

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (DAPHNIA MAGNA)

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% tolyltriazole.

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 Method 202

Type: Acute static

GLP: No

Year completed: 1991

Species: *Daphnia magna*

Supplier: In-house cultures.

Analytical monitoring: Temperature, pH, conductivity, and DO

Exposure period: 48-hours

Test organism age: <24 hours old

Statistical method: Not applicable. Survival was greater than 50% at all test concentrations.

Test conditions:

Dilution water: Filtered natural well water

Dilution water chemistry:

Conductivity: 860 $\mu\text{mhos/cm}$

pH: 7.6

Lighting: A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of $20 \mu\text{Es}^{-1}\text{m}^{-2}$.

Stock and test solution preparation: A 1000 mg/L primary stock solution was prepared in dilution water. Aliquots were then added directly to dilution water in test vessels to create test solutions.

Exposure vessels: 250 mL Pyrex glass beakers containing 200 mL of test solution and a water depth of ~6 cm.

Number of replicates: 4

Number of daphnids per replicate: 5

Number of concentrations: Five plus a blank control

Water chemistry during the study:**Conductivity range (0-48 hours):**860 – 880 μ mhos/cm (control exposure)860 – 880 μ mhos/cm (1000 mg/L exposure)**pH range (0-48 hours):**

7.6 – 8.3 (control exposure)

7.7 – 8.3 (1000 mg/L exposure)

Temperature range (0-48 hours): 20 - 21 °C

19.0 – 20.2 °C (control exposure)

19.0 – 20.1 °C (1000 mg/L exposure)

Dissolved oxygen range (0-48 hours):

8.5 – 9.2 mg/L (control exposure)

8.7 – 9.3 mg/L (1000 mg/L exposure)

RESULTS

Nominal concentrations: Bk control, 150, 250, 400, 600, and 1000 mg/L**Element values:** 48-hour EC_{50} = >1,000 mg/L

Element values based on nominal concentrations

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**

The FC-206CE 48-hour EC_{50} was determined to be >1000 mg/L, the highest concentration tested. No mortality or sublethal effects were observed at this concentration.**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133**DATA QUALITY**

Reliability: Klimisch ranking 2. Testing 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**

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

OTHER

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Study Title

Static Acute Toxicity of FC-206CE
to the Daphnid, *Daphnia magna*

Authors

Timothy J. Ward
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Study Completed

June, 1991

Performing Laboratory

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I. SUMMARY

The acute toxicity of FC-206CE to the daphnid, *Daphnia magna*, is described in this final report. The test was conducted for 3M Company for 48 hours during June 10 to 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 temperature of $20 \pm 1^\circ\text{C}$. The dilution water was filtered natural well water collected at Hampton, New Hampshire. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. 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.

Daphnids used in the test were produced from an in-house culture and were less than 24 hours old at the start of the test. After 48 hours of exposure the control daphnids had an average wet weight (blotted dry) of 0.0006 g, resulting in a loading rate of 0.015 g/L. All daphnids were in good condition at the beginning of the study.

The test substance was not toxic to daphnids at tested concentrations. Exposure of daphnids to the test substance resulted in a 48 hour EC50 greater than 1,000 mg/L FC-206CE.

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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 the daphnid, a freshwater invertebrate. 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 2936E) was delivered to EnviroSystems on May 23, 1991. It was contained in a 4 oz. plastic bottle that was labelled with the following information: "J1884-2, FC206CE". 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 archived at EnviroSystems for a minimum of 10 years.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was collected from wells at EnviroSystems in Hampton, New Hampshire. Water was stored in 500-gallon polyethylene tanks where it was aerated. Results of chemical analysis of a representative sample of water are presented in Table 1.

TEST ORGANISM:

Juvenile daphnids employed as test organisms were from a single source and were identified using an appropriate taxonomic key. Daphnids used in the test were produced from an in-house culture and were less than 24 hours old at the start of the test. Control daphnids were weighed at the conclusion of the toxicity test. Prior to testing, daphnids were maintained in 100% dilution water under static conditions in 4 liter glass jars. During acclimation daphnids were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. During the acclimation period 24 hours prior to the test initiation the temperature was 19.5°C. Daphnids were fed yeast, trout chow, and/or the freshwater alga *Selenastrum capricornutum* once or twice daily before the test.

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Table 1. Chemical characterization of a representative sample of natural well water used as dilution water for toxicity test

Parameter	Unit of Measurement	Reporting Limit	Measured Value
pH	pH units	--	7.6
Conductivity	umhos/cm	--	860
Organochlorine pesticides	ug/L	0.5	ND
Organophosphorus pesticides	ug/L	0.5	ND
Polychlorinated biphenyls	ug/L	0.5	ND

Notes: 1. None detected at or above the reporting limit.
 2. pH and conductivity were measured in dilution water collected from a control test vessel prior to introduction of organisms and pesticide and PCB data is collected during routine biannual water quality testing.

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TOXICITY TESTING:

The definitive toxicity test was performed during June 10 to 12, 1991. It is based on procedures of the OECD (1984). The test was conducted at a target temperature of $20 \pm 1^\circ\text{C}$ with five concentrations of test substance and a dilution water control. A 1,000 mg/L stock solution was formulated 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.

