

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

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 Method 203 and U.S. EPA Method EG-9

Type: Acute Flow-through

GLP: No

Year completed: 1990

Species: *Pimephales promelas*

Supplier: Commercial supplier.

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

Exposure period: 96-hours

Statistical methods: The LC₅₀ values were calculated using a computer program by Stephan, 1983.

Test fish age: Juveniles

Length and weight: Average length = 34 mm
Average weight = 0.32 g (wet)

Loading: 0.21 g/L

Pretreatment: None.

Test conditions:

Dilution water: Filtered natural well water

Dilution water chemistry:

Hardness: 180 mg/L as CaCO₃

Conductivity: 1300 µmhos/cm

pH: 8.3

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 primary stock solution was prepared by dissolving 600 g of test substance with 1000 mL dilution water. Exposure concentrations were prepared by serial dilution of the stock solutions within the proportional diluter.

Concentrations dosing rate: Continuous

Flow-through rate: Average of 6.7 complete volume changes per 24 hours.

Stability of the test chemical solutions: Not noted.

Exposure vessels: 20 L glass aquaria (20x40x25 cm) containing 15 L of test solution with a water depth of approx. 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):**
 - 8.0 – 8.5 (control exposure)
 - 7.8 – 8.3 (1000 mg/L exposure)
- Temperature range (0-96 hours):**
 - 22.4 – 22.7 °C (control exposure)
 - 22.3 – 22.5 °C (1000 mg/L exposure)
- Conductivity range: (0-96 hours)**
 - 1300 - 1500 µmhos/cm (control exposure)
 - 1300 - 1500 µmhos/cm (1000 mg/L exposure)
- Dissolved oxygen range (0-96 hours):**
 - 8.0 – 8.7 mg/L (control exposure)
 - 3.0 – 6.5 mg/L (1000 mg/L exposure)

RESULTS

Nominal concentrations: Bk control, 140, 240, 400, 610, 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 in both replicates. 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., of 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

Flow Through Acute Toxicity of FC-787
to the Fathead Minnow, *Pimephales promelas*

Authors

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Study Completed

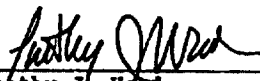
September 20, 1990

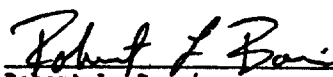
Performing Laboratory

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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. At the sponsors request, aeration was not employed to maintain dissolved oxygen concentrations above 80% saturation. The average length of fish was outside the range specified in the protocol. The randomization of test specimens can not be verified by the raw data. 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 9069-3 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 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	--
8/ 7/90	8/ 7/90	9/27/90
8/13/90	8/13/90	12/10/90
12/08/90	12/10/90	12/10/90

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V. SUMMARY

The acute toxicity of FC-787 to the fathead minnow, *Pimephales promelas*, is described in this final report. The test was conducted under flow through conditions for 3M Company for 96 hours during August 3 to 7, 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 9069-3.

The test was performed under flow through conditions with five concentrations of test substance and a dilution water control at a temperature of $23 \pm 2^{\circ}\text{C}$. The dilution water was filtered natural well water collected at Hampton, New Hampshire. Aeration was not employed to maintain dissolved oxygen concentrations above 80% saturation. Nominal concentrations of FC-787 were: 0 mg/L (control), 140 mg/L, 240 mg/L, 400 mg/L, 610 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 and acclimated under test conditions for 14 days. After 96 hours of exposure the control fish had an average total length of 3.4 cm and an average wet weight (blotted dry) of 0.32 g, resulting in a loading rate of approximately 0.21 g/L. All fish were in good condition at the beginning of the study.

The test substance was nontoxic to fathead minnows at tested concentrations. Exposure of fathead minnows to the test substance resulted in a 96 hour LC50 of $> 1,000 \text{ mg/L FC-787}$.

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 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.

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: "FC-787 Lot 101 surfactants/butyl carbtal, 5-10-90" and a 1 liter plastic bottle labelled as "FC 787, Lot 101, (1-12047)". 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 adjusted to a hardness of 180 mg/L as CaCO_3 and 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 and acclimated at the EnviroSystems facility for 14 days. Control fish were weighed and measured 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 prior to the test initiation the temperature ranged from 21.1° to 22.5°C, and the dissolved oxygen concentration was maintained at or above 8.0 mg/L. Fish were fed a commercial fish food (EnviroSystems lot number TM01) once or twice daily except for the 48 hour period 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	--	8.3
Conductivity	umhos/cm	--	1300
Hardness: initial final	mg/L as CaCO ₃	--	180 200
Organochlorine pesticides	ug/L	0.5	ND
Organophosphorus pesticides	ug/L	5.0	ND
Polychlorinated biphenyls	ug/L	0.5	ND

Notes: 1. ND = not detected above the reporting limit.
 2. The values for pH, conductivity, and hardness were derived from a sample of dilution water collected at the beginning of the toxicity test (hardness was also determined at the conclusion of the test) and all other values were derived from samples of dilution water that were analyzed as part of a routine, bi-annual water testing program.

