

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-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 202

Type: Acute static

GLP: No

Year completed: 1991

Species: *Daphnia magna*

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

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

Exposure period: 48-hours

Test organism age: <24 hrs. old

Statistical method: LC₅₀ values calculated using a computer program from Stephan, 1983.

Test conditions:

Dilution water: Filtered natural well water.

Dilution water chemistry:

Conductivity: 700 µmhos/cm

pH: 7.9

Lighting: Cool-white fluorescent lights at 12 µEin/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 primary stock solution was prepared by dissolving 2.0 g of test substance in 2000 mL of dilution water. Exposure concentrations were prepared by addition of the appropriate volume of stock solution.

Exposure vessels: 250 mL beakers with 200 mL exposure solution and a water depth of approximately 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:

pH range (0-48 hours):

7.9 – 8.3 (control exposure)
8.0 – 8.3 (1000 mg/L exposure)

Temperature range (0-48 hours):

19.7 – 20.6 °C (control exposure)
19.4 – 20.6 °C (1000 mg/L exposure)

Conductivity range: (0-48 hours)

640 - 700 µmhos/cm (control exposure)
640 - 660 µmhos/cm (1000 mg/L exposure)

Dissolved oxygen range (0-48 hours):

8.3 – 8.8 mg/L (control exposure)
8.0 – 9.9 mg/L (1000 mg/L exposure)

RESULTS

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

Element values: 48-hour EC₅₀ = >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 EC₅₀ was determined to be >1000 mg/L, the highest concentration tested. 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. This 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 test was conducted by EnviroSystems Division, Resource Analysts, Inc., of Hampton, NH at the request of the 3M Company, Lab Request number H2864-9, 1991.

OTHER

Last changed: 6/27/00

Amended 8/9/91

Study Title

Static Acute Toxicity of FC 203
to the Daphnid, Daphnia magna

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

July 29, 1991

Report Amended

August 9, 1991

Performing Laboratory

EnviroSystems Division
Resource Analysts, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

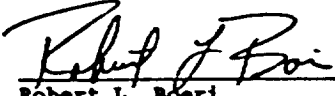
EnviroSystems Study Number 90174-3
Page 1 of 17

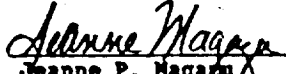
Resource Analysts, Inc., Subsidiary of MILLIPORE

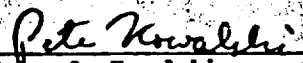
I. GOOD LABORATORY PRACTICE STATEMENT

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.

 7/29/91
Timothy J. Ward
Author and Study Director
AMENDED 8/1/91 

 7-30-91
Robert L. Boeri
Coauthor

 7-30-91
Jeanne P. Magara
Aquatic Toxicologist

 7-30-91
Peter L. Kowalski
Aquatic Toxicologist

 7-30-91
Ellen J. Stanford
Aquatic Toxicologist

II. QUALITY ASSURANCE STATEMENT

Submitted by: EnviroSystems Division
Resource Analysts, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

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 90174-3 and the Product Registration Aquatic Toxicology Laboratory Standard Operating Procedures Manual. The toxicity test was performed by Ellen Stanford, 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
11/14/90	11/15/90	—
1/10/91	1/10/91	7/30/91
2/ 7/91	2/ 7/91	7/30/91
7/30/91	7/30/91	7/30/91
8/9/91	8/9/91	8/14/91 Amended

Arthur P. Pashie 7-30-91
Quality Assurance Representative

Amended: *Arthur P. Pashie* 8-14-91

III. TABLE OF CONTENTS

SECTION:	Page
I. Good Laboratory Practice Statement	2
II. Quality Assurance Statement	3
III. Table of Contents	4
IV. Index of Tables	5
V. Summary	6
VI. Introduction	7
VII. Methods and Materials	7
VIII. Results	11
IX. References	14
Appendix A. Water Quality Data from Toxicity Test	15

