

TOXICITY TO AQUATIC PLANTS

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143 or as the major component of FC-1015. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample is FC-1015. It's purity was not sufficiently characterized, though current information indicates is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205".

The following summary applies to the test sample as a mixture of the test substance in water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance. Data was used to compare toxicity of the branched/straight chain ammonium perfluorooctanoate homologue mixture in FC-143 vs. what is supposed to be the 100% straight carbon chain ammonium perfluorooctanoate in FC-1015.

METHOD:

Method: OECD 201, USEPA – TSCA, Guideline 797.1050

Type (test type): Acute Static

GLP: No

Year study performed: 1996

Species: *Selenastrum capricornutum*

Source: Originally obtained from the University of Texas at Austin on January 10, 1996, maintained in culture medium at T.R. Wilbury Laboratories, Inc, Marblehead, Massachusetts.

Element basis: Algal cell counts (cells/ml), cell dry weights.

Exposure period: 96 hours

Statistical Methods: Probit analysis

Analytical monitoring: pH and temperature.

Test Conditions:

Algal Nutrient Medium: Sterile enriched nutrient medium per USEPA 1978 guideline. This nutrient medium provided all mineral nutrients essential for algal growth and also served as the diluent for all algal operations. The pH of this synthetic algal medium was adjusted to 7.5 prior to use in assays.

Stock and test solution preparation: A 3330 mg/L stock solution was prepared by diluting 3.33 grams of test substance in 1 liter of water. Aliquots

were then added directly to dilution water in test vessels to create test solutions.

Exposure vessels: 250 ml Erlenmeyers containing 100 ml test solution and capped with inverted glass beakers.

Agitation: Shaken continuously at 100 rpm.

Number of replicates: 3

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

Number of concentrations: five plus a blank control

Lighting: 400 foot-candles from continuous cool-white fluorescent lighting.

Water Chemistry:

pH range (0-96 hours):

7.5 – 10.8 (control exposure)

7.4 – 7.6 (3,330 mg/L exposure)

Test temperature range (0-96 hours):

24.0 – 24.2 °C (incubator)

RESULTS

Nominal concentrations: Blk control, 210, 430, 830, 1670, and 3330 mg/L

Algal Growth Response EC₅₀ values

Exposure (Contact) Hours	Cell-Count mg/L (95% C.I.)	Average Specific Growth Rate mg/L (95% C.I.)
24	2510 (1340->3330)	1700 (673->3300)
48	>3330	>3330
72	2040 (1190->3330)	>3330
96	1980 (1710-2360)	>3330

Element values are based on nominal concentrations.

Reversibility of Growth Inhibition: Aliquots of the 3330 mg/L test solution were diluted with algal medium and cultured for 72 hours. Based on growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The values reported apply to that mixture and not the test substance.

CONCLUSIONS

FC-1015 exhibits a 96-hour EC₅₀ cell count value of 1980 (1710-2360 mg/L) and a 96-hour E C₅₀ growth rate value of >3330 mg/L.

The 96-hour No Observed Effect Concentration (NOEC) is 210 mg/L for cell count and 430 mg/L for growth rate. The Lowest Observed Effect Concentration (LOEC) is 430 mg/L for cell count and 830 mg/L for growth rate. 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 = 2. This study meets the criteria for quality testing. However, the study lacks information on purity of the test substance and actual measurements of the amount of test substance in solution. Also, no explanation is given as to why the 48-hour values were in excess of the 24 hour values.

REFERENCES

Test was conducted by T.R. Wilbury Laboratories, Inc., of Marblehead, Massachusetts at the request of the 3M Company, Lab Request number P1624, 1996.

OTHER

Last changed: 5/24/00

P1624

Study Title

Growth and Reproduction Toxicity Test With FC-1015
And The Freshwater Alga, *Selenastrum capricornutum*

Guideline Number

U.S. EPA-TSCA, Guideline 797.1050
OECD, Guideline 201

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Study Completed

May 15, 1996

Sponsor

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III. SUMMARY

The toxicity of FC-1015 to the freshwater alga, *Selenastrum capricornutum*, is described in this final report. The test was conducted for 3M Company for 96 hours from April 19 to 23, 1996 at T.R. Wilbury Laboratories, Inc. in Marblehead, Massachusetts. It was conducted according to procedures of the U.S. Environmental Protection Agency (1993) and the OECD (1984). FC-1015 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 $24 \pm 2^\circ\text{C}$. Nominal concentrations of FC-1015 were 0 (control), 210, 430, 830, 1,670, and 3,330 mg/L. The dilution water was sterile enriched media adjusted to a pH of 7.5.

