

## 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-780B. (1-Octanesulfonic acid) (CAS # 2795-39-3).

**Remarks:** The 3M production lot number was 2. The test sample is FC-780B, identified by the laboratory as "3M Sample 1-3". Current information indicates it is a mixture of 0.58% PFOS, 14% diethylene glycol monobutyl ether, 76.7% water, 5.5% urea, 1.17% Hydroxy foamer, 1.15% sodium octyl sulfate, 0.8% polyoxyethylene monooctylphenyl ether, 0.05% sodium lauryl sulfate, and 0.05% benzotriazole.

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

METHOD:

**Method:** Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, EPA-660/3-75-009, 1975.

**Type:** Acute flow-through

**GLP:** No

**Year completed:** 1980

**Species:** *Pimephales promelas*

**Supplier:** Fattig Fish Hatchery, Brady, NE.

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

**Exposure period:** 96-hours

**Statistical methods:** LC<sub>50</sub> values and 95% confidence intervals calculated using a computer program by Stephan, et al. 1978.

**Test fish age:** Juveniles

**Length and weight:** Average length = 27 mm  
Average weight = 0.34 g (wet)

**Loading:** Not given.

**Pretreatment:** None.

**Test conditions:**

**Dilution water:** Filtered well water

**Dilution water chemistry:**

**Hardness:** 255 mg/L as CaCO<sub>3</sub>

**Alkalinity:** 368 mg/L as CaCO<sub>3</sub>

**pH:** 7.8

**Conductivity:** 50 µmhos/cm

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

**Stock and test solution preparation:** The stock solution was prepared in deionized water on a volume/volume basis.

**Flow-through rate:** Approximately seven volume exchanges of water every 24 hours.

**Stability of the test chemical solutions:** Not noted

**Exposure vessels:** Glass 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):**
  - 7.9 – 8.2 (control exposure)
  - 8.0 – 8.1 (1500 mg/L exposure)
- Temperature range (0-96 hours):**
  - 21 – 22 °C (control exposure)
  - 21 – 22 °C (1500 mg/L exposure)
- Dissolved oxygen range (0-96 hours):**
  - 7.9 – 9.1 mg/L (control exposure)
  - 7.9 – 8.6 mg/L (1500 mg/L exposure)

## **RESULTS**

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**Nominal concentrations:** Blank control, 90, 200, 380, 770, and 1500 mg/L.

**Element values:** 96-hour  $LC_{50}$  = >1500 mg/L  
96-hour NOEC = 380 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**

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The test sample 96-hour  $LC_{50}$  for fathead minnow was determined to be >1500 mg/L. The 96-hour No Observed Effect Concentration (NOEC) was 380 mg/L.

The 1500 mg/L concentration resulted in a 15% mortality.

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

## **DATA QUALITY**

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**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 Analytical BioChemistry Laboratories, Inc., Columbia, MO at the request of the 3M Company, St. Paul, MN, 1980.

#### OTHER

**Last changed: 6/28/00**



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®

Dynamic Acute Bioassay Report  
#26241

Submitted To:

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Attn: Mr. Moe Elnabarawy  
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3M FC-780B lot 2

Dynamic 96-Hour Acute Toxicity  
of 3M Sample 1-3  
to Fathead Minnows (Pimephales promelas)

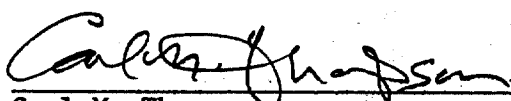
September 29, 1980

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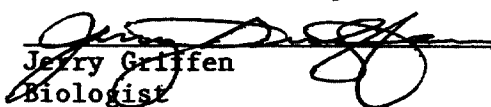
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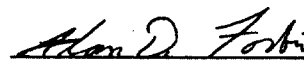
Date



