

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

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

Remarks: The 3M product lot number was 3086. The test sample is FC-203S (F2132-1). Current information indicates it is a mixture of 1.34% PFOS, 35% diethylene glycol butyl ether, 37.85% water, 2.66% Sultone foamer, 3% sodium octyl sulfate, 0.10% 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 the toxicity of the fluorochemical component of the test sample.

METHOD:

Method: U.S. EPA

Type: Acute flow-through

GLP: No

Year completed: 1988

Species: *Salmo gairdneri*

Supplier: Mt. Lassen Trout Farm, Red Bluff, California.

Analytical monitoring: Temperature, pH, and DO

Exposure period: 96-hours

Statistical methods: LC₅₀ values calculated using the Stephan et al. computer program, 1978.

Test fish age: Juveniles

Length and weight: Average length = 39 mm
Average weight = 0.86 g (wet)

Loading: 0.57 g fish/L

Pretreatment: Unknown

Test conditions:

Dilution water: Blended water which is a mixture of reverse osmosis and ABC well water.

Dilution water chemistry:

Hardness: 40 - 48 mg/L as CaCO₃

Alkalinity: 44 - 56 mg/L as CaCO₃

Lighting: A daily photoperiod of 16 hours light and 8 hours dark was maintained throughout the testing period.

Stock and test solution preparation: The primary stock solution of 160,000 mg/L was prepared by dissolving 160 g of test material in 1 L of deionized water. Test solutions were intermittently introduced to the test vessels via a

proportional diluter system with a flow rate of 1 L of test solution at an average rate of 8 times per hour.

Flow-through rate: Approximately 6.4 times per day.

Stability of the test chemical solutions: Not noted

Exposure vessels: Aquaria containing 30 L of test solution

Number of replicates: 1

Number of fish per replicate: 20

Number of concentrations: five plus a blank control

Water chemistry during the study:

pH range (0-96 hours):

7.4 – 7.8 (control exposure)

7.4 – 7.8 (400 mg/L exposure)

Temperature range (0-96 hours):

13 - 13 °C (control exposure)

12 - 13 °C (400 mg/L exposure)

Dissolved oxygen range (0-96 hours):

7.7 – 8.9 mg/L (control exposure)

7.2 – 9.0 mg/L (400 mg/L exposure)

RESULTS

Nominal concentrations: Bk control, 25, 50, 100, 200, and 400 mg/L

Element values: 96-hour LC_{50} = >400 mg/L

96-hour NOEC = 400 mg/L

Element values based on nominal concentrations

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

The testing laboratory was unable to conduct the study at concentrations greater than 400 mg/L due to insufficient sample size.

CONCLUSIONS

The test sample 96-hour LC_{50} for rainbow trout was determined to be >400 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, sample purity was not properly characterized and the study lacks

analytical confirmation of the amount of fluorochemical proportion in the solution. The first five pages of the final report are missing.

REFERENCES

Test was conducted by ABC Labs, of Columbia, MO at the request of the 3M Company, 1988.

OTHER

Last changed: 6/28/00

Internal Correspondence/3M

cc: K. W. Livermore - 236-2A-01
M. T. Pike - 236-3A-01
D. P. Neuman - 236-2A-01
M. T. Elnabarawy - 21-2W-05

TO: D. R. RICKER - 236-1B-10
FROM: E. A. REINER - ENVIRONMENTAL LAB/EE & PC - 21-2W-05
SUBJECT: ~~CONFIDENTIAL - INTERNAL USE ONLY~~
DATE: DECEMBER 28, 1988

In response to your request, a salmon fry bioassay was conducted on FC-203S Lot 3086. The results of this bioassay are attached. The highest concentration tested was 400 mg/l and there were no adverse effects at this concentration. According to ABC Labs, this was the highest concentration that could be tested with the amount of sample provided.

Hopefully, these results are adequate to meet your needs. If you have any questions or concerns, please feel free to contact me on 778-5079.



