

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

TEST SUBSTANCE

Identity: Mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-203CF-X or FC-203CF. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was 101. The test sample is FC-203CF. Current information indicates it is a mixture of 1% PFOS, 20% diethylene glycol monobutyl ether, 70.2% water, 2.75% Acrylic foamer, 3% sodium octyl sulfate, 1% triethanolamine, 2% sulfonated alkyl ether salts, 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 203.

Type: Acute static

GLP: No

Year completed: 1991

Species: *Pimephales promelas*

Supplier: Aquatic Research Organisms, Division of Resource Analysts, Inc., Hampton, NH.

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

Exposure period: 96-hours

Statistical methods: No calculations performed, based on study results.

Test fish age: Juveniles

Length and weight: Average length = 34 mm
Average weight = 0.23 g (wet)

Loading: 0.15 g/L

Pretreatment: None

Test conditions:

Dilution water: Filtered natural well water

Dilution water chemistry:

Hardness: 80 mg/L as CaCO₃

Conductivity: 830 µmhos/cm

pH: 7.7

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

Stock and test solution preparation: The test material was added directly to the dilution water on a weight/volume basis.

Concentrations dosing rate: Once.

Stability of the test chemical solutions: Not noted.

Exposure vessels: 20 L glass aquaria (40x20x25 cm) containing 15 L of test solution and an approximate water depth of 18 cm.

Number of replicates: 2

Number of fish per replicate: 10.

Number of concentrations: five plus a blank control

Water chemistry during the study:

pH range (0-96 hours):

7.1 – 7.7 (control exposure)

7.5 – 7.7 (1000 mg/L exposure)

Temperature range (0-96 hours):

21.8 – 22.7 °C

Conductivity range (0-96 hours):

830 – 850 µmhos/cm (control exposure)

850 – 910 µmhos/cm (1000 mg/L exposure)

Dissolved oxygen range (0-96 hours):

6.8 – 8.1 mg/L (control exposure)

2.5 – 8.5 mg/L (1000 mg/L exposure)

RESULTS

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

Element values: 96-hour LC₅₀ = >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 sample 96-hour LC₅₀ for fathead minnow was determined to be >1000 mg/L.

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, 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, Incorporated, of Hampton, NH at the request of the 3M Company, St. Paul, MN, Lab Request number J1884-3, 1991.

OTHER

Last changed: 6/28/00

Study Title

Static Acute Toxicity of FC-203CF to the
Fathead Minnow, *Pimephales promelas*

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

June, 1991

Performing Laboratory

EnviroSystems Division
Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842

I. SUMMARY

The acute toxicity of FC-203CF to the fathead minnow, *Pimephales promelas*, is described in this final report. The test was conducted for 3M Company for 96 hours during June 3 to 7, 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 $22 \pm 1^\circ\text{C}$. 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-203CF 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.

Fish used in the test were purchased from a commercial supplier (Aquatic Research Organisms, Hampton, New Hampshire) and acclimated under test conditions for more than 15 days. After 96 hours of exposure the control fish had an average total length of 34 mm and an average wet weight (blotted dry) of 0.23 g, resulting in a loading rate of approximately 0.15 g/L. All fish were in good condition at the beginning of the study.

Exposure of fathead minnows to the test substance resulted in a 96 hour LC50 greater than 1,000 mg/L FC-203CF. Two of the twenty fish exposed to 600 mg/L at 72 hours and all of the fish exposed to 1,000 mg/L from 24 to 96 hours were lethargic and exhibited a loss of equilibrium. No other sublethal effects were observed in any test vessel during the 96 hour exposure.

II. TABLE OF CONTENTS

SECTION:	PAGE
I. Summary	2
II. Table of Contents	3
III. Index of Tables	4
IV. Introduction	5
V. Methods and Materials	5
VI. Results	8
VII. References	11
Appendix A. Water Quality Data from Toxicity Test	12

III. INDEX OF TABLES

	PAGE
Table 1. Chemical characterization of a representative sample of natural well water used as dilution water for toxicity test	6
Table 2. Survival data from toxicity test	9
Table 3. Median lethal concentrations (LC50s) from toxicity test	10
Table A.1. Conductivity, pH, temperature, and dissolved oxygen concentration measured during toxicity test	13

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-203CF to the fathead minnow, a freshwater fish. 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-203CF (EnviroSystems Sample Number 2937E) was delivered to EnviroSystems on May 23, 1991. It was contained in a 4 oz plastic bottle that was labelled with the following information: "J1884-3, FC206CF". FC-203CF (an orange-brown 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 grams) 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 fathead minnows employed as test organisms were from a single source and were identified using an appropriate taxonomic key. They were purchased from a commercial supplier (the Aquatic Research Organisms Division of Resource Analysts, Inc., Hampton, New Hampshire) and acclimated at the EnviroSystems facility for more than 15 days. Control fish were weighed at the conclusion of the toxicity test. Prior to testing, fish were maintained in 100% dilution water under flow through conditions in an all glass aquarium. During acclimation fish 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 14 days prior to the test initiation the temperature ranged from 21.81° to 23.6°C, and the dissolved oxygen concentration was maintained above 7.6 mg/L. Fish were fed a commercial fish food (EnviroSystems lot number TH01) 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	--	7.7
Conductivity	umhos/cm	--	830
Hardness	mg/L as CaCO ₃	--	80
Organochlorine pesticides	ug/L	0.5	ND
Organophosphorus pesticides	ug/L	0.5	ND
Polychlorinated biphenyls	ug/L	0.5	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 May 24 to 28, 1991. The test was conducted at 1, 10, 100, and 1,000 mg/L and after 96 hours there was 100% survival at all concentrations.