Exposure of algae to the test substance for 96 hours resulted in a median effective concentration (EC50) of 1,980 mg/L FC-1015 (95% confidence interval = 1,710 to 2,360 mg/L) when calculated using the number of cells/ml, and >3,330 mg/L when calculated using the average specific growth rate. The 96 hour no observed effect concentration (NOEC) is 210 mg/L and the lowest observed effect concentration (LOEC) is 430 mg/L when calculated using the number of cells/ml. The NOEC is 430 mg/L and the LOEC is 830 when calculated with the average specific growth rate.

IV. GENERAL INFORMATION

Material Tested:	FC-1015
Sponsor:	3M Company Environmental Laboratory 935 Bush Avenue Building 2-3E-09 St. Paul, Minnesota 55106
Test Substance Shipped From:	3M Company Environmental Laboratory 935 Bush Avenue Building 2-3E-09 St. Paul, Minnesota 55106
Reported Purity:	100%
Experimental Start Date:	April 19, 1996
Experimental Completion Date:	April 23, 1996
Archive Location for the Raw Data and a Copy of the Final Report:	T.R. Wilbury Laboratories, Inc. Marblehead, Massachusetts

V. 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 was conducted following U.S. EPA (TSCA) and OECD guidelines but at the Sponsor's request it was not conducted according to Good Laboratory Practice (GLP) rules. 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.

VI. METHODS AND MATERIALS

TEST SUBSTANCE:

The sample of FC-1015 (T.R. Wilbury Laboratories Sample Number 604) was delivered to T.R. Wilbury Laboratories on April 4, 1996. The sample was delivered in a metal can within a cardboard box at ambient temperature. The test substance was contained in 1 250-ml plastic bottle. The label attached to the bottle contained the following information: "FC-1015, (LR P1624), See MSDS, Susan A. Beach, 2-3E-09, (612) 778-7452, 4/2/48".

FC-1015 (a clear liquid) was supplied by 3M Company, St Paul, Minnesota. Prior to use the test substance was stored in the dark at room temperature. 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 is 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 prior to use. Characterization of a representative sample of water used to formulate 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 January 10, 1996. 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 an 8 day old culture. Identification of the culture organisms was verified using an appropriate taxonomic key.

Table 1. Chemical characterization of a representative sample of water used to formulate test media for the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and PC-1015.

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.

TOXICITY TEST:

The toxicity test was performed from April 19 to 23, 1996, according to procedures of the U.S. Environmental Protection Agency (1993) and the OECD (1984). A summary of the test conditions is presented in Table 2.

A screening test was not conducted because nominal concentrations for the definitive test were provided by the sponsor. 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 were: 0 mg/L (control), 210, 430, 830, 1,670, and 3,330 mg/L. A 3,330 mg/L stock solution was formulated by adding 3.33 g of test substance to a 1,000 ml Class A volumetric flask and filling the flask to the mark with sterile algal media. The stock solution was mixed and appropriate amounts were added directly to dilution water in test vessels to formulate test media.

Algae were distributed among three replicates of each treatment at the rate of approximately 10,000 cells/ml. The test was performed in 250 ml glass Erlenmeyer flasks that contained 100 ml of test solution. Test vessels were capped with inverted glass beakers and 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 400 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.

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 3,330 mg/L vessel were transferred to a vessel containing 100 ml of fresh media without FC-1015 and incubated for 72 hours under test conditions).

Temperature of the incubator was measured and recorded daily (thermometer number 3794), and pH (Beckman model PHI 12 meter; instrument number 144) 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.

Table 2. Test conditions summary for the toxicity test with FC-1015 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:	24.0-24.2°C
Photoperiod:	24 hours light and 0 hours dark.
Light Intensity:	400 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.

