

ACUTE TOXICITY TO FISH (BLUEGILL SUNFISH)

TEST SUBSTANCE

Identity: A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-783. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot was not noted. The test sample is FC-783 (cc784-1). Current information indicates it is a mixture of 1.0% PFOS, 2.0% Sultone foamer, 2.3% sodium octyl sulfate, 1.6% polyoxyethylene monooctylphenol ether, 0.1% sodium lauryl sulfate, 25.0% diethylene glycol monobutyl ether, 0.05% tolytriazole, and 67.95% water.

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: Standard Methods, 1975, and USEPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, 1975.

Type: Acute static

GLP: No

Year completed: 1979

Species: *Lepomis macrochirus*

Supplier: Osage Catfisheries, Inc., Osage Beach, MO.

Analytical monitoring: Dissolved oxygen, pH, ammonia, and temperature.

Exposure period: 96-hours

Statistical methods: LC50 values calculated using the statistical methods of Litchfield and Wilcoxon, 1949.

Test fish age: Juveniles

Length and weight: Average length = 28.6 mm
Average weight = 0.60 g (wet)

Loading: 0.2 g/L.

Pretreatment: None.

Test conditions:

Dilution water: Well water

Dilution water chemistry:

Hardness: 255 mg/L as CaCO₃

Alkalinity: 368 mg/L as CaCO₃

pH: 8.2

Lighting: 16 hour daylight photoperiod.

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: 5 gallon glass vessels containing 15 L of test solution.

Number of replicates: 2

Number of fish per replicate: 5

Number of concentrations: five plus a blank control

Water chemistry during the study:

pH range (0-96 hours):

8.2 – 8.3 (control exposure)

8.2 – 8.3 (1000 mg/L exposure)

Temperature range (0-96 hours): 22-22 °C

Total ammonia range (0-96 hours):

0.68 – 1.1 mg/L (control exposure)

0.60 – 1.1 mg/L (1000 mg/L exposure)

Dissolved oxygen range (0-96 hours):

5.0 – 8.7 mg/L (control exposure)

3.8 – 8.7 mg/L (1000 mg/L exposure)

RESULTS

Nominal concentrations: Bk control, 100, 180, 320, 560, and 1000 mg/L.

Element values: 96-hour LC_{50} = >1000 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.

CONCLUSIONS

The FC-783 96-hour LC_{50} for bluegill sunfish was determined to be >1000 mg/L, the highest concentration tested. A 20% mortality was observed.

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.

REFERENCES

This study was conducted by Analytical BioChemistry Laboratories, Inc.,
Columbia, MO at the request of the 3M Company, 3M Lab Request number
4896S, 1979.

OTHER

Last changed: 6/26/00

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Static Acute Bioassay Report
#23897

Submitted To:

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Acute Toxicity of 3M Sample No. 6 (FC 783)
to Bluegill Sunfish (Lepomis macrochirus)

June 19, 1979

002597

Submitted By: Analytical BioChemistry Laboratories, Inc.
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SUMMARY

The acute toxicity of 3M Sample No. 6 to bluegill sunfish (*Lepomis macrochirus*) was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1). Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits.

As a quality check, the bluegill sunfish were challenged with a reference compound, Antimycin A. The observed 96 hour LC_{50} and 95% confidence limits (C.I.) were within the 95% confidence limits reported in the literature (2), indicating that the fish were in good condition.

The results of the four day static fish toxicity study using bluegill sunfish are summarized below.

<u>Compound</u>	<u>96-hour LC_{50} (95% C.I.)</u>
3M Sample No. 6	>1,000 mg/l
Antimycin A	0.000029 mg/l (0.000021-0.000040 mg/l)

A 96 hour exposure of test fish to a logarithmic series of concentrations from 100 to 1,000 mg/l resulted in only 20 percent mortality at the highest concentration.

Also, the results indicated a 96 hour, no observed effect concentration of 560 mg/l.