IV. INDEX OF TABLES

	Page
Table 1. Chemical characterization of a representative sample of natural well water used as dilution water for toxicity test	8
Table 2. Survival data from toxicity test	12
Table 3. Median effective concentrations (EC50s) from toxicity test	13
Table A.1. Conductivity, pH, temperature, and dissolved oxygen concentration measured during toxicity test	16

Amended 8/9/91

V. SUMMARY

The acute toxicity of FC 203 to the daphnid, Daphnia magna, is described in this final report. The test was conducted for 3M Company for 48 hours during March 19 to 21, 1991, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Ellen Stanford, Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward according to the protocol developed for EnviroSystems Study Number 90174-3.

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 test substance 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 0.0017 g, resulting in a loading rate of 0.004 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 median effective concentration (EC50) greater than 1,000 mg/L FC 203, the highest tested concentration.

Amended 8/9/91

VI. INTRODUCTION

This report was amended to include data from the final definitive toxicity test that was conducted during March 19 to 21, 1991. The study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of FC 203 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 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". The test substance was supplied by 3M Company, St. Paul, Minnesota. Prior to use it 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 19.6°C. Daphnid cultures were fed yeast/trout chow and/or the freshwater alga Selenastrum capricornutum daily before the test.

EnviroSystems Study Number 90174-3

Page 7 of 17

Amended 8/9/91

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	--	7.9
Conductivity	umhos/cm	--	700
Organochlorine pesticides	ug/L	2	ND
Organophosphorus pesticides	ug/L	5.0	ND
Polychlorinated biphenyls	ug/L	0.6	ND

Notes: 1. ND = Not detected above the reporting limit.

2. The pH and conductivity values were determined in a sample of dilution water collected from a control test vessel at the beginning of the toxicity test. Additional chemical parameters were measured in dilution water during October, 1990 as part of routine biannual water quality analysis.

Amended 8/9/91

TOXICITY TESTING:

A screening test with the test substance was conducted during December 5 to 7, 1990. Nominal concentrations of test substance were: 0.1, 1.0, 10, 100, and 1,000 mg/L. After 48 hours of exposure at least 70% survival occurred at all tested concentrations. A definitive test was attempted during January 9 to 11, 1991. Nominal concentrations of test substance were: 150, 250, 400, 600, and 1,000 mg/L. After 48 hours of exposure sublethal effects were noted at all tested concentrations (sublethal effects were not seen at 1,000 mg/L during the screening test) and the test was repeated.

The definitive toxicity test was performed during March 19 to 21, 1991, according to EnviroSystems Test Protocol 90174-3 (Static, Acute Toxicity Test With FC 203 And The Daphnid, Daphnia magna), which was signed by the Study Director on November 16, 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 2.0 g of test substance with dilution water and adjusting the final concentration to 2,000 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 FC 203.

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 $12 \text{ uEs}^{-1}\text{m}^{-2}$. 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 sublethal effects and 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-3), 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 2265) were measured and recorded daily in each test chamber that contained live animals.

Amended 8/9/91

STATISTICAL METHODS:

Results of the toxicity test could not be interpreted by standard statistical techniques (Stephan, 1983) because less than 50% of the daphnids exposed to the highest concentration were killed or immobilized. Nominal concentrations of test substance and the number of daphnids killed or immobilized were used to determine the EC50 value.

Amended 8/9/91

VIII. RESULTS

All test vessels containing FC 203 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 0.0017 g at the end of the test. Loading rate during the toxicity test was approximately 0.004 g/L. Exposure of daphnids to the reference toxicant sodium dodecyl sulfate resulted in a 48 hour median lethal concentration (LC50) of 7.9 mg/L.

The 24 and 48 hour EC50s for daphnids exposed to FC 203 are presented in Table 3. The 48 hour EC50 was greater than 1,000 mg/L FC 203, the highest tested concentration.