EAR/mb

Attachment: Bioassay Results on FC-203S

SUMMARY

A 96-hour acute flow-through toxicity study of ~~FC-203~~ to rainbow trout (*Salmo gairdneri*) was conducted for 3M Company from October 14, 1988 to October 18, 1988. Twenty fish per test concentration plus control were used in a nominal dosing regime of 25, 50, 100, 200, and 400 mg/l. Water chemistry measurements of dissolved oxygen, pH and temperature were consistent throughout the term of the experiment and were within the protocol specifications.

From the data collected during this study, a 96-hour LC₅₀ with 95% confidence limits was calculated to be >400 mg/l based on the nominal concentrations of FC-203. Neither mortality nor sublethal/behavioral effects occurred at any exposure concentration or control. As determined by this study, a 96-hour no-effect concentration of FC-203 toxicity to rainbow trout was 400 mg/l based on a lack of mortality or abnormal responses.

INTRODUCTION

3M Company contracted the Aquatic Toxicology Division of Analytical Bio-Chemistry Laboratories, Inc., to conduct a five concentration plus control flow-through toxicity test to determine the toxicity of FC-203 to rainbow trout (Salmo gairdneri). The primary objective of this study was to estimate the acute toxicity of FC-203 to rainbow trout by determining the median lethal concentration (LC_{50}), if possible, and examining sublethal/behavioral responses during the course of the test. This was accomplished by exposing 20 rainbow trout per concentration and control and monitoring survival and sublethal/behavioral responses over a 96 hour exposure duration.

The term LC_{50} , as used in this report, is that concentration of FC-203 causing 50% mortality after a specified exposure period. The study was conducted from October 14, 1988 to October 18, 1988 following ABC Protocol Number 8007 (APPENDIX) as approved by Mohammed Elnabarawy. 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, as well as the recommended statistical analyses, used in this study were those described by the U. S. Environmental Protection Agency (1).

I. Test Fish

The 120 rainbow trout (Salmo gairdneri) (lot #3788) used in this study were obtained from Mt. Lassen Trout Farm in Red Bluff, California. The fish were reared and maintained at ABC Laboratories in ABC soft blended water and were fed newly hatched brine shrimp or a commercially available fish food daily. The laboratory environment was maintained on a 16-hour daylight photoperiod. Fish culture techniques were basically those described by Brauhn et al. (2). A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, is included in Appendix I.

Seventy-two hours before the initiation of the test, fish were removed from the culture tank and placed in the temperature acclimation unit. During this time, the fish were held without food. At test initiation, rainbow trout were randomly placed in the testing unit. The rainbow trout used as the control group during this study had a mean weight of 0.86 (± 0.26) g and a mean standard length of 39 (± 4.2) mm when measured at the end of the test. The biomass loading rate was 0.57 g/l. These measurements are provided in Appendix I.

II. Test System

A proportional diluter system described by Mount and Brungs (3) and a Hamilton Model 420 syringe dispenser was used

for the intermittent introduction of FC-203 test solutions and diluent water into each test chamber. Five concentrations of the test material and control comprised the test design. Each concentration and control held twenty fish. Chemical characteristics, typical of ABC soft blended water, are provided in Table 1.

The diluter delivered one liter of test solution or control water to the test vessels at an average rate of 8.0 times per hour over the course of the study. This flow rate was sufficient to replace the 30 liter volume within the test chambers 6.4 times per day. The test aquaria were immersed in a circulating water bath that was thermostatically held at $12 \pm 1^\circ\text{C}$.

III. Test Material

The FC-203 (F 2132-1) (Lot No. 3086) was received from 3M on September 8, 1988 in good condition. The sample was observed to be an amber liquid and was stored at room temperature. A diluter stock solution (160,000 mg/l) was prepared by dissolving 160 g of FC-203 in 1.0 liter of deionized water. The injector volume was adjusted to deliver 5.0 ml of stock to two liters of dilution water. The resulting nominal concentration regime of FC-203 was 25, 50, 100, 200, and 400 mg/l.