INTRODUCTION

This static bioassay was performed at the aquatic bioassay laboratory of Analytical BioChemistry Laboratories, Inc., Columbia, Missouri, for 3M Company, from June 14 to June 18, 1979, as authorized in a letter from 3M Company on June 6, 1979 (Appendix I). The purpose of this test was to determine the 24, 48 and 96 hour LC₅₀ levels for 3M Sample No. 6 to bluegill sunfish (Lepomis macrochirus). A preliminary range-finding study was conducted from June 12 to June 14, 1979, to determine the concentration range from the definitive bioassay. The study was performed following the procedures outlined in ABC Protocol Number 7601 (Appendix I).

METHODS AND MATERIALS

The procedures for static bioassay, as described in Standard Methods for Examination of Water and Wastewater (3) and Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1), were used in this experiment. The bluegill sunfish used in the test were obtained from Osage Catfisheries, Inc. in Osage Beach, Missouri. The fish were identified to species using the taxonomic keys developed by Eddy (4). All test fish were held in culture tanks on a 16 hour daylight photoperiod and observed for at least fourteen days prior to testing. Fish culture techniques used were basically those described by Brauhn et. al. (5). A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, is included in Appendix I. During this period, the fish received a standard commercial fish food (Rangen's) daily until 48 hours prior to testing at which time feeding was discontinued. The bluegill sunfish used for this experiment had a mean weight of 0.60 g and a mean standard length of 28.6 mm. Weight and length measurements were made on a representative group of fish prior to the test and are included in Appendix I.

The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of laboratory well water. Characteristics of this dilution water are shown in Table 1.

These vessels were kept in a water bath at 22°C (±1.0). The test fish were acclimated to the dilution water and test temperature and held without food 48 hours prior to testing.

A 48 hour range-finding test was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 500 and 1,000 mg/l. Based on the results of this preliminary testing and results of toxicity tests with fathead minnows (Pimephales promelas) by 3M Company, five concentrations of the test compound, ranging in a logarithmic series from 100 to 1,000 mg/l, with ten fish per concentration were selected for definitive bioassay. The test was conducted with duplicate five gallon glass vessels containing five fish each as authorized by 3M Company (Appendix I). The fish were added to the test chambers by stratified random assignment within 30 minutes after addition of toxicant aliquots.

The 3M Sample No. 6 standard was received on June 8, 1979, in good condition. The sample upon receipt was observed to be a yellow liquid and was stored at 22°C. Sample purity was not provided; therefore, the test concentrations were prepared based on the total compound. The test concentrations were prepared by transferring appropriate weights directly to the test chamber. All standard weights and dilution values are listed in Appendix I.

RESULTS

Table 2 presents the predicted LC₅₀ values and 95% confidence intervals for 3M Sample No. 6 and the reference test against Antimycin A, a piscicide. These values were obtained by employing the statistical method described by Litchfield and Wilcoxon (6). Mortality rates, test concentrations, and water quality data are presented in Table 3.

The dissolved oxygen concentration which stayed between 40 and 100% saturation was considered adequate for testing. The pH values remained consistent with the control throughout the test. The ammonia concentrations were below the toxic limit (7).

The study was conducted following the intent of the Good Laboratory Practice Regulations (8) and the final report was reviewed by Analytical BioChemistry Laboratories' Quality Assurance Unit. All original raw data was provided to 3M Company, with a copy retained at Analytical BioChemistry Laboratories.

TABLE 1

Chemical Characteristics of Well Water at
ABC's Aquatic Bioassay Laboratory.

<u>Parameter</u>	<u>Concentration</u>
Dissolved Oxygen	9.3 ppm
pH	8.2
Hardness (CaCO ₃)	255 ppm
Alkalinity (CaCO ₃)	368 ppm
Conductivity	50 umhos/cm
Total Ammonia (NH ₃)	<0.05 ppm
NO ₃ -N	0.15 ppm
Ortho-Phosphate	0.10 ppm
Aluminum	<0.01 ppm
Arsenic	<0.001 ppm
Cadmium	<0.001 ppm
Chromium	0.001 ppm
Cobalt	<0.001 ppm
Copper	<0.01 ppm
Iron	0.012 ppm
Lead	0.009 ppm
Mercury	<0.0001 ppm
Nickel	0.0157 ppm
Zinc	<0.01 ppm
DDVP	<40 ng/l
Diazinon	<20 ng/l
Disyston	<20 ng/l
Methyl Parathion	<80 ng/l
Malathion	<110 ng/l
Ethyl Parathion	<80 ng/l