Jerry Griffen  
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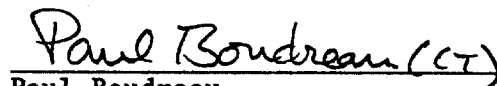
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Alan D. Forbis  
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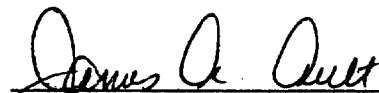


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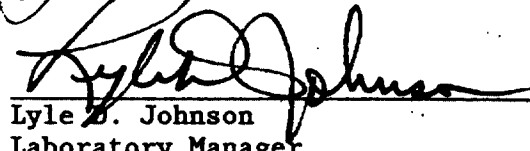
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## SUMMARY

A dynamic 96-hour toxicity study was conducted to determine the acute toxicity of 3M Sample 1-3 to fathead minnows (Pimephales promelas). The methods employed were those outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (2). 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 were: 90, 200, 380, 770 and 1,500 mg/l.

As a quality check, the fathead minnows 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 (5), indicating that the fish were in good condition.

The observed mortality data for the study gave the following estimated  $LC_{50}$  values.

TABLE 1: The estimated  $LC_{50}$  values with their 95% confidence limits for the 96-hour fathead minnow toxicity study of 3M Sample 1-3.

| <u>Hours of Exposure</u> | <u>3M Sample 1-3 (mg/l)</u> |                              |
|--------------------------|-----------------------------|------------------------------|
|                          | <u><math>LC_{50}</math></u> | <u>95% Confidence Limits</u> |
| 24                       | >1500                       | ----                         |
| 48                       | >1500                       | ----                         |
| 72                       | >1500                       | ----                         |
| 96                       | >1500                       | ----                         |

In addition to the above  $LC_{50}$  values, the results indicated a 96-hour, no observed effect concentration of 380 mg/l.



## INTRODUCTION

3M Company contracted the Aquatic Toxicology Division of Analytical BioChemistry Laboratories, Inc., to conduct a five-concentration plus control, 96-hour, flow-through bioassay to determine the toxicity of 3M Sample 1-3 to fathead minnows (Pimephales promelas). The primary objective of the study was to define the acute toxicity of 3M Sample 1-3 by determining the median lethal concentrations ( $LC_{50}$ 's) during the course of the test. The study was authorized by Mr. Moe Elnabarawy, 3M Company, in a protocol dated September 15, 1980 (Appendix I) and was conducted from September 25 to September 29, 1980.

The term  $LC_{50}$  as used in this report is that concentration of 3M Sample 1-3 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).

### I. Test Fish

The fathead minnows used in the test were obtained from Fattig Fish Hatchery in Brady, Nebraska. 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 prophylactic and therapeutic disease treatments, is included in Appendix I. 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 0.34 g and a mean standard length of 27 mm. Weight and length measurements were made on the control group of fish at termination of the test and are included in Appendix I. As a quality check, the fathead minnows were challenged with a reference compound, Antimycin A. The observed 96 hour  $LC_{50}$  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 Appendix I.

### II. Test System

A proportional diluter system described by Mount and Brungs (6), with the modifications of McAllister et. al. (7), was used for

the intermittent introduction of 3M Sample 1-3 and diluent water into the test aquaria. Aerated well water (Table 2) was delivered to the glass aquaria at a rate of 150 ml/minute/aquarium, an amount which was sufficient to replace the 30 liter test volume at least 7 times in a 24 hour period. The test aquaria were immersed in a circulating water bath held at 22°C (±1°) by submerged heating elements.

### III. Test Compound

The 3M Sample 1-3 standard was received on September 10, 1980, in good condition. The sample upon receipt was observed to be an amber liquid and was stored at room temperature (22°C). The stock solution was prepared in deionized water on a volume/volume basis and corrected for specific gravity to a weight/volume basis (mg/l). Standard weights and dilution volumes can be found in Appendix I.