IV. Test Procedure - Biological

A preliminary static 96-hour range-finding test was conducted to determine a concentration range of FC-203 to use in the definitive test. This preliminary test was conducted from September 15, 1988 to September 19, 1988 using 1.0, 10, and 100 mg/l exposure concentrations. During this test, neither mortality nor abnormal effects were observed at any level. These results were discussed with the sponsor's representative and it was agreed that the definitive flow-through test would be run at a nominal high concentration of 400 mg/l of FC-203.

The definitive test was initiated after the test solutions had been flowing through the aquaria for approximately 48 hours. Twenty rainbow trout were impartially distributed to each test container. Observations for mortality and sublethal responses were made once every 24 hours during the 96 hour test period.

V. Test Procedure - Chemical and Physical

Temperature ($^\circ\text{C}$), dissolved oxygen (mg/l), and pH were measured in the control, low, middle and highest concentration containing surviving fish at 0, 48 and 96 hours of testing. These results are provided in Appendix I of this report.

VI. Statistical Analyses

The results of the definitive study were statistically analyzed (4) for 24, 48, 72 and 96-hour LC_{50} values and their corresponding 95% confidence limits. All calculations were performed on an IBM PC-XT computer using the nominal concentrations of FC-203. Because no mortality occurred, neither an LC_{50} or 95% confidence limits were determined.

RESULTS

Water chemistry parameters measured at 0, 48 and 96 hours were within the specified limits for conducting aquatic toxicity tests (1). Dissolved oxygen ranged from 7.2 to 9.0 mg/l, and pH ranged from 7.3 to 7.8 (Table 2). Percent water saturation of dissolved oxygen given the above measurements ranged from 70% to 89% at temperatures of 12 and 13°C, respectively.

From the data collected during this flow-through study, the 24-, 48-, 72- and 96-hour LC_{50} values were estimated to be >400 mg/l (Table 3). As shown in Table 4, no mortality or abnormal effects were seen at any exposure level or control. For this reason, the no effect concentration for the acute toxicity of FC-203 to rainbow trout was 400 mg/l, the maximum concentration tested.

The study was conducted following the intent of the Good Laboratory Practice Regulations (5) and the preliminary report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data will be provided to 3M Company with the final report, with a copy retained at Analytical Bio-Chemistry Laboratories.

TABLE 1

Chemical Characteristics of Soft Blended Water Used
by ABC's Aquatic Toxicology Division^a

Parameter	Concentration
Temperature	18 - 23°C
Dissolved Oxygen ^b	8.0 - 9.3 mg/l
pH	7.1 - 7.9
Hardness (CaCO ₃)	40 - 48 mg/l
Alkalinity (CaCO ₃)	44 - 56 mg/l
Conductivity	100 - 160 µmhos/cm
Suspended Solids	0 - 1.4 mg/l
Aluminum	<0.2 mg/l
Arsenic	<0.001 mg/l
Boron	0.181 mg/l
Cadmium	<0.005 mg/l
Chromium	<0.010 mg/l
Cobalt	0.169 mg/l
Copper	<0.025 mg/l
Iron	0.127 mg/l
Lead	<0.003 mg/l
Mercury	<0.0004 mg/l
Nickel	0.048 mg/l
Silver	<0.001 mg/l
Zinc	<0.020 mg/l

^aBlended water is a mixture of reverse osmosis (R.O.) and ABC well water to achieve a final hardness of between 40 - 48 mg/l.

^bAfter aeration

Note: The ranges of these selected parameters are from the time period April through June, 1988 or from the most recent quarterly screen for contaminants. All raw data supporting these ranges are on file at ABC Laboratories.

