

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-120, or 3M Sample J2905-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafuoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and butyl cellosolve (approximately 25% test substance, 37.5% butyl cellosolve and 37.5% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test sample was from 3M Lot 164.

METHOD

Method: OECD 202.

Test type: Static acute

GLP: No

Year Completed: 1992

Species: *Daphnia magna*

Analytical monitoring: pH, dissolved oxygen, conductivity, temperature. Test substance concentration was not monitored.

Statistical methods: EC₅₀ values calculated using Binomial/nonlinear interpolation

Test daphnid source: In-house cultures, EnvironSystems Division, Resource Analysts, Incorporated, Hampton, NH

Test daphnid age at study initiation: < 24-hours

Test conditions

Dilution water: Aerated, filtered natural well water

Dilution water chemistry (from a representative sample):

hardness: 166 mg/L as CaCO₃

pH: 7.2

Conductivity: 540 µmhos/cm

Stock and test solutions preparation: A primary stock solution was prepared in dilution water to yield a test sample concentration of 1000 mg/L. All test solutions were made by diluting the appropriate amount of stock solution with dilution water.

Exposure vessels: 250 mL glass beakers containing 200 mL of test solution.

Number of replicates: 4

Number of daphnids per replicate: 5

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0-48 hours):

8.7 – 9.3 mg/L (control exposure)

8.8 – 9.1 mg/L (100 mg/L exposure)

pH range (0-48 hours):

7.2 – 8.1 (control exposure)

7.2 – 8.2 (100 mg/L exposure)

Test temperature range (0 – 48 hours)

19.4 – 20.8°C

Element basis: mortality and immobilization

RESULTS

Nominal concentrations: Bk control, 15, 25, 40, 60, 100 mg/L

Element value: 24-hour $EC_{50} \Rightarrow 100$ mg/L

48-hour $EC_{50} = 44$ (25 – 60) mg/L

Statistical Evaluation: The EC_{50} value and 95% confidence interval was calculated using the binomial/nonlinear interpolation method.

Mortality of controls: None

Remarks: Values reported are for the test sample (mixture). No calculations were made to adjust for the concentration of the test substance in the test sample.

Cumulative percent mortality and immobilization:

Nominal Test Conc., mg/L	24-hours	48-hours
Neg. Control	0	0
15	5	15
25	5	20
40	10	35
60	0	60
100	25	90

CONCLUSIONS

The test sample 48-hour EC_{50} for *Daphnia magna* was determined to be 44 mg/L with a 95% confidence interval of 25 to 60 mg/L.

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Testing was conducted on the "as-sold" mixture of the subject material in water (~25% subject material). The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component.

REFERENCES

This study was conducted by EnviroSystems Division Resource Analysts, Incorporated, Hampton, New Hampshire on behalf of the 3M Company.

OTHER

Last changed: 5/18/00

Study Title

Static Acute Toxicity of FC-120
to the Daphnid, *Daphnia magna*

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

February, 1992

Performing Laboratory

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I. SUMMARY

The acute toxicity of FC-120 to the daphnid, *Daphnia magna*, is described in this final report. The test was conducted for 3M Company for 48 hours during February 5 to 7, 1992, 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-120 were: 0 mg/L (control), 15, 25, 40, 60, and 100 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 0.0002 g, resulting in a loading rate of 0.005 g/L. All daphnids were in good condition at the beginning of the study.

Exposure of daphnids to the test substance resulted in a 48 hour EC50 of 44 mg/L FC-120, with a 95% confidence interval of 25 to 60 mg/L. Six of the surviving daphnids exposed to 60 mg/L and all of the surviving daphnids exposed to 100 mg/L were immobilized at 48 hours. No other sublethal effects were observed in any test vessel.

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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-120 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-120 (EnviroSystems Sample Number 5103E) was delivered to EnviroSystems on November 13, 1991. It was contained in a 250 ml plastic bottle that was labelled with the following information: "J2904-I, FC-120, Lot 164, 2-Butoxy Ethanol." FC-120 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 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 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 20.3°C. Daphnids were fed yeast, trout chow, and/or the freshwater alga *Selenastrum capricornutum* once or twice daily before the test.



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	--	7.2
Conductivity	umhos/cm	--	540
Hardness	mg/L as CaCO ₃	--	166
Organochlorine pesticides	ug/L	0.5	ND
Organophosphorus pesticides	ug/L	2	ND
Polychlorinated biphenyls	ug/L	1	ND

Notes: 1. ND= not detected at or above the reporting limit.
 2. pH, hardness, and conductivity were measured in dilution water collected from the control test vessel prior to introduction of organisms and pesticide and PCB data is collected during routine biannual water quality testing.

TOXICITY TESTING:

A screening test with the test substance was conducted during January 21 to 23, 1992. Nominal concentrations of test substance were 1, 10, 100, and 1,000 mg/L. After 48 hours of exposure there was 0% survival at 100 and 1,000 mg/L and 80% survival or greater at all lower tested concentrations. A definitive toxicity test was attempted from January 29 to 31, 1992. Nominal concentrations of test substance were 150, 250, 400, 600, and 1,000 mg/L. This test was repeated because less than 50% survival occurred at all tested concentrations.

