

TOXICITY TO AQUATIC PLANTS

TEST SUBSTANCE

Identity: Mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-203CF-X or FC-203CF. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was 101. The test sample is FC-203CF. Current information indicates it is a mixture of 1% PFOS, 20% diethylene glycol monobutyl ether, 70.2% water, 2.75% Acrylic foamer, 3% sodium octyl sulfate, 1% triethanolamine, 2% sulfonated alkyl ether salts, and 0.05% tolyltriazole.

The following summary applies to a mixture with incompletely characterized concentrations of impurities. Data may not accurately reflect toxicity of the fluorochemical component of the test sample.

METHOD:

Method: OECD Guideline 201

Type: Acute Static

GLP: No

Year study performed: 1991

Species: *Selenastrum capricornutum*

Source: In-house culture. Originally from the Culture Collection of Algae at the University of Texas at Austin, Texas.

Element basis: Algal cell counts (cells/ml), growth rate

Exposure period: 96 hours

Statistical Methods: Statistical analyses were performed using the computer program of Stephan, 1983.

Analytical monitoring: pH, conductivity, and temperature

Test Conditions:

Algal Nutrient Medium: Nutrient medium per U.S. EPA, 1978. 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 8.0.

Stock and test solution preparation: A primary 1000 mg/L stock solution was prepared in algal medium, and added directly to the exposure vessels.

Exposure vessels: 250 mL Erlenmeyer flasks containing 100 mL test solution and capped with inverted glass beakers.

Agitation: Continuous

Number of replicates: 3.

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

Number of concentrations: five plus a blank control

Lighting: Continuous lighting at 44 $\mu\text{Ein}/\text{sec}/\text{m}^2$ using cool-white fluorescent lamps.

Water Chemistry:

pH range (0 – 96 hours):

8.0 – 10.8 (control exposure)

8.2 – 8.3 (1000 mg/L exposure)

Test temperature range (0 – 96 hours):

21.9 – 22.3°C (incubator)

RESULTS

Nominal concentrations: Blank control, 62.5, 125, 250, 500, and 1000 mg/L

96-Hr. Algal Growth Response values

Nominal Exposure Conc. (mg/L)	Cell-Count (cells/mL)	Avg. Specific Growth rate	% Change from Control
Control	602,000	0.043	---
62.5	451,000	0.040	7
125	207,000	0.032	23
250	12,000	0.002	95
500	0	0.000	>100
1000	0	0.000	>100

Element values: 96-hour E_rC_{50} = 143 (130 – 156) mg/L

96-hour NOEC = 62.5 mg/L

Element values are based on nominal concentrations.

Reversibility of Growth Inhibition: Not conducted.

Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical proportion alone.

CONCLUSIONS

The test substance exhibits a 96-hour E_rC_{50} of 143 mg/L with a 95% confidence interval of 130 to 156 mg/L. The 96-hour No Observed Effect Concentration (NOEC) was 62.5 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking = 2. The study meets the criteria for quality testing. However, the sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

This test was conducted by EnviroSystems Division, Resource Analysts, Incorporated, of Hampton, NH at the request of the 3M Company, St. Paul, MN, Lab Request number J1884-3, 1991.

OTHER

Last changed: 6/28/00

Study Title

Static Acute Toxicity of FC-203CF
to the Alga, *Selenastrum capricornutum*

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Study Completed

August, 1991

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I. SUMMARY

The acute toxicity of FC-203CF to the alga, *Selenastrum capricornutum*, is described in this final report. The test was conducted for 3M Company for 96 hours during July 15 to 19, 1991, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Ellen Stanford, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 22.1°C. The dilution water was synthetic media prepared with sterile deionized water. Flasks were incubated on a rotary shaker. Light was adjusted to 44 uEin/sec/m² with a photoperiod of 24 hours light and 0 hours dark. Nominal concentrations of FC-203CF were: 0 mg/L (control), 62.5 mg/L, 125 mg/L, 250 mg/L, 500 mg/L, and 1,000 mg/L. Nominal concentrations were used for all calculations.

Algae used in the test was from an in-house culture that was maintained under test conditions. Exposure of algae to the test substance resulted in a 72 hour EC50 of 130 mg/L FC-203CF and a no observed effect concentration (NOEC) of 62.5 mg/L. The 96 hour EC50 and NOEC are 143 mg/L and 62.5 mg/L, respectively.

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IV. INTRODUCTION

This study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of FC-203CF to a freshwater alga. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the test.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

FC-203CF (EnviroSystems Sample Number 2937E) was delivered to EnviroSystems on May 23, 1991. It was contained in a 4 oz. plastic bottle that was labelled with the following information: "J1884-3, FC-203CF". FC-203CF (an orange-brown liquid) was supplied by 3M Company, 935 Bush Avenue, St. Paul, Minnesota. Prior to use the test material was stored in the dark at room temperature. A reserve sample (approximately 1.0 gram) will be retained at EnviroSystems for a minimum of 10 years.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was sterile enriched media (U.S. EPA, 1978) with a pH of approximately 8.0. Results of chemical analysis of a representative sample of deionized water used to prepare the media are presented in Table 1.

TEST ORGANISM:

Algae used for the test was from a culture originally procured from the Culture Collection of Algae at the University of Texas at Austin and delivered to EnviroSystems on May 14, 1991. The identity of the culture was verified using an appropriate taxonomic key. The culture was transferred to sterile enriched media identical to media used for this test and maintained at test conditions.

TOXICITY TESTING:

A screening test with the test substance was conducted June 24 to 28, 1991. Nominal concentrations of the test material were: 1 mg/L, 10 mg/L, 100 mg/L, and 1,000 mg/L. After 96 hours the number of cells per ml at 1,000 mg/L was less than 50% of the control and at all lower concentrations the number of cells per ml was 50% of the control or greater.

