	2	10	20	132	568	1,260
	3	10	23	114	614	1,356
	mean	10	22	127	573	1,249
	% control	--	--	--	--	--
4.4	1	10	14	112	584	1,236
	2	10	32	128	554	1,270
	3	10	16	144	584	1,064
	mean	10	21	128	574	1,190
	% control	100	95	101	100	95
1,000	1	10	31	136	608	1,370
	2	10	26	144	580	1,338
	3	10	24	106	512	1,334
	mean	10	27	129	567	1,347
	% control	100	123	102	99	108

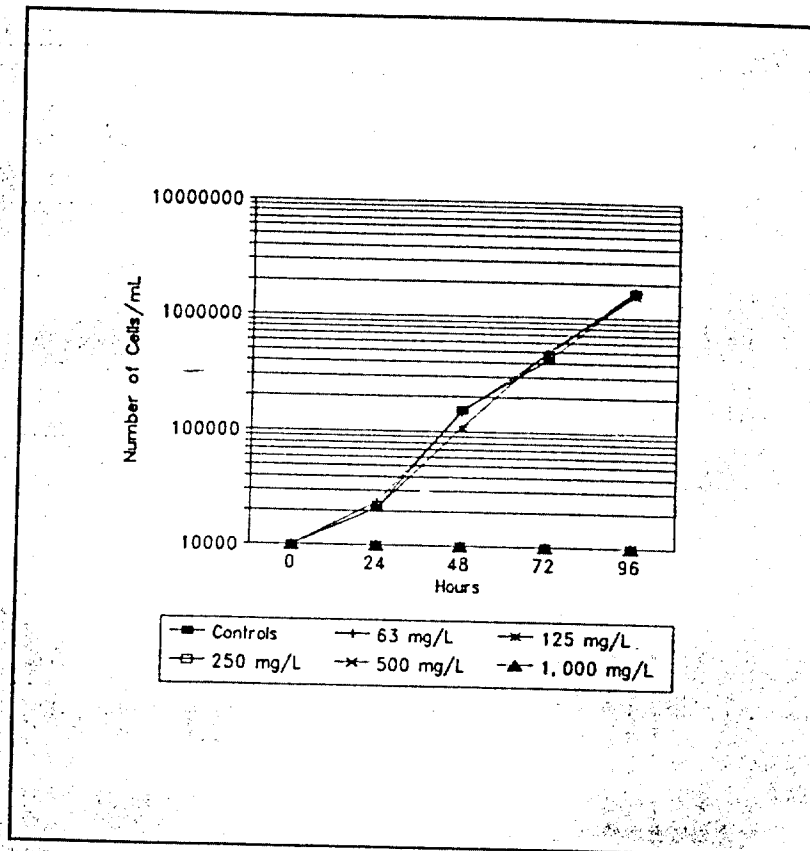


Figure 1. Growth of *Selenastrum capricornutum* exposed to N2803-3 for 96 hours.

Table 5. Average specific growth rate and percent change from the control from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and N2803-3.

Nominal Concentration of N-2803-3 (mg/L)		Average Specific Growth Rate				Percent Change From Control			
as-Tested	as-Received	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr
0 (control)		0.031	0.057	0.052	0.053	--	--	--	--
63	32	0.036	0.056	0.054	0.054	-16	2	-4	-2
125	63	0.031	0.050	0.055	0.053	0	12	-6	0
250	130	0.000	0.000	0.000	0.000	100	100	100	100
500	250	0.000	0.000	0.000	0.000	100	100	100	100
1,000	500	0.000	0.000	0.000	0.000	100	100	100	100

Table 6. Effective concentrations (EC50s) from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and M2803-3.

Time	EC Value (mg/L)	95 Percent Confidence Limits (mg/L)
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Calculated based on Test Substance as-Tested (50% M2803-3 and 50% Isopropanol)

1. Calculated Using the Number of Cells/ml

72 hours	EC50 = 180	125 - 250
96 hours	EC50 = 180	125 - 250

2. Calculated Using the Average Specific Growth Rate

72 hours	EC50 = 180	125 - 250
96 hours	EC50 = 180	125 - 250

Calculated based on Test Substance as-Received (M2803-3 without Isopropanol)

1. Calculated Using the Number of Cells/ml

72 hours	EC50 = 90	63 - 130
96 hours	EC50 = 90	63 - 130

2. Calculated Using the Average Specific Growth Rate

72 hours	EC50 = 90	63 - 130
96 hours	EC50 = 90	63 - 130

Note: EC10 and EC90 values could not be calculated using the data from this toxicity test.

X. REFERENCES

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XI. SIGNATURE PAGE

Jeanne Magazu 1/24/96
 Jeanne P. Magazu
 Study Director
 Aquatic Toxicologist

Peter L. Kowalski 1-24-96
 Peter L. Kowalski
 Aquatic Toxicologist

Peter C. Nevius 1/24/96
 Peter C. Nevius
 Biologist

Jacqueline M. Nevius 1-24-96
 Jacqueline M. Nevius
 Biologist

For: Kaven M. Stevens 1/24/96
 For: Kaven M. Stevens
 Biologist

Robert L. Boeri 1-24-96
 Robert L. Boeri
 Coauthor

Timothy J. Ward 1/24/96
 Timothy J. Ward
 Author

XII. PROTOCOL AMENDMENTS AND DEVIATIONS

PROTOCOL AMENDMENTS:

The protocol was amended to specify that the test substance would be combined in a 50:50 ratio with isopropanol and this solution would be considered the test substance at 100% active ingredient and to list nominal concentrations for the definitive study. No other amendments were made to the protocol.

PROTOCOL DEVIATIONS:

The 24 and 48 hour EC10, EC50, and EC90 values were not calculated. The 72 and 96 hour EC10 and EC90 values and the slope of the dose response curve could not be calculated with the data from this test. The pH of test media was 7.4 rather than 7.5 at 0 hr. These deviations did not affect the outcome of this test and no other deviations were made from the protocol.

Appendix A: WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Temperature measured during the toxicity test with N2803-3 and the freshwater alga, *Selenastrum capricornutum*.

Hour of Exposure	Temperature of Incubator (°C)
0	24.0
24	23.6
48	23.5
72	23.8
96	23.5

Table A.2. pH values measured during the toxicity test with M2803-3 and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration (mg/L)		Replicate	pH	
as-Tested	as-Received		Initial	Final
Test with M2803-3				
0	(control)	1	7.4	10.3
		2	7.4	10.3
		3	7.4	10.3
63	32	1	6.8	10.3
		2	6.8	10.3
		3	6.8	10.3
125	63	1	6.4	10.3
		2	6.3	10.3
		3	6.3	10.3
250	130	1	4.0	4.1
		2	4.0	4.1
		3	4.0	4.1
500	250	1	3.4	3.4
		2	3.4	3.4
		3	3.4	3.4
1,000	500	1	3.0	3.0
		2	2.9	3.0
		3	2.9	3.0