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, when possible. The probit method (Stephan, 1983) was used for calculating EC10, EC50, and EC90 values (the probit method was used despite cautionary statements because EC50 values compared well to values compared to other methods). All calculations were performed using the number of cells/ml and the average specific growth rates and nominal concentrations of FC-1015. 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.

VII. RESULTS

Insoluble material was not observed in test vessels during the test. The algal population grew well, resulting in an average of 1,471,000 cells/ml in the control after 96 hours (Table 3). Water quality throughout the test was within acceptable limits (Appendix A). The range of incubator temperatures was 24.0 to 24.2°C (Table A.1). The pH of test media was not significantly affected by the test substance at the beginning of the test (Table A.2).

Biological data generated by the acute toxicity test with FC-1015 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 is 2.2 based on cells/ml at 96 hours). The EC10, EC50, and EC90 values for algae exposed to FC-1015 are presented in Table 5. The 96 hour EC50 (and associated 95 percent confidence limits) is 1,980 mg/L (1,710 and 2,360 mg/L) when calculated using the number of cells per ml, and >3,330 mg/L when calculated using the average specific growth rate. No effects (size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells) were noted during the test. The 96 hour NOEC is 210 mg/L and the LOEC is 430 mg/L FC-1015, when calculated using the number cells/ml and the 96 hour NOEC is 430 mg/L and the LOEC is 830 mg/L FC-1015, when calculated using the average specific growth rate.

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 3,330 mg/L vessel was transferred to a vessel containing fresh media without FC-1015 and incubated for 72 hours under test conditions. Algae increased from <10,000 cells/ml to 830,000 cells/ml, indicating that the effect of FC-1015 at this concentration was algistatic rather than algicidal.

Table 3. Cell growth data from the toxicity test with FC-1015 and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of FC-1015 (mg/L)	Replicate	Number of Cells/ml x 10 ³ (hour)				
		0	24	48	72	96
0 (control)	1	10	32	136	664	1,480
	2	10	32	146	662	1,518
	3	10	34	128	696	1,416
	mean	10	33	137	674	1,471
210	1	10	30	154	724	1,442
	2	10	30	146	650	1,496
	3	10	34	144	712	1,466
	mean	10	31	148	695	1,468
	% control	100	94	108	103	100
430	1	10	30	138	620	1,338
	2	10	40	140	598	1,280
	3	10	28	148	662	1,452
	mean	10	33	142	627	1,357
	% control	100	100	104	93	92
830	1	10	30	156	400	1,190
	2	10	24	160	484	1,096
	3	10	28	150	436	1,168
	mean	10	27	155	440	1,151
	% control	100	82	113	65	78
1,670	1	10	20	140	404	798
	2	10	22	142	402	862
	3	10	16	138	380	870
	mean	10	19	140	395	843
	% control	100	58	102	59	57
3,330	1	10	16	88	280	460
	2	10	12	84	232	454
	3	10	14	80	250	488
	mean	10	14	84	254	467
	% control	100	42	61	38	32