TABLE 2

Water Quality Measurements During the Acute Flow-Through
Toxicity Test of FC-203 to Rainbow Trout (Salmo gairdneri)

Nominal Concentration (mg/l)	Water Quality								
	0-hour			48-hour			96-hour		
	Temp. °C	D.O. ^a mg/l	pH ^b	Temp. °C	D.O. mg/l	pH	Temp. °C	D.O. mg/l	pH
Control	13	8.5	7.7	13	7.7	7.8	13	8.9	7.4
25	13	8.6	7.7	13	7.7	7.7	13	9.0	7.4
50									
100	13	9.0	7.7	13	7.9	7.7	13	8.9	7.3
200									
400	13	9.0	7.8	12	7.2	7.8	13	8.6	7.4

^aDissolved oxygen concentrations - Dissolved Oxygen Probe (YSI Model 54).

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

NOTE: Altitude-adjusted dissolved oxygen saturation at the test temperatures of 12 and 13°C is 10.3 and 10.1 mg/l, respectively.

TABLE 3

The Estimated LC₅₀ Values with 95% Confidence Limits for the 96-Hour Toxicity Study of FC-203 to Rainbow Trout (Salmo gairdneri)

Period of Exposure, Hours	(mg/l)	
	LC ₅₀	95% Confidence Limits
24, 48, 72, 96	>400 ^a	--

^aNo mortality occurred at any exposure concentration; therefore, the LC₅₀ was considered greater than the maximum exposure concentration. No confidence limits could be determined.

TABLE 4

Mortality and Behavioral Observations During
the Acute Flow-Through Toxicity Test of FC-203 to
Rainbow Trout (Salmo gairdneri)

Nominal Test Concentration mg/l (ppm)	No. Placed in test	24-Hours		48-Hours		72-Hours		96-Hours	
		Mort.	Observations	Cumm. Mort.	Observations	Cumm. Mort.	Observations	Cumm. Mort.	Observations
Control	20	0	20 N	0	20 N	0	20 N	0	20 N
25	20	0	20 N	0	20 N	0	20 N	0	20 N
50	20	0	20 N	0	20 N	0	20 N	0	20 N
100	20	0	20 N	0	20 N	0	20 N	0	20 N
200	20	0	20 N	0	20 N	0	20 N	0	20 N
400	20	0	20 N	0	20 N	0	20 N	0	20 N

Key to Observations: N = Normal

LITERATURE CITED

- (1) 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.
- (2) 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.
- (3) Mount, D. I. and W. A. Brungs. 1967. A Simplified Dosing Apparatus for Fish Toxicological Studies. Water Res. 1:21-29.
- (4) 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.
- (5) Organization for Economic Cooperation and Development. 1981. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30(Final):7-28.

QUALITY ASSURANCE STATEMENT

Quality Assurance Statement for preliminary report #37242 entitled, "Acute Flow-Through Toxicity of FC-203 to Rainbow Trout (Salmo gairdneri)", for Mo Elnabarawy, 3M Company, St. Paul Minnesota.

In accordance with ABC Laboratories' intent that all studies conducted at our facilities are designed and function in compliance with good laboratory practice regulations and the protocols for individual laboratory studies, an inspection of the report for FC-203 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 procedure audit was conducted on October 12, 1988 with results reported to the study director on the same day. Audit findings were reported to ABC Laboratories' management on November 1, 1988. A final inspection of all data and records on November 14, 1988 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 the Quality Assurance Unit at your convenience.

Lowell G. Sensintaffar 12-2-88
Lowell G. Sensintaffar Date
Quality Assurance Officer

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC report #37242 entitled, "Acute Flow-Through Toxicity of FC-203 to Rainbow Trout (Salmo gairdneri)", for Mo Elnabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories' intent that all aquatic toxicity tests conducted by our facility follow good laboratory practices, ABC's study director for the above test herein confirms that the study was conducted in compliance with Principles of Good Laboratory Practice Annex 2, C(81).

All original raw data will be provided to 3M Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

Jane H. Bowman
Jane H. Bowman
ABC Study Director

12-2-88
Date