

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-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The 3M production lot number was Belgium 2367. The test sample is FC-600, referred to by the laboratory as "3M-I". 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: EPA-660/3-75-009 and Std. Methods (APHA), 1980, 15th Ed.

Test type: Acute flow-through

GLP: No

Year Completed: 1983

Species: *Pimephales promelas*

Supplier: In-house cultures maintained at ABC Laboratories, Inc. Columbia, MO.

Analytical monitoring: Temperature, pH, DO, and ammonia

Exposure period: 96-hours

Statistical methods: LC₅₀ values and 95% confidence intervals calculated using binomial, the moving average, or the probit tests.

Test fish age: Juveniles.

Average Length and weight: Length = 30 mm
Weight = 0.52 g

Loading: 0.35 g/L

Pretreatment: None.

Test conditions:

Dilution water: Well water.

Dilution water chemistry:

pH:	7.8
Hardness (as CaCO₃):	255 mg/L
Alkalinity (as CaCO₃):	368 mg/L
Conductivity:	50 µmhos/cm

Lighting: A daily photoperiod of 16 hours light and 8 hours dark was maintained.

Stock and test solution preparation: A 381,000 mg/L stock solution was prepared by diluting 381 grams of test sample to 1-L with deionized water. Test solutions were prepared by a diluter delivering one nominal concentration of the test sample and control water.

Flow-through rate: At least 5 volume replacements in a 24 hour period.

Stability of the test chemical solution: Not noted.

Exposure vessels: Aquaria with a maintained test solution volume of 15 liters.

Number of replicates: two

Number of fish per replicate: 10

Number of concentrations: 1 plus a blank control, in duplicate

Water chemistry during the study:

pH range (0-96 hours):

8.0 – 8.1 (control exposure)

7.8 – 8.2 (1,000 mg/L exposure)

Temperature range (0-96 hours):

21°C (for all aquaria)

Dissolved oxygen range (0-96 hours):

7.6 – 8.8 mg/L (control exposure)

2.7 – 9.2 mg/L (1,000 mg/L exposure)

Remarks: The dissolved oxygen concentrations for the 1,000 mg/L exposure aquaria fell to 5.7 and 2.7 mg/L after 96 hours. Since fathead minnows are generally more tolerant of low DO concentrations, it was not considered to be a significant factor in the results of the test.

RESULTS

Nominal concentrations: Bk control 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
1,000	0	0	5	35

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.

Behavior observations during the test indicated that mortality was preceded by surfacing and loss of equilibrium.

A reference test was conducted using Antimycin A. Test results were within published literature values.

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 the criteria for quality testing. However, the sample purity was not properly characterized and it lacks analytical confirmation of the amount of fluorochemical proportion in solution. Additionally, DO concentrations fell significantly below acceptable levels at the 1,000 mg/L exposure.

REFERENCES

This study was conducted by ABC Laboratories, Inc. of Columbia, MO at the request of the 3M Company, 3M Lab Request number 8875 and 9063, 1983.

OTHER

Last changed: 6/28/00



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FC-600

Belgium lot 236
~~US lot 628~~

LR 8875

LR 9063

Flow-Through Acute Toxicity Report
#30175

Submitted To:

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Dynamic Acute Toxicity of 3M-I
to Fathead Minnows (Pimephales promelas)

March 29, 1983

002043

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SUMMARY

A dynamic 96-hour toxicity study was conducted to determine the acute toxicity of 3M-I to fathead minnows (Pimephales promelas). The methods employed were those outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits.

A flow-through proportional diluter system was used to maintain constant test concentrations. Nominal exposure concentrations of 3M-I were duplicate 1,000 mg/l and duplicate controls.

As a quality check, the fathead minnows were challenged with a reference compound, Antimycin A. The observed 96-hour LC₅₀ and 95% confidence limits (C.I.) were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition.

The observed mortality data for the study indicated that the LC₅₀ would be >1,000 mg/l.

INTRODUCTION

3M Company contracted the Aquatic Toxicology Division of Analytical Bio-Chemistry Laboratories, Inc., to conduct a duplicate 1,000 mg/l concentration plus duplicate control, 96-hour, flow-through bioassay to estimate the toxicity of 3M-I to fathead minnows (Pimephales promelas). The primary objective of the study was to define the acute toxicity of 3M-I by estimating the median lethal concentrations (LC₅₀'s) during the course of the test. The study was authorized by M. T. Elnabarawy, 3M Company, in a protocol dated February 21, 1983 (Appendix) and was conducted from March 10 to March 14, 1983.

The term LC₅₀ as used in this report is that concentration of 3M-I causing 50% mortality after a specified exposure period. This report presents the results of the toxicity study and describes the technical approach used to meet the project objectives.

METHODS AND MATERIALS

The biological methods used for the 96-hour flow-through toxicity test are basically those described in Standard Methods for the Examination of Water and Wastewater (1) and Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (2). The study was conducted following the procedures outlined in ABC Protocol No. 8007 (Appendix).

