

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-787 or FC-787X. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was 101. The test sample is FC-787. Current information indicates it is a mixture of 0.73% PFOS, 10% diethylene glycol monobutyl ether, 79.74% water, 5% ethylene glycol, 1.47% propane Sultone foamer, 0.73% sodium octyl sulfate, 0.73% sodium decyl sulfate, 0.75% xanthan gum, 0.75% magnesium sulfate, 0.10% N-(3-chloroallyl)hexaminium chloride, and 0.05% benzotriazole.

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 Guideline 202.

Type: Acute static

GLP: No

Year completed: 1990

Species: *Daphnia magna*

Supplier: In-house cultures maintained at EnviroSystems Division, Resource Analysts, Inc., Hampton, NH.

Analytical monitoring: pH, temperature, conductivity, and dissolved oxygen.

Exposure period: 48-hours

Test organism age: <24-hours old

Statistical method: The EC₅₀ values were calculated using a computer program by Stephan, 1983.

Test conditions:

Dilution water: Filtered natural well water

Dilution water chemistry:

Hardness: 180 mg/L as CaCO₃

Conductivity: 1300 µmhos/cm

pH: 8.1

Lighting: Cool-white fluorescent lights at 18 µE/sec/m². A daily photoperiod of 16 hours light and 8 hours dark was maintained throughout the testing period.

Stock and test solution preparation: A 1000 mg/L stock solution was prepared by dissolving 1 g of test substance in 1000 mL dilution water. Exposure concentrations were prepared by addition of stock solution to the test vessels.

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

Number of replicates: 2

Number of daphnids per replicate: 10.

Number of concentrations: five plus a blank control

Water chemistry during the study:

pH range (0-48 hours):

8.0 – 8.1 (control exposure)

8.0 – 8.3 (1000 mg/L exposure)

Temperature range (0-48 hours):

19.8 – 20.9 °C (control exposure)

20.0 – 20.9 °C (1000 mg/L exposure)

Conductivity range: (0-48 hours)

1300 – 1300 µmhos/cm (control exposure)

1200 – 1300 µmhos/cm (1000 mg/L exposure)

Dissolved oxygen range (0-48 hours):

8.5 – 9.0 mg/L (control exposure)

8.0 – 9.0 mg/L (1000 mg/L exposure)

RESULTS

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

Element values: 48-hour EC_{50} = >1000 mg/L

Element values based on nominal concentrations

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 48-hour EC_{50} was determined to be >1000 mg/L. No mortality or sublethal effects were observed.

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 study was conducted by EnviroSystems Division, Resource Analysts, Inc., Hampton, NH at the request of the 3M Company, St. Paul, MN, Lab Request number H1609, 1990.

OTHER

Last changed: 6/28/00.

Study Title

Static Acute Toxicity of FC-787
to the Daphnid, *Daphnia magna*

Authors

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Study Completed

September 4, 1990

Performing Laboratory

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I. GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to OECD Good Laboratory Practice Regulations. Neither the Study Director nor the sponsor are aware of any circumstances that would affect the integrity of this study. The test substance was stored at room temperature, test specimens were added to test vessels indiscriminately and test vessels were randomized, and the test was conducted with 2 rather than 4 replicates. No deviations existed that significantly affected the validity of the study.

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II. QUALITY ASSURANCE STATEMENT

Submitted by: EnviroSystems Division
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Certification: Data presented in this report were derived by methods and with materials identified in the section of the report entitled "Methods and Materials." The test was performed in accordance with EnviroSystems Protocol 9070-3, with the noted exceptions and the Product Registration Aquatic Toxicology Laboratory Standard Operating Procedures Manual. The toxicity test was performed by Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward. The original raw data and final report will be archived at Resource Analysts, Inc. for at least 10 years.

All data transcribed from the raw data to the report were checked for accuracy, and all data were verified by Quality Assurance Auditors. Quality assurance audits were performed on

Audit Date	Reported to Study Director	Reported to Management
5/ 5/90	5/ 7/90	--
7/25/90	7/25/90	8/ 6/90
8/14/90	8/14/90	10/25/90
10/25/90	10/25/90	10/29/90

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V. SUMMARY

The acute toxicity of FC-787 to the daphnid, *Daphnia magna*, is described in this final report. The test was conducted for 3M Company for 48 hours during July 23 to 25, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward according to the protocol developed for EnviroSystems Study Number 9070-3.

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 from wells at Hampton, New Hampshire. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of FC-787 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 0.0002 g, resulting in a loading rate of 0.01 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 $> 1,000$ mg/L FC-787.

VI. INTRODUCTION

This study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of FC-787 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.

