

TOXICITY TO AQUATIC PLANTS

TEST SUBSTANCE

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

Remarks: The 3M production lot number was not noted. The test sample is FC-203. Current information indicates it is a mixture of 1.34% PFOS, 35% diethylene glycol butyl ether, 37.85% water, 20% ethylene glycol, 2.66 % Sultone foamer, 3% sodium octyl sulfate, 0.1% sodium lauryl sulfate, 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 cultures. Originally obtained from the Culture Collection of Algae at the University of Texas at Austin.

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

Exposure period: 96 hours

Statistical Methods: The EC50 values were calculated using the computer program of Stephan, 1983. The NOEC was calculated using Dunnett's test.

Analytical monitoring: pH 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 1000 mg/L primary stock solution was prepared in nutrient medium. Aliquots were then added directly to the 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: 35 $\mu\text{Ein}/\text{sec}/\text{m}^2$ from continuous cool-white fluorescent lighting.
Water Chemistry:
 pH range (0-96 hours):
 7.8 – 9.4 (control exposure)
 7.8 – 8.0 (1000 mg/L exposure)
 Test temperature range (0-96 hours):
 23.3 – 23.4 °C (incubator)

RESULTS

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

96-Hour Algal Growth Response Values

Nominal Conc. (mg/L)	Mean Cell-Count (cells/mL)	Average Specific Growth Rate	Percent Change in Growth Rate from Control (96 hrs.)
Control	399,000	0.038	---
62.5	645,000	0.044	-16
125	347,000	0.037	3
250	69,000	0.020	47
500	51,000	0.017	55
1000	12,000	0.002	95

Algal Growth Response E_rC_{50} values:

96-hour E_rC_{50} = 354 (323 – 389) mg/L
 96-hour NOEC = 500 mg/L

Element values are based on nominal concentrations.

Reversibility of Growth Inhibition: Not give.

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} growth rate value of 354 (323 – 389) mg/L. The 96-hour No Observed Effect Concentration (NOEC) is 500 mg/L for growth rate.

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, 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, Inc., or Hampton, NH at the request of the 3M Company, Lab Request number H2864-9, 1991.

OTHER

Last changed: 6/27/00

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Study Title

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

Authors

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Study Completed

June, 1991

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I. SUMMARY

The acute toxicity of FC 203 to the alga, *Selenastrum capricornutum*, is described in this final report. The test was conducted for 3M Company for 96 hours during May 6 to 10, 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 23.6°C. The dilution water was synthetic media prepared with sterile deionized water. Flasks were incubated on a rotary shaker. Light was adjusted to 35 $\mu\text{Ein}/\text{sec}/\text{m}^2$ with a photoperiod of 24 hours light and 0 hours dark. Nominal concentrations of FC 203 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 338 mg/L FC 203 and a no observed effect concentration (NOEC) of 250 mg/L. The 96 hour EC50 and NOEC are 354 mg/L and 500 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 203 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 203 (EnviroSystems Sample Number 2699E) was delivered to EnviroSystems on November 15, 1990. It was contained in a 250 mL glass bottle that was labelled with the following information: "H 2864-9, FC 203". FC 203 (a clear amber 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 1.1 grams) will be archived 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 April 12, 1990. 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:

The definitive toxicity test was performed during April 22 to 26, 1991. It is based on procedures of the OECD (1984). 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.

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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.

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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 $35 \mu\text{Es}^{-1}\text{m}^{-2}$.

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 PHI 12 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 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.

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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 203 are presented in Table 4. The 72 hour EC50 is 338 mg/L, and the 72 hour NOEC is 250 mg/L FC 203. The 96 hour EC50 is 354 mg/L and the 96 hour NOEC is 500 mg/L.

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Table 2. Growth data from toxicity test

Nominal Concentration (mg/L)	rep.	Number of Cells per Milliliter x 10 ³ (hour)				
		0	24	48	72	96
0.0 (control)	1	10	26	94	188	394
	2	10	32	34	172	418
	3	10	30	116	282	386
	mean	10	29	81	214	399
62.5	1	10	30	92	346	604
	2	10	52	94	256	596
	3	10	48	92	296	734
	mean	10	43	93	299	645
125	1	10	26	52	100	350
	2	10	26	76	82	214
	3	10	34	46	94	476
	mean	10	29	58	92	347
250	1	10	28	48	62	76
	2	10	26	62	42	82
	3	10	20	42	64	50
	mean	10	25	51	56	69
500	1	10	32	32	30	58
	2	10	44	28	28	58
	3	10	28	64	26	36
	mean	10	35	41	28	51
1,000	1	10	24	36	28	12
	2	10	20	36	24	10
	3	10	26	32	30	14
	mean	10	23	35	27	12

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)	0.045	0.044	0.043	0.038	0	0	0	0
62.5	0.062	0.047	0.047	0.044	-38	-7	-9	-16
125	0.044	0.037	0.031	0.037	2	16	28	3
250	0.038	0.034	0.024	0.020	16	23	44	47
500	0.052	0.030	0.014	0.017	-16	32	67	55
1,000	0.036	0.026	0.014	0.002	20	41	67	95

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

Exposure period	EC50	95 percent confidence limits	Calculation method
24 hours	>1,000 mg/L	---	---
48 hours	>1,000 mg/L	---	---
72 hours	338 mg/L	293 - 395 mg/L	moving average
96 hours	354 mg/L	323 - 389 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.

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Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

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Table A.1. Temperature measured during toxicity test

Day of Exposure	Temperature of Incubator, °C
0	23.3
1	23.3
2	23.3
3	23.4
4	23.4

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Table A.2. pH measured during toxicity test

Nominal concentration (mg/L)	Replicate	pH	
		initial	final
0 (control)	1	7.8	8.0
	2	7.8	8.0
	3	7.8	9.4
62.5	1	7.7	9.5
	2	7.7	10.0
	3	7.7	9.5
125	1	7.7	8.7
	2	7.7	8.7
	3	7.7	8.7
250	1	7.7	8.3
	2	7.7	8.3
	3	7.7	8.3
500	1	7.6	8.3
	2	7.7	8.2
	3	7.7	8.1
1,000	1	7.8	8.0
	2	7.8	8.0
	3	7.8	8.0

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