Amended 8/9/91

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	4	0	0	0
	2	5	5	5	0	0	0
	3	5	5	4	0	0	0
	4	5	5	5	0	0	0
250	1	5	5	4	0	0	0
	2	5	5	4	0	0	0
	3	5	5	5	0	0	0
	4	5	5	4	0	0	0
400	1	5	4	3	0	0	0
	2	5	5	4	0	0	0
	3	5	4	1	0	0	0
	4	5	5	5	0	0	0
600	1	5	5	5	0	0	0
	2	5	2	2	0	0	0
	3	5	4	4	0	0	0
	4	5	2	2	0	0	0
1,000	1	5	5	4	0	0	0
	2	5	5	3	0	0	0
	3	5	5	5	0	0	0
	4	5	3	2	0	0	0

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

Amended 8/9/91

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

EnviroSystems Study Number 90174-3
Page 15 of 17

Resource Analysts, Inc. Subsidiary of MILLIPORE

Amended 8/9/91

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

Nominal concentration (mg/L)	Rep.	Conductivity (umhos/cm)			pH		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	700	660	640	7.9	8.3	8.0
	2	700	660	650	7.9	8.3	8.0
	3	700	660	650	7.9	8.3	8.0
	4	700	660	660	7.9	8.3	8.0
150	1	660	630	650	8.0	8.2	8.0
	2	660	630	650	8.0	8.2	8.0
	3	660	630	650	8.0	8.2	8.0
	4	660	640	660	8.0	8.2	8.0
250	1	660	640	660	8.0	8.2	8.0
	2	660	640	660	8.0	8.2	8.0
	3	660	640	660	8.0	8.2	8.0
	4	660	640	660	8.0	8.2	8.0
400	1	660	640	660	8.0	8.2	8.0
	2	660	640	660	8.0	8.2	8.0
	3	660	640	660	8.0	8.2	8.0
	4	660	640	660	8.0	8.2	8.0
600	1	660	640	660	8.0	8.3	8.0
	2	660	640	670	8.0	8.3	8.0
	3	660	640	660	8.0	8.3	8.0
	4	660	640	670	8.0	8.3	8.0
1,000	1	660	640	660	8.0	8.3	8.0
	2	660	640	660	8.0	8.3	8.0
	3	660	640	660	8.0	8.3	8.0
	4	660	640	660	8.0	8.3	8.0

Amended 8/9/91

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	20.3	19.8	19.7	8.5	8.8	8.3
	2	20.3	19.9	20.1	8.4	8.8	8.3
	3	20.2	19.9	20.5	8.5	8.8	8.5
	4	20.4	19.8	20.6	8.5	8.8	8.3
150	1	20.4	19.7	19.4	8.8	8.7	8.1
	2	20.3	19.8	19.3	8.7	9.0	8.2
	3	20.3	19.7	19.2	8.6	9.2	8.0
	4	20.3	19.3	19.3	8.7	9.2	8.1
250	1	20.3	19.3	19.3	8.6	9.4	8.0
	2	20.2	19.3	19.3	8.6	9.4	8.0
	3	20.1	19.4	19.3	8.5	9.5	8.1
	4	20.3	19.7	19.3	8.6	9.6	8.1
400	1	20.4	19.8	19.4	8.7	9.6	8.1
	2	20.3	19.7	19.6	8.5	9.7	8.1
	3	20.2	19.9	19.4	8.5	9.7	8.1
	4	20.3	19.8	19.5	8.5	9.6	8.2
600	1	20.3	19.6	19.2	8.4	9.6	8.2
	2	20.4	19.7	19.8	8.4	9.7	8.2
	3	20.4	19.8	19.7	8.4	9.8	8.0
	4	20.2	19.5	19.8	8.4	9.6	8.1
1,000	1	20.3	19.4	19.7	8.3	9.8	8.1
	2	20.3	20.2	20.6	8.4	9.9	8.0
	3	20.3	19.7	19.8	8.4	9.7	8.1
	4	20.3	19.4	19.7	8.3	9.7	8.0