VII. METHODS AND MATERIALS

TEST SUBSTANCE:

FC-787 (EnviroSystems Sample Number 2469E) was delivered to EnviroSystems on May 15, 1990. It was contained in a 1 gallon plastic bottle that was labelled with the following information: "Lot 101 surfactants/butyl carbtal, 5-10-90". FC-787 was supplied by 3M Company, St. Paul, Minnesota. Prior to use the test material was stored in the dark at room temperature. A reserve sample 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 20.0°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	--	8.1
Conductivity	umhos/cm	--	1300
Hardness	mg/L as CaCO ₃	--	180
Organochlorine pesticides	ug/L	0.5	ND ^a
Organophosphorus pesticides	ug/L	5.0	ND ^a
Polychlorinated biphenyls	ug/L	0.5	ND ^a

^aNot detected above the reporting limit.

TOXICITY TESTING:

A screening test with the test substance was conducted during July 20 to 22, 1990. Nominal concentrations of test substance were 10 mg/L, 50 mg/L, 100 mg/L, 500 mg/L, and 1,000 mg/L. After 48 hours of exposure there was 50% survival at 1,000 mg/L, 90% survival at 500 mg/L, and 100% survival at all other tested concentrations.

The definitive toxicity test was performed during July 23 to 25, 1990, according to EnviroSystems Test Protocol 9070-3 (Static, Acute Toxicity Test With FC-787 And The Daphnid, *Daphnia magna*), which was signed by the Study Director on July 20, 1990. 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 prepared by combining 1.0 g of test substance with dilution water and adjusting the final concentration to 1,000 ml 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 two 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 $18 \text{ uEs}^{-1}\text{m}^{-2}$. 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. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-18), pH (Beckman model PHI 12 meter; instrument number PRL-4), conductivity (Labcomp SCT meter, instrument number PRL-9), and temperature (ASTM mercury thermometer; thermometer number 2211) 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 greater than 50% survival occurred at all tested concentrations.

VIII. RESULTS

All test vessels containing FC-787 appeared clear initially and remained free of insoluble material 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 0.01 g/L. Exposure of daphnids to the reference toxicant sodium dodecyl sulfate resulted in a 48 hour LC50 and EC50 of 24.8 mg/L.

The 24 and 48 hour EC50s for daphnids exposed to FC-787 are presented in Table 3. The test substance was nontoxic to daphnids at tested concentrations, resulting in a 48 hour EC50 of > 1,000 mg/L FC-787.

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	10	10	10	0	0	0
	2	10	10	10	0	0	0
100	1	10	10	10	0	0	0
	2	10	10	9	0	0	0
150	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
250	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
400	1	10	10	10	0	0	0
	2	10	10	9	0	0	0
600	1	10	10	10	0	0	0
	2	10	10	10	0	0	0

Note: No daphnids 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	> 1,000 mg/L	--	--
48 hours	> 1,000 mg/L	--	--

IX. REFERENCES

OECD. 1981. Decision of the Council Concerning the Mutual Acceptance of Data in the Assessment of Chemicals. Annex 2. OECD Principles of Good Laboratory Practice. C (81) 30 (Final).

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 calculation of LC50 values. 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	1300	1300	1300	8.1	8.0	8.1
	2	1300	1300	1300	8.1	8.0	8.1
150	1	1300	1300	1200	8.0	8.1	8.3
	2	1300	1300	1200	8.0	8.1	8.3
250	1	1300	1300	1200	8.0	8.1	8.3
	2	1300	1300	1200	8.0	8.1	8.3
400	1	1300	1300	1200	8.0	8.1	8.3
	2	1300	1300	1200	8.0	8.1	8.3
600	1	1300	1300	1200	8.0	8.1	8.3
	2	1300	1300	1200	8.0	8.1	8.3
1,000	1	1300	1300	1200	8.0	8.2	8.3
	2	1300	1300	1200	8.0	8.2	8.3

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	20.9	20.0	19.8	8.5	9.0	8.9
	2	20.9	20.0	19.8	8.5	9.0	8.9
150	1	20.9	20.0	19.8	8.5	9.0	8.7
	2	20.9	20.0	20.0	8.5	9.0	8.7
250	1	20.9	20.0	19.9	8.5	9.0	8.7
	2	20.9	20.0	19.9	8.5	9.0	8.7
400	1	20.9	20.0	19.9	8.5	9.0	8.3
	2	20.9	20.0	19.9	8.5	9.0	8.3
600	1	20.9	20.0	19.9	8.5	9.0	8.3
	2	20.9	20.0	19.9	8.5	9.0	8.3
1,000	1	20.9	20.0	20.0	8.3	9.0	8.0
	2	20.9	20.0	20.0	8.3	9.0	8.0