

## ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

### TEST SUBSTANCE

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

**Remarks:** The 3M production lot number was 30034. The test sample is FC-203CF. Current information indicates it is a mixture of 1% PFOS, 20% diethylene glycol monobutyl ether, 70.2% water, 2.75% Acrylic foamer, 3% sodium octyl sulfate, 1% triethanolamine, 2% sulfonated alkyl ether salts, and 0.05% tolyltriazole.

***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:** Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians (ASTM E729-88a), 1995

**Type:** Acute static renewal

**GLP:** No

**Year completed:** 1999

**Species:** *Pimephales promelas*

**Supplier:** In-house culture. Original brood stock from Aquatic Biosystems, Inc., Fort Collins, CO.

**Analytical monitoring:** Temperature, pH, conductivity, and dissolved oxygen.

**Exposure period:** 96-hours

**Statistical methods:** The  $LC_{50}$  values were calculated by Trimmed Spearman-Kärber method. The NOEC values were calculated by Fisher's Exact test using Toxstat statistical software, Version 3.5.

**Test fish age:** Juveniles, 26 to 28 days old

**Length and weight:** Average length = 17 mm  
Average weight = 0.037 g (wet)

**Loading:** 0.12 g/L

**Pretreatment:** None

**Test conditions:**

**Dilution water:** Dechlorinated City of Duluth, MN tap water, post-carbon filter. The water was aerated for 24 hours prior to the test.

**Dilution water chemistry:**

**Hardness:** 42 mg/L as  $CaCO_3$

**Alkalinity:** 54 mg/L as  $CaCO_3$

**pH:** 8.03

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

**Stock and test solution preparation:** The test material was added directly to the dilution water on a volume/volume basis.

**Concentrations dosing rate:** Renewal at 48 hours.

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

**Exposure vessels:** 4 L wide mouth glass jars containing 3 L test solutions.

**Number of replicates:** 2

**Number of fish per replicate:** 10.

**Number of concentrations:** five plus a blank control

**Water chemistry during the study:**

**pH range (0-96 hours):**

7.80 – 8.03 (control exposure)

7.33 – 7.89 (720 mg/L exposure)\*

**Temperature range (0-96 hours):** 24.8 – 25.6 °C

**Conductivity range (0-96 hours):**

125 – 132 µmhos/cm (control exposure)

143 – 159 µmhos/cm (720 mg/L exposure)\*

**Dissolved oxygen range (0-96 hours):**

7.4 – 8.3 mg/L (control exposure)

5.2 – 8.4 mg/L (720 mg/L exposure)\*

\* 720 mg/L (intermediate concentration) exposure values used

because the highest two concentrations resulted in total mortality by 24 hours.

## **RESULTS**

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**Nominal concentrations:** Blank control, 259, 432, 720, 1200, and 2000 mg/L

**Element values:** 96-hour  $LC_{50}$  = 839 (766 – 920) mg/L  
96-hour NOEC = 720 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 839 mg/L with a 95% confidence interval of 766 to 920 mg/L. The 96-hour No Observed Effect Concentration (NOEC) is 720 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, sample purity was not properly characterized and it lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

## REFERENCES

This test was conducted by AScl Corporation, Duluth Environmental Testing Division, Duluth, MN at the request of the 3M Company, St. Paul, MN, Lab Request number W1127, 1999.

## OTHER

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

**STUDY TITLE**

ACUTE TOXICITY OF FC-203CF TO  
FATHEAD MINNOW (*Pimephales promelas*)

**DATA STANDARD**

ASTM E 729 - 88a

**STUDY COMPLETED**

February 22, 1999

**TESTING FACILITY**

ASCI Corporation  
Environmental Testing Division  
4444 Airpark Boulevard  
Duluth, MN 55811

Tel. No. (218) 722-4040  
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**STUDY IDENTIFICATION NUMBERS**