TOXICITY TESTING:

A screening test with the test substance was conducted under static conditions during July 20 to 24, 1990. The nominal concentrations of test substance were 10, 50, 100, 500, and 1,000 mg/l FC-787. After 96 hours there was 100% survival at all tested concentrations.

The definitive toxicity test was performed during August 3 to 7, 1990, according to EnviroSystems Test Protocol 9069-3 (Acute Flow Through Toxicity of FC-787 To The Fathead Minnow *Pimephales promelas*), which was signed by the Study Director on July 10, 1990. It is based on procedures of the OECD (1984).

The test was conducted at a target temperature of $23 \pm 2^\circ\text{C}$ with five concentrations of test substance and a dilution water control. A stock solution was prepared by combining 600.0 g of test substance and deionized water and adjusting the total volume to 1,000 ml. Nominal concentrations of the test material were: 0 mg/L (control), 140 mg/L, 240 mg/L, 400 mg/L, 610 mg/L, and 1,000. The test substance was supplied to the test vessels under flow through conditions by an intermittent flow proportional diluter. All parts of the diluter that contacted the test substance were fabricated from glass or Teflon. During the test the diluter was activated 806 times, resulting in an average of 6.7 media exchanges per 24 hours in each test vessel.

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 a water bath 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 that provided a light intensity of $18 \mu\text{Es}^{-1}\text{m}^{-2}$. 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. 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 2160) 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 100% survival occurred at all tested concentrations.

VIII. RESULTS

Test vessels containing 0 mg/l to 400 mg/L FC-787 were initially clear and remained free of insoluble material throughout the test. Test vessels containing 610 mg/l and 1,000 mg/L FC-787 contained foam on the test media surface 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 3.4 cm, and a wet weight (blotted dry) of 0.32 g, resulting in a loading rate of approximately 0.21 g/L.

The 24, 48, 72, and 96 hour LC50s for fish exposed to FC-787 are presented in Table 3. Because toxicity did not occur at any tested concentration, the 96 hour LC50 value is >1,000 mg/L FC-787.

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
140	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
240	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
610	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
1,000	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0

Note: Sublethal effects that were not observed were loss of reflex, excitability, discoloration, lethargy, and change in behavior.

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	--	--

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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 203, Fish Acute Toxicity 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

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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	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	1300	1500	1500	1300	1300	8.3	8.1	8.5	8.1	8.1
	2	1300	1500	1500	1300	1300	8.3	8.0	8.5	8.1	8.2
140	1	1300	1500	1500	1300	1300	8.2	8.0	8.5	8.0	8.1
	2	1300	1500	1500	1300	1300	8.2	8.0	8.5	8.0	8.1
240	1	1300	1500	1500	1300	1300	8.2	8.0	8.4	8.0	8.0
	2	1300	1500	1500	1300	1300	8.2	8.0	8.4	8.0	8.0
400	1	1300	1500	1500	1300	1300	8.1	7.9	8.4	7.9	8.0
	2	1300	1500	1500	1300	1300	8.1	7.9	8.4	7.9	7.9
600	1	1300	1500	1500	1300	1300	8.1	7.9	8.4	7.9	7.9
	2	1300	1500	1500	1300	1300	8.1	7.9	8.4	7.9	7.9
1,000	1	1300	1500	1500	1300	1300	8.1	7.9	8.3	7.8	7.9
	2	1300	1500	1500	1300	1300	8.1	7.9	8.3	7.8	7.9

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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	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	22.4	22.4	22.5	22.7	22.4	8.2	8.3	8.0	8.6	8.0
	2	22.5	22.5	22.5	22.7	22.5	8.2	8.0	8.0	8.7	8.3
140	1	22.4	22.5	22.4	22.5	22.5	7.7	7.8	7.7	6.1	7.5
	2	22.5	22.4	22.5	22.5	22.5	7.3	7.4	7.5	7.9	7.2
240	1	22.4	22.5	22.4	22.5	22.5	7.3	7.5	7.3	7.6	6.7
	2	22.4	22.4	22.5	22.5	22.5	7.3	7.1	7.1	7.1	6.7
400	1	22.5	22.5	22.6	22.6	22.5	6.8	6.3	6.5	6.5	5.2
	2	22.5	22.4	22.5	22.5	22.5	7.0	6.3	6.3	6.4	5.5
610	1	22.5	22.4	22.5	22.5	22.5	7.0	5.8	5.8	6.2	4.5
	2	22.5	22.5	22.6	22.5	22.5	6.8	5.8	5.6	6.2	4.7
1,000	1	22.5	22.5	22.5	22.5	22.4	6.4	4.7	5.0	4.8	3.0
	2	22.5	22.4	22.5	22.4	22.3	6.5	5.0	5.0	5.1	3.0