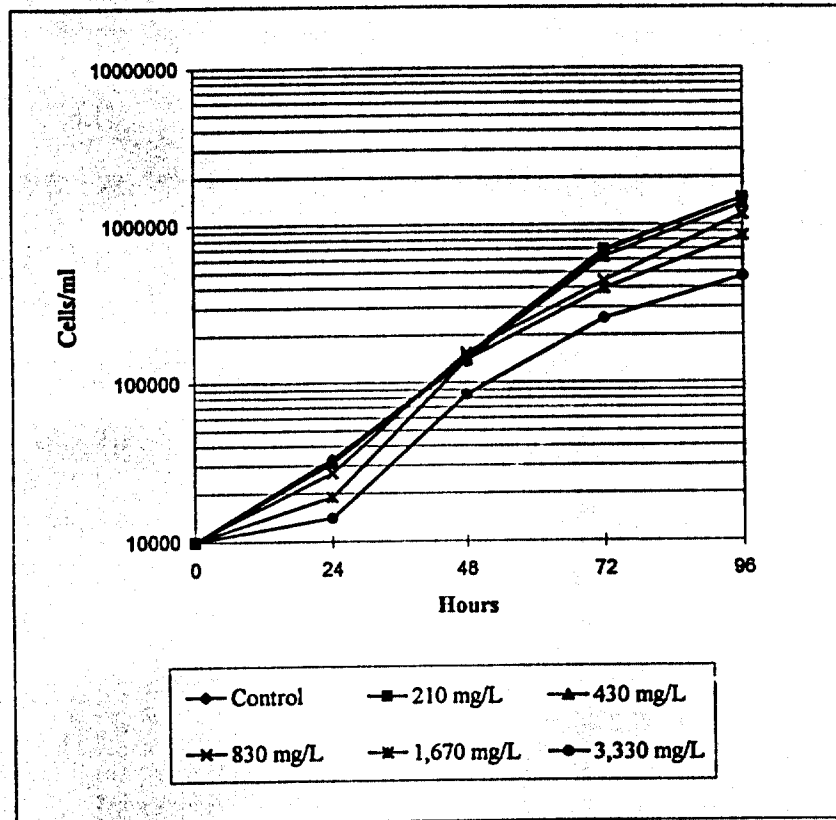


Figure 1. Growth of *Selenastrum capricornutum* exposed to FC-1015 for 96 hours.

Table 4. Average specific growth rate and percent change from the control from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and FC-1015.

Nominal Concentration of FC-1015 (mg/L)	Average Specific Growth Rate				Percent Change From Control			
	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr
0 (control)	0.050	0.055	0.058	0.052	--	--	--	--
210	0.047	0.056	0.059	0.052	6	-2	-2	0
430	0.050	0.055	0.057	0.051	0	0	2	2
830	0.041	0.057	0.053	0.049	18	-4	9	6
1,670	0.027	0.055	0.051	0.046	46	0	12	12
3,330	0.014	0.044	0.045	0.040	72	20	22	23

Table 5. Effective concentrations (EC10s, EC50s, EC90s) from the toxicity test with the freshwater alga, *Selenastrum capricornutum*, and FC-1015.

Time	EC Value (mg/L)	95 Percent Confidence Limits (mg/L)
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Calculated Using the Number of Cells/ml

24 hours	EC10 = 524 EC50 = 2,510 EC90 = >3,330	<210 - 1,010 1,340 - >3,330 >3,330
48 hours	EC50 = >3,330	>3,330
72 hours	EC10 = 448 EC50 = 2,040 EC90 = >3,330	<210 - 814 1,190 - >3,330 >3,330
96 hours	EC10 = 533 EC50 = 1,980 EC90 = >3,330	417 - 643 1,710 - 2,360 >3,330

Calculated Using the Average Specific Growth Rate

24 hours	EC10 = 541 EC50 = 1,700 EC90 = >3,330	<210 - 1,110 673 - >3,330 2,260 - >3,330
48 hours	EC50 = >3,330	>3,330
72 hours	EC10 = 1,330 EC50 = >3,330 EC90 = >3,330	970 - 1,770 >3,330 >3,330
96 hours	EC10 = 1,430 EC50 = >3,330 EC90 = >3,330	1,070 - 1,860 >3,330 >3,330

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IX. SIGNATURE PAGE

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Appendix A: WATER QUALITY DATA FROM THE TOXICITY TEST

Table A.1. Temperature measured during the toxicity test with FC-1015 and the freshwater alga, *Selenastrum capricornutum*.

Hour of Exposure	Temperature of Incubator (°C)
0	24.0
24	24.1
48	24.2
72	24.0
96	24.0

Table A.2. pH values measured during the toxicity test with FC-1015 and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of FC-1015 (mg/L)	Replicate	pH	
		Initial	Final
0 (control)	1	7.5	10.7
	2	7.5	10.8
	3	7.5	10.8
210	1	7.4	9.9
	2	7.4	9.8
	3	7.4	10.0
430	1	7.4	6.5
	2	7.4	6.4
	3	7.4	6.4
830	1	7.4	6.1
	2	7.4	7.0
	3	7.4	7.4
1,670	1	7.4	7.6
	2	7.4	7.6
	3	7.4	7.4
3,330	1	7.4	7.6
	2	7.4	7.5
	3	7.4	7.6