

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-206CE. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was not given. The test sample is FC-206CE (5/23/91). It's purity was not sufficiently characterized, though current information indicates it is a mixture of 0.92% PFOS, 15% diethylene glycol butyl ether, 76.09% water, 4% urea, 1.28% Sultone foamer, 1.55% sodium octyl sulfate, 1.05% polyoxyethylene monooctylphenyl ether, 0.06% sodium lauryl sulfate, and 0.05% tolytriazole..

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

METHOD:

Method: OECD 201 and EPA-600/9-78-18

Type: Acute Static

GLP: No

Year study performed: 1991

Species: *Selenastrum capricornutum*

Source: Originally obtained from the Culture Collection at the University of Texas at Austin, maintained in culture medium at EnviroSystems, Hampton, NH.

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

Exposure period: 96 hours

Statistical Methods: Performed using standard statistical techniques (Stephan, 1983). EC₅₀ values were calculated using the binomial or moving average method. 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 prepared in deionized water. 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 algal medium. Aliquots were added directly to algal medium in test vessels to create test solutions.

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

Agitation: Continuous rotary shaker, rate of rotation not given.

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 $50 \text{ uEs}^{-1}\text{m}^{-2}$ using cool-white fluorescent lights.

Water Chemistry:

pH range (0-96 hours):

8.2 – 9.9 (control exposure)

7.9 – 8.3 (1000 mg/L exposure)

Test temperature range (0-96 hours):

23.0 – 23.4 °C (incubator)

RESULTS

Nominal concentrations: Bk control, 150, 250, 400, 600, and 1000 mg/L

Algal Growth Response at 96 Hours

Nominal Conc., mg/L	Avg. Cell Count (cells/mL)	Avg. Specific Growth Rate	% Change ⁽¹⁾
Bk. Control	479,000	0.040	0
150	718,000	0.045	-12
250	415,000	0.039	2
400	242,000	0.033	17
600	0	0.000	100
1000	0	0.000	100

(1) Average growth rate in control compared to test concentration growth rate.

Element values:

96-hour E_rC_{50} : 456 (400-600) mg/L

96-hour NOEC: 400 mg/L

Element values are based on nominal concentrations.

Reversibility of Growth Inhibition: Not determined.

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

CONCLUSIONS

FC-206CE exhibits a 96-hour E_{C50} of 456 mg/L with a 95% Confidence Interval of 400 to 600 mg/L. The 96-hour No Observed Effect Concentration (NOEC) was 400 mg/L.

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 the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

Test was conducted by EnviroSystems Division of Resource Analysts Incorporated, Hampton, NH at the request of the 3M Company, St. Paul, MN, Lab Request number J1884-2, 1991.

OTHER

Last changed: 6/26/00

Study Title

Static Acute Toxicity of FC-206CE
to the Alga, *Selenastrum capricornutum*

Authors

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Study Completed

July, 1991

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I. SUMMARY

The acute toxicity of FC-206CE to the alga, *Selenastrum capricornutum*, is described in this final report. The test was conducted for 3M Company for 96 hours during July 8 to July 12, 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 23.2°C. The dilution water was synthetic media prepared with sterile deionized water. Flasks were incubated on a rotary shaker. Light was adjusted to 50 $\mu\text{Ein}/\text{sec}/\text{m}^2$ with a photoperiod of 24 hours light and 0 hours dark. Nominal concentrations of FC-206CE were: 0 mg/L (control), 150 mg/L, 250 mg/L, 400 mg/L, 600 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 418 mg/L FC-206CE and a no observed effect concentration (NOEC) of 150 mg/L. The 96 hour EC50 and NOEC are 456 mg/L and 400 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-206CE 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-206CE (EnviroSystems Sample Number 2936A) was delivered to EnviroSystems on May 23, 1991. It was contained in a 40 oz. plastic bottle that was labelled with the following information: "J1884-2, FC-206CE". FC-206CE (a yellow 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:

The definitive toxicity test was performed during July 8 to 12, 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), 150 mg/L, 250 mg/L, 400 mg/L, 600 mg/L, and 1,000 mg/L.

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.

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 50 $\mu\text{Es}^{-1}\text{m}^{-2}$.

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 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-206CE are presented in Table 4. The 72 hour EC50 is 418 mg/L, and the 72 hour NOEC is 150 mg/L FC-206CE. The 96 hour EC50 is 456 mg/L and the 96 hour NOEC is 400 mg/L.

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	12	120	144	372
	2	10	18	106	218	450
	3	10	18	136	196	615
	mean	10	16	121	186	479
150	1	10	18	36	104	620
	2	10	30	38	130	750
	3	10	12	26	105	785
	mean	10	20	33	113	718
250	1	10	20	25	90	515
	2	10	12	20	65	415
	3	10	12	15	85	315
	mean	10	15	20	80	415
400	1	10	14	25	45	200
	2	10	24	10	70	260
	3	10	14	30	55	265
	mean	10	17	22	57	242
600	1	10	20	0	0	0
	2	10	26	0	0	0
	3	10	22	0	0	0
	mean	10	23	0	0	0
1,000	1	10	18	10	0	0
	2	10	16	10	0	0
	3	10	12	0	0	0
	mean	10	15	7	0	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)	0.020	0.052	0.041	0.040	0	0	0	0
150	0.029	0.025	0.034	0.045	-45	52	17	-12
250	0.017	0.015	0.029	0.039	15	71	29	2
400	0.022	0.017	0.024	0.033	-10	67	41	17
600	0.035	0.000	0.000	0.000	-75	100	100	100
1,000	0.017	-0.007	0.000	0.000	15	86	100	100

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	< 150 mg/L	-	--
72 hours	418 mg/L	250 - 600 mg/L	Binomial/Nonlinear Interpolation
96 hours	456 mg/L	400 - 600 mg/L	Binomial/Nonlinear Interpolation

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	23.2
1	23.0
2	23.0
3	23.4
4	23.2

Table A.2. pH measured during toxicity test

Nominal concentration (mg/L)	Replicate	pH	
		initial	final
0 (control)	1	8.2	9.8
	2	8.2	9.8
	3	8.2	9.9
150	1	8.2	9.1
	2	8.2	9.1
	3	8.2	9.1
250	1	8.2	9.1
	2	8.2	8.7
	3	8.2	9.1
400	1	8.2	8.8
	2	8.2	8.7
	3	8.3	8.6
600	1	8.3	8.1
	2	8.3	8.2
	3	8.2	8.3
1,000	1	8.3	7.9
	2	8.3	8.0
	3	8.3	8.0