ASCI Study ID# 5010-068

3M Company Lab Request# W1127

## 1.0 GENERAL INFORMATION

|                                        |                                                                                                                                              |                                                                                                            |
|----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| Study Title                            | Acute Toxicity of FC-203CF to Fathead Minnow ( <i>Pimephales promelas</i> ).                                                                 |                                                                                                            |
| Data Standard                          | ASTM Designation: E 729-88a.                                                                                                                 |                                                                                                            |
| Sponsor                                | 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (651) 778-7452.              |                                                                                                            |
| Sponsor's Representative               | Susan Beach, 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (651) 778-7452. |                                                                                                            |
| Sponsor's Lab Request#                 | W1127.                                                                                                                                       |                                                                                                            |
| Testing Facility                       | ASCI Corporation<br>Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.                       |                                                                                                            |
| Principal Investigator                 | Joe Dierkes                                                                                                                                  |                                                                                                            |
| Project Director                       | Joe Amato                                                                                                                                    |                                                                                                            |
| Testing Facility Director              | Donald Mount                                                                                                                                 |                                                                                                            |
| Definitive Test Dates                  | February 9-13, 1999.                                                                                                                         |                                                                                                            |
| Data Validation and Report Review      | Alan Mozol                                                                                                                                   | Report Signature:<br> |
| Retention of Raw Data and Final Report | Two years from the date study is completed.                                                                                                  |                                                                                                            |
| Location of Raw Data and Final Report  | ASCI Corporation Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.                                        |                                                                                                            |

## 2.0 OBJECTIVE(S)

To determine the 96-hour 10% and 50% lethal concentrations ( $LC_{10}$  and  $LC_{50}$ ), and the 96-hour no observable effect concentration (NOEC) of the test substance, FC-203CF, for fathead minnow (*Pimephales promelas*) under static-renewal test conditions.

This study was conducted according to ASTM Designation: E 729-88a, Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians, 1995.

## 3.0 TEST METHODS

|                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Test Substance | <p><i>Properties:</i> FC-203CF, amber solution, specific gravity = 1.0 (water = 1), stable, and miscible with water.</p> <p><i>Test concentrations:</i> 0 (dilution water control) 259, 432, 720, 1200, and 2000 mg/L. Test solution prepared by volume addition of test substance to dilution water followed by stirring.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Test Organisms | <p><i>Source:</i> Healthy, juvenile fathead minnow (<i>Pimephales promelas</i>) were obtained from ASCI in-house cultures. Original brood stock for the culture was obtained from Aquatic Biosystems, Inc., Fort Collins, Colorado.</p> <p><i>Holding/acclimation:</i> The batch of fish acclimated for this study was hatched on January 12, 13, and 14, 1999. The fish were acclimated to test conditions for 14 days. For the 14 days preceding test initiation, mortality was 0%, and the fish were not fed for 26 hours before test initiation. Age of the organisms at test initiation was 26 to 28 days. The fish mean length was <math>1.7 \pm 0.2</math> cm, and mean wet weight was <math>37 \pm 12</math> mg. Loading rate was calculated to be 0.12 g of fish per L test solution.</p> <p><i>Use in test:</i> Used 10 organisms/replicate and 2 replicates/test concentration.</p> |
| Dilution Water | <p><i>Source:</i> Dilution water was carbon treated City of Duluth, MN, tap water. The most recent chemical analysis is in Raw Data Package. The same batch of dilution water was used for the entire acclimation period and the toxicity test. The batch of dilution water was aerated continuously during storage.</p> <p><i>Characteristics:</i> At test time, the dilution water had a total hardness 42 mg/L, an alkalinity of 54 mg/L (both as <math>CaCO_3</math>) and a pH of 8.03.</p>                                                                                                                                                                                                                                                                                                                                                                                                |

|                       |                                                                                                                                                                                                                                                                                                                                                  |
|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Exposure Chambers     | <p><i>Type:</i> 4-L wide mouth clear glass jar.</p> <p><i>Use in test:</i> 3 L of dilution water or test solution/chamber. During the test, kept covered, except when experimental observations or solution renewals were made. Test solutions were renewed at the 48-hour test interval, and aeration was not supplied during the exposure.</p> |
| Incubation            | <p><i>Duration:</i> 96 hours.</p> <p><i>Daily photoperiod:</i> 16 h light and 8 h dark, maintained using cool-white fluorescent lamps.</p> <p><i>Temperature:</i> 25.0° ± 1°C.</p>                                                                                                                                                               |
| Observations          | <p><i>Biological:</i> Daily- Test organisms with abnormal behavior and signs of stress, and dead test organisms.</p> <p><i>Water chemistry:</i> (1) At test initiation and solution renewal- total hardness and alkalinity, (2) daily- temperature, DO, conductivity, and pH. All determinations were made according to APHA methods (1998).</p> |
| Endpoint Calculations | <p><i>LC<sub>10</sub>:</i> US EPA ICp Analysis.</p> <p><i>LC<sub>50</sub>:</i> Spearman-Kärber Method.</p> <p><i>NOEC:</i> Fisher's Exact Test (Toxstat Version 3.5).</p>                                                                                                                                                                        |