The definitive toxicity test was performed during July 15 to 19, 1991. It is based on procedures of the OECD (1984).

Table 1. Chemical characterization of a representative sample of deionized water used to prepare test media for toxicity test

Parameter	Unit of Measurement	Reporting Limit	Measured Value
Organochlorine pesticides	ug/L	2	ND
Organophosphorus pesticides	ug/L	0.5	ND
Polychlorinated biphenyls	ug/L	0.5	ND

Note: ND = not detected at or above the reporting limit.

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. A 1,000 mg/L stock solution was prepared in dilution water. The stock solution was added directly to dilution water contained in the test vessels without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L (control), 62.5 mg/L, 125 mg/L, 250 mg/L, 500 mg/L, and 1,000 mg/L.

Algae was randomly and equally 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. The flasks were capped with inverted glass beakers. Test vessels were randomly arranged on a rotary shaker in an incubator during the 96 hour test (a random numbers table was used to select the location of each vessel). A 24 hour light and 0 hour dark photoperiod was maintained with cool-white fluorescent lights that provided a light intensity of $44 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

The number of algal cells/ml in each test vessel was determined visually every 24 hours by means of direct microscopic counts with a hemacytometer. The pH (Beckman model pH 51 meter) was determined in each test flask at the beginning and end of the test, and temperature (ASTM mercury thermometer) was measured and recorded daily in the incubator.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques (Stephan, 1983). The binomial or moving average method was used by the author to calculate EC50 values using nominal concentrations of test substance. The NOEC was calculated using Dunnett's test, which includes a parametric one-way analysis of variance (ANOVA). The average specific growth rate was calculated as the natural log of the number of cells per ml at 24, 48, 72, or 96 hours minus the natural log of the number of cells per ml at 0 hours divided by the exposure period. The percent change from control is calculated by subtracting the sample average specific growth rate from the control average specific growth rate, dividing that value by the average specific growth rate in the control and multiplying that value by 100. The percent change from control was used to compute EC50 and NOEC values.

VI. RESULTS

Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Appendix A, respectively, and the percent of control and average specific growth rate information is presented in Table 3.

The 24, 48, 72, and 96 hour EC50s for algae exposed to FC-203CF are presented in Table 4. The 72 hour EC50 is 130 mg/L, and the 72 hour NOEC is 62.5 mg/L FC-203CF. The 96 hour EC50 is 143 mg/L and the 96 hour NOEC is 62.5 mg/L.

Table 2. Growth data from toxicity test

Nominal Concentration (mg/L)	rep.	Number of Cells per Milliliter $\times 10^3$ (hour)				
		0	24	48	72	96
0.0 (control)	1	10	28	128	310	620
	2	10	28	116	246	512
	3	10	38	146	288	674
	mean	10	31	130	281	602
62.5	1	10	20	88	218	470
	2	10	22	44	162	388
	3	10	22	66	156	494
	mean	10	21	66	179	451
125	1	10	20	44	52	220
	2	10	18	80	58	198
	3	10	22	58	74	202
	mean	10	20	61	61	207
250	1	10	16	14	12	16
	2	10	22	20	12	12
	3	10	18	20	10	8
	mean	10	19	18	11	12
500	1	10	12	6	4	0
	2	10	20	16	2	0
	3	10	16	4	4	0
	mean	10	16	9	3	0
1,000	1	10	10	4	8	0
	2	10	10	12	4	0
	3	10	10	4	4	0
	mean	10	10	7	5	0

Table 3. Percent change from control and average specific growth rate from toxicity test

Nominal Concentration of Test Substance (mg/L)	Average specific growth rate				Percent change from control			
	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr
0.0 (control)	0.048	0.053	0.046	0.043	0	0	0	0
62.5	0.031	0.040	0.040	0.040	35	24	13	7
125	0.029	0.038	0.025	0.032	40	28	46	23
250	0.027	0.012	0.001	0.002	44	77	98	95
500	0.020	-0.002	-0.016	0.000	58	>100	>100	>100
1,000	0.000	-0.009	-0.009	0.000	100	>100	>100	>100

Table 4. Median effective concentrations (EC50s) from toxicity test

Exposure period	EC50	95 percent confidence limits	Calculation method
24 hours	212 mg/L	182 - 244 mg/L	Moving Average
48 hours	156 mg/L	140 - 174 mg/L	Moving Average
72 hours	130 mg/L	120 - 141 mg/L	Moving Average
96 hours	143 mg/L	130 - 156 mg/L	Moving Average

VII. REFERENCES

OECD. 1984. Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 201, Alga, Growth Inhibition Test. Adopted June 4, 1984.

Stephan, C.E. 1983. Computer program for calculation of LC50 values. Personal communication.

U.S. EPA. 1978. The *Selenastrum capricornutum* Printz Algal Assay Bottle Test. EPA-600/9-78-018. July, 1978.

Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Temperature measured during toxicity test

Day of Exposure	Temperature of Incubator, °C
0	21.9
1	22.1
2	22.1
3	22.0
4	22.3

Table A.2. pH measured during toxicity test

Nominal concentration (mg/L)	Replicate	pH	
		initial	final
0 (control)	1	8.0	10.8
	2	8.0	10.8
	3	8.1	10.6
62.5	1	8.2	10.4
	2	8.2	10.1
	3	8.3	10.2
125	1	8.3	9.0
	2	8.3	9.0
	3	8.3	9.0
250	1	8.3	8.5
	2	8.3	8.5
	3	8.3	8.5
500	1	8.3	8.4
	2	8.3	8.4
	3	8.3	8.4
1,000	1	8.2	8.3
	2	8.2	8.3
	3	8.2	8.3