

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-600. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The 3M production lot number was not noted. The test sample is FC-600 (Belgium AFFF). Current information indicates it is a mixture of 1.0% PFOS, 81.20% water, 12.00% diethylene glycol butyl ether, 1.00% sodium octyl sulfate, 2.00% propane Sultone foamer, 1.00% sodium decyl sulfate, 0.85% xanthan gum, 0.1% N-(3-chloroallyl) hexaminium chloride, 0.80% starch, and 0.05 % tolyltriazole.

The following summary applies to a mixture with incompletely characterized concentration of impurities. Data may not accurately reflect the toxicity of the fluorochemical component of the test sample.

METHOD:

Method: Procedures closely followed those outlined in EPA-560/6-82-002, OECD, and ASTM Proposed standard practices for conducting acute toxicity tests with fish, macroinvertebrates and amphibians, E729-80.

Test type: Acute flow-through

GLP: No

Year Completed: 1982 (study) and 1983 (report)

Species: *Pimephales promelas*

Supplier: Commercial supplier in Brady, Nebraska.

Analytical monitoring: Temperature, pH, and DO

Exposure period: 96-hours

Statistical methods: LC₅₀ values and 95% confidence intervals could not be calculated. There was no mortality during the test.

Test fish age: Juveniles

Average Length and weight: Length = 3.8 + 0.45 cm
Weight = 0.42 ± 0.11 g

Loading: 0.47 g/L

Pretreatment: Not noted.

Test conditions:

Dilution water: Carbon-filtered well water, 3M well #2, St. Paul, MN.

Dilution water chemistry:

Dissolved Oxygen:	95 – 98% saturation
pH:	7.9 – 8.1
Hardness (as CaCO₃):	250 mg/L
Alkalinity (as CaCO₃):	230 mg/L

Conductivity: 340 μ mhos/cm

Lighting: Cool white fluorescent lights provided a daily photoperiod of 16 hours light and 8 hours dark with a 30 minute transition period was maintained during testing. Intensity was measured at 60 – 70 foot-candles at the water surface.

Stock and test solution preparation: A 40,000 mg/L stock solution was prepared daily by diluting 240 grams of test sample to 6-L with dilution water. Test solutions were prepared by a diluter delivering five nominal concentration of the test sample and control water.

Flow-through rate: Approximately five volume replacements per 24 hour period.

Stability of the test chemical solution: Not noted.

Exposure vessels: 24 cm diameter by 25 cm deep cylindrical glass aquaria with a maintained test solution volume of 9 liters.

Number of replicates: two

Number of fish per replicate: 10

Number of concentrations: 5 plus a blank control

Water chemistry during the study:

pH range (0-96 hours):

7.5 – 8.2 (control exposure)

7.5 – 8.2 (1,000 mg/L exposure)

Temperature range (0-96 hours):

18 – 20°C (for all aquaria)

Dissolved oxygen range (0-96 hours):

6.7 – 9.0 mg/L (control exposure)

6.1 – 9.0 mg/L (1,000 mg/L exposure)

RESULTS

Nominal concentrations: Bk control, 100, 280, 320, 560, and 1,000 mg/L

Percent mortality (based on 20 fish per concentration)

Nominal Concentration (mg/L)	Percentage mortality			
	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
100	0	0	0	0
280	0	0	0	0
320	0	0	0	0
560	0	0	0	0
1000	0	0	0	0

Element value: 24-hour LC₅₀: >1,000 mg/L
48-hour LC₅₀: >1,000 mg/L
72-hour LC₅₀: >1,000 mg/L
96-hour LC₅₀: >1,000 mg/L

Element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The value reported applies to that mixture and not the fluorochemical proportion alone.

No secondary effects were observed during the 96-hour flow through test.

CONCLUSIONS

The FC-600 96-hour LC₅₀ for *Pimephales promelas* was determined to be >1,000 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 2. The study meets all 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 solutions.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, 3M Lab Request number 9595, 1982.

3M Technical Report "Aquatic Toxicity Testing: FC-600 (Belgium-AFFF) LR 9595." M. T. Elnabarawy, Project 9970012600, Commercial Chemicals, Report Number 82, July 21, 1983

OTHER

Last changed: 6/28/00

TECHNICAL REPORT SUMMARY

TO: TECHNICAL COMMUNICATIONS CENTER - 201-2C-12

(Important - If report is printed on both sides of paper, send two copies to TCC.) Guidelines on reverse side.