### 3.0 RESULTS

|                                                                                                  |                                                               |
|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------|
| Test Substance Toxicity Endpoints and (95% Confidence Intervals). Pertinent data are in Table 1. | 24-h <i>LC<sub>50</sub></i> : 906 mg/L (95% CI 862-952 mg/L). |
|                                                                                                  | 48-h <i>LC<sub>50</sub></i> : 839 mg/L (95% CI 766-920 mg/L). |
|                                                                                                  | 72-h <i>LC<sub>50</sub></i> : 839 mg/L (95% CI 766-920 mg/L). |
|                                                                                                  | 96-h <i>LC<sub>50</sub></i> : 839 mg/L (95% CI 766-920 mg/L). |
|                                                                                                  | 96-h <i>LC<sub>10</sub></i> : 576 mg/L (95% CI Not Reliable). |
|                                                                                                  | 96-h <i>NOEC</i> : 720 mg/L.                                  |

|                                      |                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ranges of Water Chemistry Parameters | <p><i>Hardness (mg/L as CaCO<sub>3</sub>): 42-44.</i></p> <p><i>Alkalinity (as CaCO<sub>3</sub> mg/L): 46-52.</i></p> <p><i>Temperature (°C): 24.8-25.6.</i></p> <p><i>DO (mg/L): 5.2-8.4. Maintained via solution renewal at the 48-hour test interval.</i></p> <p><i>pH: 7.33-8.03.</i></p> <p><i>Specific conductivity (µmhos/cm): 125-177.</i></p> |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

#### 4.0 TEST QA/QC

| QA/QC Criteria                                                                                                        | Data                                                                              |
|-----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Mortality in control exposure must not be more than 10%.                                                              | 0% mortality.                                                                     |
| The lowest DO concentration at test termination must be at least 60% of the air saturation value at test temperature. | The lowest DO concentration measured was 63% of the air saturation value at 25°C. |
| Test duration must be 96 h.                                                                                           | The test duration was 96 h.                                                       |
| Reference Substance Toxicity Test.                                                                                    | Reference toxicity testing data is included in the Raw Data Package.              |

#### 5.0 REFERENCES

American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, D.C.

ASTM. 1995. Section 11. Volume 11.05. Designation: E 729-88a. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.

**TABLE 1. ACUTE TOXICITY OF FC-203CF TO FATHEAD MINNOW (*P. promelas*)**

| Test Substance<br>Concentration (mg/L) | Cumulative Number of Dead Organisms and Percent Mortality <sup>a</sup> |          |          |          |
|----------------------------------------|------------------------------------------------------------------------|----------|----------|----------|
|                                        | 24 h                                                                   | 48 h     | 72 h     | 96 h     |
| 0 (control)                            | 0 (0)                                                                  | 0 (0)    | 0 (0)    | 0 (0)    |
| 259                                    | 0 (0)                                                                  | 0 (0)    | 0 (0)    | 0 (0)    |
| 432                                    | 0 (0)                                                                  | 0 (0)    | 0 (0)    | 0 (0)    |
| 720                                    | 1 (5)                                                                  | 4 (20)   | 4 (20)   | 4 (20)   |
| 1200                                   | 20 (100)                                                               | 20 (100) | 20 (100) | 20 (100) |
| 2000                                   | 20 (100)                                                               | 20 (100) | 20 (100) | 20 (100) |

<sup>a</sup> Percentages are in parenthesis.

**LETHAL AND NO-EFFECT CONCENTRATIONS WITH  
CORRESPONDING 95% CONFIDENCE INTERVALS**

| LC's and NOEC         | Data (mg/L) <sup>a</sup> | 95% Confidence Interval |
|-----------------------|--------------------------|-------------------------|
| 24-h LC <sub>50</sub> | 906                      | 862-952                 |
| 48-h LC <sub>50</sub> | 839                      | 766-920                 |
| 72-h LC <sub>50</sub> | 839                      | 766-920                 |
| 96-h LC <sub>50</sub> | 839                      | 766-920                 |
| 96-h LC <sub>10</sub> | 576                      | Not reliable            |
| 96-h NOEC             | 720                      | Not applicable          |

<sup>a</sup> Values based on nominal test concentrations.