Twenty daphnids were randomly and equally distributed among four replicates of each treatment. The test was performed in 250 ml glass beakers that contained 200 ml of test solution (water depth was approximately 6 cm). Test vessels were randomly arranged in an incubator during the 48 hour test (a random numbers table was used to select the location of each vessel). A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of $20 \text{ uEs}^{-1}\text{m}^{-2}$. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Daphnids were not fed during the test.

The number of surviving organisms and the occurrence of immobilization (inability to swim within 15 seconds after gentle agitation) or other sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24 and 48 hours. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-3), pH (Beckman model PHI 12 meter; instrument number PRL-51), conductivity (Labcomp SCT meter, instrument number PRL-50), and temperature (ASTM mercury thermometer; thermometer number 2265) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test could not be interpreted by standard statistical techniques (Stephan, 1983) because 50% survival or greater occurred in all tested concentrations.

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VI. RESULTS

All test vessels containing FC-206CE were initially clear and remained clear throughout the test. Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Appendix A, respectively. One hundred percent survival occurred in the control exposure. Control daphnids had an average wet weight (blotted dry) of 0.0006 g at the end of the test. Loading rate during the toxicity test was approximately 0.015 g/L.

The 24 and 48 hour EC50s for daphnids exposed to FC-206CE are presented in Table 3. The 48 hour EC50 was greater than 1,000 mg/L FC-206CE.

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Table 2. Survival data from toxicity test

Nominal Concentration (mg/L)	rep.	Number Alive			Number Affected		
		0 hr	24 hr	48 hr	0hr	24hr	48hr
0 (control)	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
150	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
250	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
400	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
600	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
1,000	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0

Table 3. 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	--	--

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VII. REFERENCES

OECD. 1984. Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 202, Daphnid sp., Acute Immobilization Test and Reproduction Test. Adopted April 4, 1984.

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Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

Fig. 4-6-10

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Table A.1. Conductivity, pH, temperature, and dissolved oxygen concentration measured during toxicity test

Nominal concentration (mg/L)	Rep.	Conductivity (umho/cm)			pH		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	860	870	880	7.6	7.7	8.3
	2	860	870	880	7.6	7.7	8.3
	3	860	870	880	7.6	7.7	8.3
	4	860	870	880	7.6	7.7	8.3
150	1	860	860	900	7.5	7.7	8.3
	2	860	860	900	7.5	7.7	8.3
	3	860	860	900	7.5	7.7	8.3
	4	860	860	900	7.5	7.7	8.3
250	1	870	870	870	7.6	7.7	8.3
	2	870	870	870	7.6	7.7	8.3
	3	870	870	870	7.6	7.7	8.3
	4	870	870	870	7.6	7.7	8.3
400	1	870	870	860	7.6	7.7	8.3
	2	870	870	860	7.6	7.7	8.3
	3	870	870	860	7.6	7.7	8.3
	4	870	870	860	7.6	7.7	8.3
600	1	880	870	860	7.6	7.7	8.3
	2	880	870	860	7.6	7.7	8.3
	3	880	870	860	7.6	7.7	8.3
	4	880	870	860	7.6	7.7	8.3
1,000	1	880	870	860	7.7	7.7	8.3
	2	880	870	860	7.7	7.7	8.3
	3	880	870	860	7.7	7.7	8.3
	4	880	870	860	7.7	7.7	8.3

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Table A.1. Continued.

Nominal concentration (mg/L)	Rep.	Temperature (°C)			Dissolved Oxygen (mg/L)		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	20.0	19.0	19.1	8.5	9.0	9.2
	2	20.2	19.0	19.3	8.5	9.0	9.2
	3	20.1	19.1	19.4	8.5	9.0	9.2
	4	20.2	19.1	19.3	8.5	9.0	9.2
150	1	19.8	19.2	19.3	8.6	9.0	8.7
	2	19.7	19.3	19.3	8.6	9.1	8.7
	3	19.9	19.1	19.2	8.6	9.0	8.6
	4	19.9	19.2	19.3	8.6	9.2	8.7
250	1	20.0	19.2	19.4	8.7	9.2	8.8
	2	20.0	19.3	19.2	8.7	9.2	8.8
	3	20.1	19.1	19.2	8.6	9.2	8.9
	4	20.1	19.1	19.3	8.6	9.2	8.9
400	1	20.2	19.2	19.2	8.6	9.2	9.0
	2	19.9	19.1	19.1	8.6	9.2	9.1
	3	20.1	19.1	19.4	8.7	9.3	9.0
	4	20.1	19.0	19.3	8.7	9.3	9.1
600	1	20.2	19.1	19.4	8.7	9.2	9.3
	2	20.3	19.0	19.5	8.7	9.4	9.4
	3	19.8	19.2	19.3	8.7	9.4	9.2
	4	19.9	19.1	19.2	8.8	9.3	9.2
1,000	1	20.0	19.2	19.3	8.7	9.2	9.1
	2	20.1	19.1	19.4	8.7	9.3	9.0
	3	20.0	19.0	19.2	8.7	9.3	9.0
	4	19.8	19.2	19.3	8.7	9.2	9.1