Division Environmental Laboratory (EE & PC)		Dept. Number 0535
Project Commercial Chemicals		Project Number 9970012600
Report Title Aquatic Toxicity Testing: FC-600 (Belgium-AFFF) LR 9595		Report Number 82
To E. A. Reiner		Period Covered or Date 7/21/83
Author(s) M. T. Elnabarawy		Employee Number(s) 46981
Notebook Reference		No. of Pages Including Coversheet
SECURITY ☒ Open Report & Summary ☐ Closed Report-Open Summary		3M CHEMICAL REGISTRY ☐ Check box if new chemicals are reported. Use Chemical Registry Form 6092 to report all new substances.

KEYWORDS:
Lab Code

Other Keywords

EE & PC-Div.
(Env. Lab)Toxicity
(Aquatic)

CURRENT OBJECTIVE:

To determine the acute aquatic LC₅₀, following exposure to varying concentrations of FC-600 (Belgium-AFFF) under flow-through conditions.

REPORT ABSTRACT: This abstract information is distributed by the Technical Communications Center to alert 3M's Company R&D.

The acute aquatic 96-hour LC₅₀ values obtained for fathead minnows (*Pimephales promelas*) following exposure to FC-600 in replicated experiments were greater than 1,000 mg/l under flow-through conditions. The acute toxicity of FC-600 to aquatic organisms was considered insignificant under the reported test conditions.

Information Liaison
Initials: AVM

ACUTE TOXICITY OF FC-600 (Belgium-AFFF)

FATHEAD MINNOW (Pimephales promelas)

UNDER FLOW-THROUGH CONDITIONS

LR No. 9595 (9970012600)

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Environmental Laboratory
EE & PC
3M Company - 2-3E

July 20, 1983

INTRODUCTION

The purpose of this study was to estimate the acute toxicity of FC-600 (Belgium - AFFF) to fathead minnow (Pimephales promelas) under flow-through conditions. Preliminary range-finding static tests and a subsequent 96-hour flow-through test were conducted at the Environmental Laboratory, 2-3E, St. Paul, Minnesota. The acute toxicity of FC-600 was measured by estimating the LC50 value for the compound at each 24 hour interval of the test. The LC50 is defined as the concentration of the test compound in dilution water which caused mortality of 50% of the test animal population at the stated exposure interval. The flow-through test was conducted from July 11-15, 1983. The data reported herein are based on studies performed by M. T. Elnabarawy and R. R. Robideau. All raw data generated are stored at the above location.

MATERIALS AND METHODS

Procedures used in this acute toxicity test closely followed those described in the U.S. EPA protocol entitled "Environmental Effects Test Guidelines" (1982). Other test protocols considered particularly pertinent are listed in the reference section: ASTM-E729 (1980), OECD (1981). Values are reported to different levels of significance depending on the accuracy of the measuring devices involved in any one process.

The sample of FC-600, a viscous amber liquid, was received on September 15, 1982. The test material was stored in the dark at ambient temperature (21°C). The material was tested as 100% active ingredient. "Nominal" test concentrations are reported as milligrams of FC-600 per liter of test solution (mg/l).

The fathead minnows (Env. Lab Lot 2-1983) were obtained from a commercial fish supplier in Brady, Nebraska, and held in a 475-l fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness with a 30-minute transition period. All fish were fed a basic dried fish food, Tetra Min, daily except during the 48 hours prior to and during testing. There was less than 3% mortality in the test fish population during this 2-day acclimation period. The temperature in the holding tank was 20±1°C during this 2-day period (Daily Record of Fish Holding Conditions). The well water which flowed into this tank was characterized as having total hardness and alkalinity ranges as calcium carbonate (CaCO₃) of 250 mg/l and 230 mg/l, respectively, and a specific conductance of 340 micromhos per centimeter (umhos/cm) (Monthly Water Quality Analysis Logbook). Other parameters monitored in the holding tank were a pH range of 7.9-8.1, a dissolved oxygen (DO) range of 95-98% of saturation and a flow rate of a minimum of 2 tank volume replacements/day (Daily Record of Fish Holding Conditions). Test fish were maintained under these conditions for a minimum of 14 days. The specific conductance was measured with a YSI Model #31 conductivity-bridge, the pH was measured with an Orion Model 407A pH meter and combination electrode, the DO was measured with a YSI Model #54 dissolved oxygen meter and probe and the temperature was measured with an ASTM-Calibrated thermometer. Total hardness and alkalinity were measured according to APHA et al. (1980).

The toxicant-delivery system used in this study was a modified, (ACE Glass gas driven siphon type), proportional diluter similar to that described by Mount and Brungs (1967) with a 0.05 initial dilution factor. The bioassay diluter was constructed primarily of glass, #316 Stainless Steel, and perfluorocarbon plastics (Teflon). The dilution water was well water which was pumped to a polyethylene constant-head reservoir box where it was aerated with filtered air. This water was characterized as having total hardness and alkalinity ranges as CaCO_3 of 250 mg/l and 230 mg/l, respectively, a pH range of 7.9-8.1 and a specific conductance of 340 umhos/cm (Monthly Water Quality Analysis Logbook). A complete list of the physical/chemical characteristics of well water at the Envir. Lab facility, 2-3E is attached.