I. Test Fish

The fathead minnows used in the test were obtained from ABC Laboratories in-house culture. The fish were identified to species using the taxonomic keys developed by Eddy (3). All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. Fish culture techniques were basically those described by Brauhn et al. (4). A daily record of fish observations during the holding period, along with any prophylactic and therapeutic disease treatments, is included in the Appendix. During the holding period, the fish received a standard commercial fish food (Rangen's®) daily in an amount equivalent to 3 percent of their body weight. The test fish were not fed during the acclimation and test periods. The fathead minnows used for this experiment had a mean weight of 0.52 g and a mean standard length of 30 mm. Weight and length measurements were made on the control fish at test termination and are included in the Appendix. As a quality check, the fathead minnows were challenged with a reference compound, Antimycin A. The observed 96 hour LC₅₀ and 95% confidence limits were within the 95% confidence limits reported in the literature (5), indicating that the fish were in good condition. Results of this test are included in the Appendix.

II. Test System

A proportional diluter system described by Mount and Brungs (6), with a Hamilton dual syringe dispenser system was used for the intermittent introduction of 3M-I and diluent water into the test aquaria. Aerated well water (Table 2) was delivered to the glass aquaria at a rate of 52 ml/minute/aquarium, an amount which was sufficient to replace the 15 liter test volume at least 5 times in a 24 hour period. The test aquaria were immersed in a water bath held at 22°C ($\pm 1^\circ$) by submerged heating elements.

III. Test Compound

The 3M-I standard was received on February 24, 1983, in good condition. The sample upon receipt was observed to be a viscous amber liquid and was stored at room temperature. The stock solution was prepared in deionized water on a weight/volume basis. Standard weights and dilution volumes can be found in the Appendix.

IV. Test Procedure - Biological

The test was initiated on March 10, 1983, by random assignment of 10 fathead minnows to each of the 15 liter test aquaria after test solutions had been flowing through the aquaria for 24 hours. The fish were exposed to the following nominal concentrations of 3M-I: duplicate 1,000 mg/l and duplicate controls. The fish were observed for mortality and abnormal behavior initially and once every 24 hours for the 96-hour test period. Dead individuals were removed at each observation.

V. Test Procedure - Chemical and Physical

Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits (Table 3).

The nominal concentration of 1,000 mg/l was calculated by dividing the working stock concentration by the diluter dilution factor. The dilution factor was determined by dividing the toxicant mixing box volume by volume injected by toxicant delivery system. Calculations are in the Appendix.

VI. Statistical Analysis

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC₅₀ program developed by Stephan et al. (7). This program calculated the LC₅₀ statistic and its 95 percent confidence limits using the binomial, the moving average, and the probit tests. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all

possible sets of data that may be obtained. The method of calculation selected for use in this report was that which gave the narrowest confidence limits for the LC_{50} (7, 8).

RESULTS

The estimated LC_{50} for 3M-I would be $>1,000$ mg/l after 96 hours. Table 1 presents the LC_{50} values and 95% confidence intervals for Antimycin A. The 96-hour LC_{50} for Antimycin A to fathead minnows was 5.6×10^{-5} mg/l.

Mortality data is presented in Table 4 and was 35% after 96 hours. Behavior observations throughout the test indicated that mortality was preceded by surfacing and loss of equilibrium.

Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured in the control and 1,000 mg/l concentration every 24 hours (Table 3). The dissolved oxygen concentrations in the control stayed between 86 and 100 percent saturation at 22°C and was considered adequate for testing (2). The dissolved oxygen concentrations for the exposure aquaria were lower than the control (5.7 and 2.7 mg/l) after 96 hours. This appeared to be compound related but would not have adversely effected the results. Ammonia concentrations were below the toxic level (9). The pH values were consistent with the control throughout the study.

The study was conducted following the intent of the Good Laboratory Practice Regulations (10) and the final report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data was provided to 3M Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

TABLE 1: The estimated LC_{50} values with their 95% confidence limits for the 96-hour fathead minnow toxicity study of Antimycin A.

<u>Hours of Exposure</u>	<u>3M-I (mg/l)</u>	
	<u>LC_{50}</u>	<u>95% Confidence Limits</u>
24	--	--
48	6.5×10^{-5}	$4.2 \times 10^{-5} - 1.4 \times 10^{-4}$
72	6.2×10^{-5}	$4.2 \times 10^{-5} - 1.4 \times 10^{-4}$
96	5.6×10^{-5}	$4.2 \times 10^{-5} - 7.5 \times 10^{-5}$

TABLE 2: Chemical characteristics of well water at ABC's Aquatic Bioassay Laboratory.