The definitive toxicity test was performed during February 5 to 7, 1992. 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 made by combining 0.25 g of the test substance and dilution water in a 250 ml volumetric flask and adjusting the final volume to 250 mls with dilution water. Nominal concentrations of the test material were: 0 mg/L (control), 15, 25, 40, 60, and 100 mg/L.

Twenty daphnids were 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), pH (Beckman model PHI 12 meter), conductivity (Cole Parmer number 1481-60), and temperature (ASTM mercury thermometer) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques (Stephan, 1983). The 24 and 48 hour EC50 values were calculated by the binomial, moving average, or probit method. All calculations were performed by the author using nominal concentrations of the test substance.

VI. RESULTS

All test vessels containing FC-120 were 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 0.0002 g at the end of the test. Loading rate during the toxicity test was approximately 0.005 g/L.

Exposure of daphnids to the test substance resulted in a 48 hour EC50 of 44 mg/L FC-120, with a 95% confidence interval of 25 to 60 mg/L. Six of the surviving daphnids exposed to 60 mg/L and all of the surviving daphnids exposed to 100 mg/L were immobilized at 48 hours. No other sublethal effects were observed in any test vessel during the test.

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
15	1	5	5	5	0	0	0
	2	5	5	4	0	0	0
	3	5	4	3	0	0	0
	4	5	5	5	0	0	0
25	1	5	5	3	0	0	0
	2	5	5	5	0	0	0
	3	5	4	3	0	0	0
	4	5	5	5	0	0	0
40	1	5	4	4	0	0	0
	2	5	4	3	0	0	0
	3	5	5	2	0	0	0
	4	5	5	4	0	0	0
60	1	5	5	2	0	0	1
	2	5	5	1	0	0	1
	3	5	5	3	0	0	2
	4	5	5	2	0	0	2
100	1	5	3	0	0	0	0
	2	5	4	0	0	0	0
	3	5	3	1	0	0	1
	4	5	5	1	0	0	1

Note: Affected organism were immobilized (unable to swim within 15 seconds after gentle agitation).

Table 3. Median effective concentrations (EC50s) from toxicity test

Exposure period	EC50	95 percent confidence limits	Calculation method
24 hours	>100 mg/L	--	--
48 hours	44 mg/L	25 - 60 mg/L	Binomial/Nonlinear Interpolation

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.

Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.

Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

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	540	580	600	7.2	8.0	8.0
	2	550	580	590	7.2	8.1	8.0
	3	570	580	590	7.2	8.1	8.0
	4	570	580	580	7.2	8.1	8.0
15	1	580	580	590	7.2	8.1	8.0
	2	580	570	580	7.2	8.1	8.0
	3	580	570	580	7.2	8.1	8.0
	4	580	570	590	7.2	8.1	8.0
25	1	580	580	590	7.2	8.1	8.0
	2	580	580	580	7.2	8.1	8.0
	3	580	580	580	7.2	8.1	8.0
	4	580	580	580	7.2	8.1	8.0
40	1	580	580	590	7.2	8.2	8.1
	2	580	580	580	7.2	8.2	8.1
	3	580	580	580	7.2	8.2	8.1
	4	580	580	590	7.2	8.2	8.1
60	1	580	580	590	7.2	8.2	8.1
	2	580	580	590	7.2	8.2	8.1
	3	580	570	580	7.2	8.2	8.1
	4	580	580	590	7.2	8.2	8.1
100	1	580	590	590	7.2	8.2	8.1
	2	580	580	580	7.2	8.2	8.1
	3	580	580	580	7.2	8.2	8.1
	4	580	580	590	7.2	8.2	8.1

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	19.4	20.5	20.4	8.7	8.9	9.3
	2	19.4	20.3	20.3	8.8	9.0	9.2
	3	19.4	20.3	20.3	8.8	9.1	9.3
	4	19.4	20.2	20.4	8.8	9.0	9.2
15	1	19.4	20.3	20.3	8.9	9.0	9.0
	2	19.4	20.3	20.3	9.0	9.0	9.0
	3	19.4	20.2	20.2	9.0	9.0	9.0
	4	19.4	20.3	20.4	9.0	9.0	9.1
25	1	19.4	20.3	20.4	9.0	9.0	9.1
	2	19.4	20.3	20.3	8.9	9.0	9.1
	3	19.4	20.3	20.3	8.9	9.0	9.1
	4	19.5	20.2	20.3	9.0	8.9	9.1
40	1	19.4	20.6	20.3	8.9	9.0	9.1
	2	19.4	20.6	20.3	8.9	8.9	9.1
	3	19.6	20.4	20.4	8.9	8.8	9.1
	4	19.4	20.3	20.4	8.9	8.8	9.1
60	1	19.4	20.8	20.4	8.9	9.0	9.1
	2	19.4	20.8	20.4	9.0	8.8	9.1
	3	19.4	20.6	20.4	9.0	8.9	9.1
	4	19.4	20.4	20.4	8.8	8.8	9.1
100	1	19.4	20.8	20.4	9.0	9.0	9.1
	2	19.4	20.8	20.4	9.0	9.0	9.1
	3	19.4	20.8	20.4	9.0	8.8	9.1
	4	19.4	20.7	20.4	8.9	8.8	9.1