The bioassay diluter was calibrated to deliver five nominal logarithmic concentrations of FC-600; 1000, 560, 320, 180, 100 mg/l and control water containing no FC-600 to duplicate test aquaria. Each cylindrical glass test aquarium measured 24 cm in diameter and 25 cm deep with 21 cm high drains which maintained water volumes of 9 l. The diluter delivered 0.2 l of test water to each aquarium at an average rate of 225 cycles per day. This is equivalent to 5 aquarium volume replacements per 24-hour period. Illumination was provided by Cool White^R fluorescent lights for 16 hours measuring 60-70 footcandles at the water surface each day. Test temperature was continuously monitored in one of the control tanks during the 96-hr exposure test using a Taylor temperature chart-recorder.

A 10-l polyethylene bottle was utilized to deliver the toxicant stock solution to the mixing chamber of the diluter with every cycle. The stock solution of 40,000 mg/l was prepared daily by adding 240 grams of FC-600 and diluting to 6,000 ml. A total amount of 960 grams of FC-600 was used during the continuous flow study.

After establishing the desired test concentrations in the test aquaria, the FC-600 stock solution was introduced into the diluter and cycling began on July 11, 1983. The test was initiated when ten fathead minnows uniform in size and age with a mean ($N = 10$) wet weight and total length of 0.42 ± 0.11 grams and 3.8 ± 0.45 cm (Fish Weights and Lengths Log) were randomly distributed to each aquarium. A total of 20 organisms were exposed to each concentration. The fish loading ratio was calculated to be 0.47 g fish per liter of test solution.

No aeration of test solutions in all test chambers was provided during the test.

The pH, temperature and dissolved oxygen (DO) concentrations were measured at 0, 24, 48, 72, and 96 hours in both replicates of all concentrations including those of the control. Biological observations of the fish including mortality and secondary effects (behavioral observations) were made and recorded daily.

The general operation of the bioassay diluter was checked at least twice daily throughout the test.

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate 24-, 48-, 72-, and 96-hour median lethal concentrations (LC50). When applicable, the method of calculation selected is determined by the characteristics of the data base (i.e., presence or absence of test concentrations causing mortality of 100% of the test animal population, test concentrations causing mortality of a partial number of animals in the population, etc.).

RESULTS

The test material FC-600 was not acutely toxic to fathead minnows at nominal concentrations as high as 1,000 mg/l (Table 1). No FC-600 related mortality of fish exposed to 1,000 mg/l was observed during the 96 hour exposure period. The exposed fish appeared generally healthy and active. The estimated LC50 values after 24, 48, 72, and 96 hours of exposure were >1,000 mg/l, the highest concentration tested.

No control organisms died or showed signs of disease or stress during the test.

The temperature in various aquaria ranged from 19-21°C during exposure, and did not vary more than 2°C in any 12-hour period. The pH and dissolved oxygen concentrations measured during the toxicity test are presented in Aquatic Toxicity Data Sheets (attached). The pH levels remained relatively unchanged, and the dissolved oxygen concentrations did not fall below 60% saturation.

The results lead to the conclusion that FC-600 (Belgium-AFFF) is not acutely toxic to the aquatic environment as a result of exposure under comparable conditions.

REFERENCES

- APHA, AWWA, WPCF. 1980. Standard methods for the examination of water and wastewater. 15th Edition, Washington, D.C., 1134 pp.
- ASTM-Standard Practice, 1980. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, Philadelphia, PA, ASTM-E729-80.
- Lemke, A. E., W. A. Brungs and B. J. Halligan. 1978. Manual for construction and operation of toxicity-testing proportional diluters. EPA-600/3-78-072.
- Mount, D. I. and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 20-29.
- OECD. 1981. Guidelines for Testing of Chemicals. Organization for Economic Cooperation and Development, Washington, D.C.
- U.S. EPA. 1982. Environmental Effects Test Guidelines. EPA 560/6-82-002.

Table 1. Concentrations tested, corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to FC-600 under flow-through conditions. Twenty fish were exposed to each concentration.