### IV. Test Procedure-Biological

The test was initiated on September 25, 1980, by random assignment of 20 fathead minnows to each of the 30 liter test aquaria after test solutions had been flowing through the aquaria for 8 hours. The fish were exposed to the following nominal concentrations of 3M Sample 1-3: 90 mg/l, 200 mg/l, 380 mg/l, 770 mg/l and 1,500 mg/l. 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. A computerized LC<sub>50</sub> program developed by Stephan et. al. (8) was used to calculate the LC<sub>50</sub> values and their 95% confidence limits.

### 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 concentrations of 3M Sample 1-3 were determined by measuring predetermined dilutions of a stock Rhodamine B dye. A standard calibration curve was constructed to measure the absorbance (Coleman Spectrophotometer Model 55) of known dilutions of the dye that bracketed the absorbance value obtained from the proportional diluter. The dilution factor was determined by interpolation from the standard curve (Appendix I). The stock 3M Sample 1-3 concentration divided by the dilution factor determined the highest nominal concentration. The remaining concentrations were calculated by multiplying the highest concentration by the percent dilution (% difference) obtained from the calibration curve. For example:

$$\frac{631.5 \text{ g/l (stock concentration)}}{421 \text{ (dilution factor)}} \times 0.25 \left( \begin{array}{l} \% \text{ dilution for} \\ \text{aquaria \#4} \end{array} \right) =$$

$$380 \text{ mg/l} \left( \begin{array}{l} \text{aquaria \#4} \\ \text{nominal concentration} \end{array} \right)$$

The nominal concentrations of 3M Sample 1-3 calculated in this manner were: 90, 200, 380, 770 and 1,500 mg/l. The maximum test concentration was set at 1,500 mg/l, as requested by the study sponsor.

## RESULTS

Table 1 presents the predicted LC<sub>50</sub> values and 95% confidence intervals for 3M Sample 1-3. These values were obtained by employing a computerized LC<sub>50</sub> program developed by Stephan et. al. (8).

TABLE 1: The estimated LC<sub>50</sub> values with their 95% confidence limits for the 96-hour fathead minnow toxicity study of 3M Sample 1-3.

| <u>Hours of Exposure</u> | <u>3M Sample 1-2 (mg/l)</u> |                              |
|--------------------------|-----------------------------|------------------------------|
|                          | <u>LC<sub>50</sub></u>      | <u>95% Confidence Limits</u> |
| 24                       | >1500                       | ----                         |
| 48                       | >1500                       | ----                         |
| 72                       | >1500                       | ----                         |
| 96                       | >1500                       | ----                         |

In addition to the above LC<sub>50</sub> values, the results indicated a 96-hour, no observed effect concentration of 380 mg/l. Mortality data is presented in Table 4.

Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured in the control, low concentration and high concentration throughout the test (Table 3). The dissolved oxygen concentrations which stayed between 85 and 100 percent saturation were considered adequate for testing (2) and 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 BioChemistry Laboratories' Quality Assurance Unit. All original raw data was provided to 3M Company, with a copy retained at Analytical BioChemistry Laboratories.

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

| <u>Parameter</u>                              | <u>Concentration</u> |
|-----------------------------------------------|----------------------|
| Dissolved Oxygen                              | 9.3 ppm              |
| pH                                            | 7.8                  |
| Hardness (CaCO <sub>3</sub> )                 | 255 ppm              |
| Alkalinity (CaCO <sub>3</sub> )               | 368 ppm              |
| Conductivity                                  | 50 $\mu$ mhos/cm     |
| Total Ammonia (NH <sub>3</sub> )              | <0.05 ppm            |
| NO <sub>3</sub> -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            |
| Measured organophosphorus pesticides          | a                    |
| Measured organochlorine pesticides plus PCB's | a                    |

<sup>a</sup>See appendix for individual analyses.