The definitive toxicity test was performed during June 3 to 7, 1991. It is based on procedures of the OECD (1984). The test was conducted at a target temperature of $22 \pm 1^\circ\text{C}$ with five concentrations of test substance and a dilution water control. No stock solution was prepared as test material 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.

Twenty fish were randomly and equally distributed among two replicates of each treatment. The test was performed in 20 liter glass aquaria (approximately 20 cm in width, 40 cm in length, and 25 cm in height) that contained 15 liters of test solution (water depth was approximately 18 cm). Test vessels were randomly arranged in an incubator during the 96 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. Aeration was not employed to maintain dissolved oxygen concentrations above acceptable levels. Fish were not fed during the test.

The number of surviving organisms and the occurrence of 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, 48, 72, and 96 hours. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-3), pH (Beckman model PHI 12 meter; instrument number PRL-51), conductivity (Cole Parmer model number 1481-60, instrument number PRL-50), and temperature (ASTM mercury thermometer; thermometer number 1686) 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 in all tested concentrations.

VI. RESULTS

All test vessels containing FC-203CF 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 fish had an average total length of 34 mm, and an average wet weight (blotted dry) of 0.23 g at the end of the test. Loading rate during the toxicity test was approximately 0.15 g/L.

The 24, 48, 72, and 96 hour LC50s for fish exposed to FC-203CF are presented in Table 3. The 96 hour LC50 value is greater than 1,000 mg/L FC-203CF. Two of the twenty fish exposed to 600 mg/L at 72 hours and all of the fish exposed to 1,000 mg/L from 24 to 96 hours were lethargic and exhibited a loss of equilibrium. No other sublethal effects were observed in any test vessel during the 96 hour exposure.

Table 2. Survival data from toxicity test

Nominal Concentration (mg/L)	rep.	Number Alive					Number Affected				
		0hr	24hr	48hr	72hr	96hr	0hr	24hr	48hr	72hr	96hr
0 (control)	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
150	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
250	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
400	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
600	1	10	10	10	10	10	0	0	0	1	0
	2	10	10	10	10	10	0	0	0	1	0
1,000	1	10	10	9	9	9	0	10	9	9	9
	2	10	10	9	9	9	0	10	9	9	9

Note: Affected fish were lethargic and exhibited a loss of equilibrium.

Table 3. Median lethal concentrations (LC50s) from toxicity test

Exposure period	LC50	95 percent confidence limit	LC50 calculation method
24 hours	>1,000 mg/L	--	--
48 hours	>1,000 mg/L	--	--
72 hours	>1,000 mg/L	--	--
96 hours	>1,000 mg/L	--	--

VII. REFERENCES

OECD. 1984. Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 203, Fish Acute Toxicity Test. Adopted April 4, 1984.

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	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	830	830	840	840	850	7.7	7.3	7.1	7.1	7.4
	2	830	840	840	850	850	7.7	7.4	7.1	7.1	7.4
150	1	840	840	850	850	860	7.7	7.5	7.3	7.2	7.4
	2	840	840	850	850	860	7.7	7.5	7.3	7.2	7.4
250	1	840	840	850	850	870	7.7	7.5	7.3	7.3	7.5
	2	840	840	850	850	860	7.7	7.5	7.3	7.3	7.6
400	1	840	840	850	860	860	7.7	7.5	7.3	7.4	7.6
	2	840	840	850	850	860	7.7	7.5	7.4	7.4	7.5
600	1	840	840	850	860	860	7.7	7.6	7.4	7.5	7.5
	2	840	840	850	860	870	7.7	7.6	7.5	7.5	7.6
1,000	1	860	850	900	860	870	7.7	7.7	7.7	7.5	7.6
	2	880	850	900	910	910	7.7	7.7	7.7	7.5	7.5

Table A.1. Continued.

Nominal concentration (mg/L)	Rep.	Temperature (°C)					Dissolved Oxygen (mg/L)				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	22.2	22.2	22.1	22.2	22.4	8.1	8.1	7.2	7.0	6.8
	2	21.8	22.0	22.0	22.1	22.4	8.1	8.0	7.3	7.2	6.8
150	1	22.4	22.0	21.9	22.0	22.4	8.4	8.0	7.0	6.8	6.3
	2	22.4	22.1	22.1	22.1	22.5	8.4	8.0	7.0	6.8	5.2
250	1	22.0	22.7	21.8	22.2	22.3	8.7	8.0	7.0	6.8	6.2
	2	22.4	22.2	22.1	22.4	22.5	8.7	8.0	7.0	6.8	6.2
400	1	22.3	22.0	22.0	22.1	22.5	8.5	8.0	7.0	6.4	5.6
	2	22.3	22.1	22.2	22.3	22.5	8.5	8.0	7.0	6.5	5.6
600	1	22.0	22.0	22.1	22.2	22.5	8.5	8.0	7.0	6.2	4.4
	2	21.9	22.2	22.3	22.4	22.6	8.5	8.0	7.0	6.3	5.5
1,000	1	22.1	21.9	21.9	22.0	22.4	8.5	8.0	7.0	6.0	2.5
	2	22.3	22.4	22.3	22.5	22.7	8.5	8.0	7.0	6.2	2.6