Nominal Concentration (mg/l)	Percentage Mortality			
	24 hour	48 hour	72 hour	96 hour
1000	0	0	0	0
560	0	0	0	0
320	0	0	0	0
180	0	0	0	0
100	0	0	0	0
Control	0	0	0	0
LC ₅₀ (mg/l)	>1,000	>1,000	>1,000	>1,000

Table 2. WELL WATER QUALITY

SAMPLE DATE: 6/25/83

SAMPLE TAKEN: 3M Bldg. 2-3E, St. Paul

SAMPLE DATE 6/25/83 (TAKEN FROM ROOM 5)

<u>PARAMETER</u>	<u>CONCENTRATION</u>	<u>ENV. LAB WATER QUALITY BOOK REF.</u>
pH	7.9	8
Residual Chlorine	<0.02 mg/l	9
Ammonia Nitrogen	<0.1 mg/l as N	8
Conductivity	340 umhos/cm at 25°C	9
M-Alkalinity	230 mg/l as CaCO ₃	8
Total Solids	280 mg/l	8
Total Hardness	250 mg/l as CaCO ₃	8
Total Fluoride	<0.02 mg/l	9
Dilute COD	<0.4 mg/l	9
Total Coliform	Negative	10
Standard Plate Count	40 per ml	10
Aluminum as Al*	<19 ug/l	16
Arsenic as As*	1 ug/l	16
Boron as B*	15 ug/l	16
Cobalt as Co*	<5 ug/l	16
Copper as Cu*	<3 ug/l	16
Chromium as Cr*	<2 ug/l	16
Cadmium as Cd*	<0.1 ug/l	16
Iron as Fe*	11 ug/l	15
Nickel as Ni*	<7 ug/l	16
Lead as Pb*	<1 ug/l	16
Silver as Ag*	<5 ug/l	16
Zinc as Zn*	89 ug/l	15
Mercury as Hg*	0.7 ug/l	16
PCB's*	<0.01 ug/l	17
Total Organochlorine* Pesticides	<0.01 ug/l	17
Total Organophosphorus* Pesticides	<0.01 ug/l	17

*Sample Date 3/10/83

ENVIRONMENTAL LABORATORY
AQUATIC TOXICITY DATA SHEET
DEFINITIVE (FULL-SCALE TEST)

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Test 96HR ACUTE FLOW-THROUGH Material Tested AEEF-EUROPE (BELGIUM) Lot FC-600
Test Organism FATHEAD MINNOW Source BRADY, NEB Date Rec'd 12/7/82 Batch # 2-83
J.Wt. 0.42 grams Avg. Size 3.8 cm No. Organisms per dose 10 Loading Factor 0.426 g/l
Fish Disease Treatment: NONE

Test Solution Volume 9 Liters/All-Glass Tank Tank Dimensions: 35x20 cm and 25 cm deep
Test Started 4:00 PM Date Started 7/11/83 Date Completed 7/15/83
Test Solution Water Carbon-filtered well water (Temp. 18 °C., D.O. 9.0 ppm, pH 8.2)
Investigator [Signature] LC₅₀ (95% Confidence Limits) >1000 mg/l
All Signatures [Signature]

Tank No.	Initial Conc. mg/l	Total Amount Added	24 Hours			48 Hours			72 Hours			96 Hours		
			Total No. Dead	pH	D.O. ppm	Total No. Dead	pH	D.O. ppm	Total No. Dead	pH	D.O. ppm	Total No. Dead	pH	D.O. ppm
2	Control		0	7.5	6.9	0	7.5	7.4	0	7.6	6.7	0	7.5	6.9
	Control		0	7.6	7.0	0	7.6	7.4	0	7.6	6.8	0	7.6	6.7
1	1000		0	7.7	6.4	0	7.6	6.3	0	7.5	6.2	0	7.6	6.1
	1000 X		0	7.7	6.3	0	7.6	6.1	0	7.5	6.1	0	7.6	6.4
3	500		0	7.7	6.9	0	7.6	6.3	0	7.5	6.2	0	7.5	6.2
	500 X		0	7.9	6.8	0	7.6	6.5	0	7.6	6.2	0	7.5	6.1
Test Temp. °C			20°C			20°C			20°C			20°C		
Initials & Date			A.3 7/12/83			A.3 7/13/83			A.3 7/14/83			A.3 7/15/83		
Comments:														

Secondary Effects (other than Mortality):

EC₅₀

Tank No.	Symptoms or Reactions	Total Number Affected			
		24-Hr.	48-Hr.	72-Hr.	96-Hr.
Control					
	No secondary effects were noted during the 96hr flow-through test. [Signature] 7/15/83				

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sh Disease Treatment: NONE

1e Started 4:00 PM Date Started 4/11/85 Date Completed 4/15/85

Investigator MA 10, 13 LC_{50} (95% Confidence Limits) $n=2$ >1000 mg/l
 All Signature) 6, 13

Secondary Effects (other than Mortality):

EC50

[illegible]