TABLE 3: Water quality measurements during the 96-hour toxicity study of 3M Sample 1-3 to fathead minnows (Pimephales promelas).

| Hours of Exposure | Control  |                        |                 |                                   | Low Concentration |                        |                 |                                   | High Concentration <sup>a</sup> |                        |                 |                                   |
|-------------------|----------|------------------------|-----------------|-----------------------------------|-------------------|------------------------|-----------------|-----------------------------------|---------------------------------|------------------------|-----------------|-----------------------------------|
|                   | Temp. °C | D.O. <sup>b</sup> mg/l | pH <sup>c</sup> | NH <sub>3</sub> <sup>d</sup> mg/l | Temp. °C          | D.O. <sup>b</sup> mg/l | pH <sup>c</sup> | NH <sub>3</sub> <sup>d</sup> mg/l | Temp. °C                        | D.O. <sup>b</sup> mg/l | pH <sup>c</sup> | NH <sub>3</sub> <sup>d</sup> mg/l |
| 0                 | 22       | 8.5                    | 8.2             | 0.56                              | 22                | 8.5                    | 8.0             | 1.0                               | 22                              | 8.4                    | 8.0             | 1.4                               |
| 24                | 22       | 9.1                    | 8.1             | 1.6                               | 22                | 9.0                    | 8.1             | 1.3                               | 22                              | 8.6                    | 8.1             | 1.6                               |
| 48                | 21       | 8.4                    | 7.9             | 0.56                              | 21                | 8.3                    | 8.0             | 0.62                              | 21                              | 8.5                    | 8.0             | 0.48                              |
| 72                | 22       | 8.6                    | 8.1             | 1.0                               | 22                | 8.5                    | 8.1             | 0.98                              | 22                              | 8.3                    | 8.0             | 0.86                              |
| 96                | 22       | 7.9                    | 8.1             | 0.64                              | 22                | 7.7                    | 8.0             | 0.58                              | 22                              | 7.9                    | 8.0             | 0.60                              |

<sup>a</sup>High concentration water quality was determined in Tank 6 (1500 mg/l 3M Sample 1-3) throughout the study.

<sup>b</sup>Dissolved oxygen concentrations - YSI Model 54 Dissolved Oxygen meter and probe.

<sup>c</sup>pH - pH Probe (Corning Model 476022) used with a Corning Model 125 pH and mV meter.

<sup>d</sup>Ammonia concentrations - Ammonia Probe (Extech Model 8002-8) used with a Corning Model 125 pH and mV meter.

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TABLE 4: Percent mortality of fathead minnows exposed to five concentrations of 3M Sample 1-3 during a 96-hour toxicity study.

| 3M Sample 1-3<br>Nominal<br>concentration<br>(mg/l) | Hours of Exposure |    |    |    |
|-----------------------------------------------------|-------------------|----|----|----|
|                                                     | 24                | 48 | 72 | 96 |
| Control                                             | 0                 | 0  | 0  | 0  |
| 90                                                  | 0                 | 0  | 0  | 0  |
| 200                                                 | 0                 | 0  | 0  | 0  |
| 380                                                 | 0                 | 0  | 0  | 0  |
| 770                                                 | 10                | 10 | 10 | 10 |
| 1500                                                | 15                | 15 | 15 | 15 |

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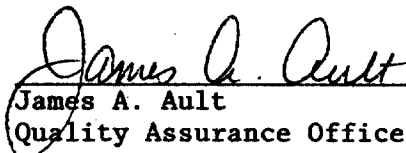
#### LITERATURE CITED

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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. 1972. Water Quality Criteria. 1972. U. S. Department of Commerce, PB-236 199. 592 p.
- (10) Food and Drug Administration. Regulations for Good Laboratory Practice. 21 CFR, Part 58.

Quality Assurance Statement for final report #26241 entitled, "Dynamic 96-Hour Acute Toxicity of 3M Sample 1-3 to Fathead Minnows (Pimephales promelas)," for Mr. Moe 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 Sample 1-3 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. An in-progress study inspection was conducted on September 26, 1980. A final inspection of all data and records on September 29, 1980, 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.

 9/30/80  
James A. Ault Date  
Quality Assurance Officer