TABLE 2

The Acute Toxicity of 3M Sample No. 6
and Antimycin A to bluegill sunfish (Lepomis macrochirus)

Compound	LC ₅₀ in milligrams/liter (ppm)		
	24 hours	48 hours	96 hours
3M Sample No. 6	>1,000	>1,000	>1,000
Antimycin A***	0.000070 (0.000056-0.000088)**	0.000040 (0.000032-0.000050)**	0.000029 (0.000021-0.000040)**

*Bioassay as conducted at 22°C (±1.0), mean weight and length, 0.60 g and 28.6 mm.

**95% confidence interval.

***Antimycin A standard obtained from Sigma Chemical Company, Type III, crystalline, Lot 125C-0152.

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TABLE 3

The mortality rates and water quality parameters during the acute toxicity test of 3M Sample No. 6 with bluegill sunfish (Lepomis macrochirus).

mg/l Concentration	Percent Mortality Hours			Water Quality											
				24 hours				48 hours				96 hours			
	24	48	96	Temp. °C	D.O.* mg/l	pH**	NH ₃ *** mg/l	Temp. °C	D.O.* mg/l	pH**	NH ₃ *** mg/l	Temp. °C	D.O.* mg/l	pH**	NH ₃ *** mg/l
Control	0	0	0	22	8.2	8.3	1.1	22	5.8	8.2	0.75	22	5.0	8.3	1.1
100	0	0	0	22	8.3	8.3	1.2	22	5.8	8.3	0.75	22	3.6	8.3	1.0
180	0	0	0												
320	0	0	0												
560	0	0	0												
1,000	20	20	20	22	8.3	8.3	1.1	22	4.7	8.2	0.62	22	3.8	8.2	1.0

*Dissolved oxygen concentrations - Dissolved Oxygen Probe (Extech Model 8012) used with an Extech Model 671 pH and mV meter.

**pH - pH Probe (Fisher Model 13-639-108) used with an Extech Model 671 pH and mV meter.

***Total ammonia concentrations - Ammonia Probe (Extech Model 8002-8) used with an Extech Model 671 pH and mV meter.

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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) Berger, B. L., R. E. Lennon and J. W. Hogan. 1969. Laboratory Studies on Antimycin A as a fish toxicant. U. S. Department of Interior, Investigations in Fish Control No. 26. 21 p.
- (3) American Public Health Association. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed. Washington, DC. 1193 p.
- (4) Eddy, Samuel. 1969. The Freshwater Fishes. 2nd ed. W. C. Brown Company, Dubuque, IA. 286 p.
- (5) 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.
- (6) Litchfield, J. T., Jr. and F. Wilcoxon. 1949. A Simplified Method of Evaluating Dose-Effect Experiments. Jour. Pharm. Exp. Ther. 96:99113.
- (7) National Academy of Sciences. 1971. Water Quality Criteria, 1972. U. S. Department of Commerce, PB-236 199. 592 p.
- (8) Food and Drug Administration. Regulations for Good Laboratory Practice. Federal Register, Vol. 43, No. 247, 59986-60025, December 22, 1978.

Quality Assurance Statement for final report #23897 entitled, "Acute Toxicity of 3M Sample No. 6 to bluegill sunfish (Lepomis macrochirus)," for Dale L. Bacon, 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 Sample No. 6 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. It was also noted that since the purity of the compound being tested had not been supplied, the LC₅₀ would have to be calculated based on nominal concentrations of the total compound. A final inspection of all data and records on June 22, 1979, 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.

<u>James A. Ault</u>	<u>7/13/79</u>
James A. Ault	Date
Quality Assurance Officer	