<u>Parameter</u>	<u>Concentration</u>
Dissolved Oxygen	9.2 ppm
pH	7.8
Hardness (CaCO ₃)	255 ppm
Alkalinity (CaCO ₃)	368 ppm
Conductivity	50 μ mhos/cm
Total Ammonia (NH ₃)	<0.10 ppm
NO ₃ -N	0.15 ppm
Ortho-Phosphate	0.10 ppm
Aluminum	<0.007 ppm
Arsenic	<0.001 ppm
Cadmium	<0.001 ppm
Chromium	<0.002 ppm
Cobalt	<0.002 ppm
Copper	<0.002 ppm
Iron	<0.004 ppm
Lead	<0.02 ppm
Mercury	<0.0001 ppm
Nickel	<0.004 ppm
Silver	<0.002 ppm
Zinc	<0.001 ppm
Measured organophosphorus pesticides	a
Measured organochlorine pesticides plus PCB's	a

^aSee appendix for individual analyses.

TABLE 3: Water quality measurements during the 96-hour toxicity study of 3M-I to fathead minnows.

Hours of Exposure	Control ^a				1,000 mg/l ^a			
	Temp. °C	D.O. ^b mg/l	pH ^c	NH ₃ ^d mg/l	Temp. °C	D.O. ^b mg/l	pH ^c	NH ₃ ^d mg/l
0	21, 21	8.8, 8.8	8.1, 8.1	0.18, 0.17	21, 21	8.9, 9.2	8.1, 8.2	0.19, 0.18
24	21, 21	8.4, 8.4	8.1, 8.0	0.26, 0.31	21, 21	8.6, 8.5	8.1, 8.0	0.41, 0.36
48	21, 21	7.6, 7.6	8.1, 8.1	0.35, 0.30	21, 21	5.1, 4.3	8.0, 7.9	0.26, 0.20
72	21, 21	8.3, 8.3	8.1, 8.1	0.22, 0.30	21, 21	5.8, 3.9	8.0, 7.8	0.18, 0.20
96	21, 21	7.9, 7.8	8.1, 8.1	0.18, 0.26	21, 21	5.6, 2.7	7.9, 7.8	0.31, 0.36

^aDuplicate aquaria.

^bDissolved oxygen concentrations - YSI Model 54 Dissolved Oxygen meter and probe.

^cpH - pH Probe (Corning Model 476182) used with a Corning Model 125 pH and mV meter.

^dAmmonia concentrations - Ammonia Probe (Orion Model 95-10) used with a Corning Model 125 pH and mV meter.

TABLE 4: Percent mortality of fathead minnows exposed to 1,000 mg/l of 3M-I during a 96-hour toxicity study.

3M-I Nominal Concentration (mg/l)	Hours of Exposure			
	24	48	72	96
Control a	0	0	0	0
Control b	0	0	0	0
1,000 a	0	0	0	10
1,000 b	0	0	10	60

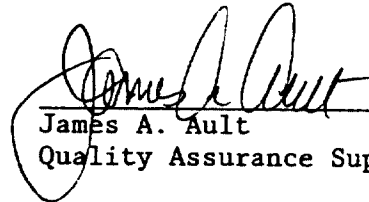
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- (2) Committee on Methods for Toxicity Tests with Aquatic Organisms (C. E. Stephan, Chairman). 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. 61 p.
- (3) Eddy, Samuel. 1969. The Freshwater Fishes. 2nd ed. W. C. Brown Company, Dubuque, IA. 286 p.
- (4) Brauhn, J. L. and R. A. Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May, 1975. 45 p.
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- (6) Mount, D. I. and W. A. Brungs. 1967. A Simplified Dosing Apparatus for Fish Toxicological Studies. Water Res. 1:21-29.
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- (8) Stephan, C. E., K. A. Busch, R. Smith, J. Burke and R. W. Andrew. 1978. A computer program for calculating an LC_{50} . U. S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.
- (9) National Academy of Sciences. 1971. Water Quality Criteria, 1972. U. S. Department of Commerce, PB-236 199. 592 p.
- (10) Food and Drug Administration. Regulations for Good Laboratory Practice in Non-Clinical Animal Studies. 21 CFR Part 58.

Quality Assurance Statement for final report #30175 entitled, "Dynamic Acute Toxicity of 3M-I to Fathead Minnows (Pimephales promelas)," for Dr. M. T. Elnabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories intent that all studies conducted at our facilities are designed and function in conformance with good laboratory practice regulations and the protocols for individual laboratory studies, an inspection of the final report for 3M-I was conducted and found to be in an acceptable form by a member of our Quality Assurance Unit. An inspection of the daily mortality rate of the test organisms prior to the initiation of the study indicated they were in good health and should not bias the observed mortality in the study. A final inspection of all data and records on March 25, 1983 indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories.

Should you have any questions relating to the information provided in this statement or the function of our Quality Assurance Unit, please contact me at your convenience.


James A. Ault
Quality Assurance Supervisor

3/30/83
Date