

## ACUTE TOXICITY TO AQUATIC INVERTEBRATES (DAPHNIA MAGNA)

### TEST SUBSTANCE

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**Identity:** A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-603EF. (1-Octanesulfonic acid) (CAS # 2795-39-3).

**Remarks:** The 3M production lot number is not noted. The test sample is FC-603EF. Current information indicates it is a mixture of 1.30% PFOS, 10% diethylene glycol butyl ether, 54.15% water, 22.5% ethylene glycol, 2.6% propanesultone foamer, 5% Alkylether sulfate, 0.1% N-(3-Chloroallyl)hexaminium chloride, 0.9% Xanthan gum, 0.9% starch, 1.0% triethanolamine, and 0.05% tolyl triazole.

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

### METHOD:

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**Method:** OECD Guideline 202

**Type:** Acute static

**GLP:** No

**Year completed:** 1996

**Species:** *Daphnia magna*

**Supplier:** In-house cultures, maintained at AScl-DETD, Duluth, MN.

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

**Exposure period:** 48-hours

**Test organism age:** <24 hours old

**Statistical methods:** The EC50 values were calculated by the Trimmed Spearman-Kärber method. The NOEC was calculated by Steel's Many-One Rank test (Toxstat software, Version 3.1).

**Test conditions:**

**Dilution water:** Dechlorinated City of Duluth, MN, tap water (post carbon filter) that was aerated for 24 hours prior to use.

**Dilution water chemistry:**

**Hardness:** 45 mg/L as CaCO<sub>3</sub>

**Alkalinity:** 43 mg/L as CaCO<sub>3</sub>

**pH:** 7.92

**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:** A 2000 mg/L primary stock solution was prepared by diluting 2000 mg test substance in 2 L of dilution water. The

remaining exposure concentrations were prepared using a 0.5 serial dilution scheme.

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

**Exposure vessels:** 250 mL borosilicate glass beakers containing 200 mL exposure solution.

**Number of replicates:** 4

**Number of daphnids per replicate:** 5

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

**Water chemistry during the study:**

**pH range (0-48 hours):**

7.69 – 8.13 (control exposure)

7.78 – 7.94 (1000 mg/L exposure)

**Temperature range (0-48 hours):** 20.1 – 22.4 °C

**Conductivity range (0-48 hours):**

152 µmhos/cm (control exposure)

147 – 155 µmhos/cm (1000 mg/L exposure)

**Dissolved oxygen range (0-48 hours):**

8.3 – 8.6 mg/L (control exposure)

7.4 – 8.7 mg/L (1000 mg/L exposure)

## **RESULTS**

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**Nominal concentrations:** Blank control, 62.5, 125, 250, 500, and 1000 mg/L.

**Element values:** 48-hour EC<sub>50</sub> = 479 (378 – 607) mg/L  
48-hour NOEC = 250 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 substance 48-hour EC<sub>50</sub> was determined to be 479 mg/L with a 95% confidence interval of 378 to 607 mg/L. The 48-hour No Observed Effect Concentration (NOEC) was determined to be 250 mg/L.

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

## **DATA QUALITY**

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**Reliability:** Klimisch ranking = 2. This study 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 solution.

#### REFERENCES

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This study was conducted by AScl Corporation, AScl-Duluth Environmental Testing Division, Duluth, MN at the request of the 3M Company, Lab Request number P2581, 1996.

#### OTHER

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**Last changed:** 6/26/00

ASel Corporation/ASel Duluth  
Environmental Testing Division  
Submitted to 3M Co. - 11-96  
ASel Study ID# 5010-068

STUDY TITLE

ACUTE TOXICITY OF FC-603EF, LIGHT WATER(TM)  
ATC 3%, AR-AFFF TO *DAPHNIA MIGNA*

DATA STANDARD

OECD GUIDELINE 202

STUDY COMPLETED

November 7, 1996

TESTING FACILITY

ASel Corporation/ASel-Duluth  
Environmental Testing Division  
4444 Airpark Boulevard  
Duluth, MN 55811

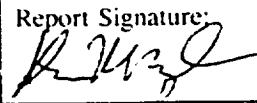
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STUDY IDENTIFICATION NUMBERS

ASel Study ID# 5010-068

3M Company Lab Request# P2581

## 1.0 GENERAL INFORMATION

|                                        |                                                                                                                                                   |                                                                                                            |
|----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| Study Title                            | Acute Toxicity of FC-603EF Light Water(TM)<br>ATC 3%, AR-AFFF <i>Daphnia magna</i> .                                                              |                                                                                                            |
| Data Standard                          | OECD Guideline 202.                                                                                                                               |                                                                                                            |
| Sponsor                                | 3M Environmental Technology and Services,<br>Building 2-3E-09, 935 Bush Avenue, St. Paul,<br>MN 55133.                                            |                                                                                                            |
| Sponsor's Representative               | Susan A. Beach, 3M Environmental<br>Technology and Services, Building 2-3E-09,<br>935 Bush Avenue, St. Paul, MN 55133.<br>Tel No. (612) 778-7452. |                                                                                                            |
| Sponsor's Lab Request#                 | P2581.                                                                                                                                            |                                                                                                            |
| Testing Facility                       | ASel Corporation/ASel-Duluth Environmental<br>Testing Division, 4444 Airpark Boulevard,<br>Duluth, MN 55811; Tel. No. (218) 722-4040.             |                                                                                                            |
| Principal Investigator                 | Joe Dierkes.                                                                                                                                      |                                                                                                            |
| Project Director                       | Joe Amato.                                                                                                                                        |                                                                                                            |
| Testing Facility Director              | Donald Mount.                                                                                                                                     |                                                                                                            |
| Definitive Test Dates                  | October 29-31, 1996.                                                                                                                              |                                                                                                            |
| 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  | ASel Corporation/ASel-Duluth Environmental<br>Testing Division, 4444 Airpark Boulevard,<br>Duluth, MN 55811; Tel. No. (218) 722-4040.             |                                                                                                            |

## 2.0 OBJECTIVE(S)

To determine the 48-h  $EC_{50}$  and 48-h NOEC (no observed effect concentration) of the test substance, FC-603EF, for *Daphnia magna*.

This study was conducted according to the ASCI study plan retained by the Sponsor and OECD Guideline 202 (OECD 1993).

## 3.0 TEST METHODS

|                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Test Substance    | <p><i>Properties:</i> FC603EF, amber-colored liquid, specific gravity ca. 1.06 (water = 1), vapor pressure ca. 15.8 mmHg, stable, and completely soluble in water.</p> <p>Test concentrations: 0 (dilution water control), 62.5, 125, 250, 500, and 1000 mg/L. High test concentration solution prepared by diluting 2000 mg of test substance in 2 L of dilution water. Lesser concentrations were prepared using a 0.5 serial dilution scheme.</p> |
| Test Organisms    | <p><i>Source:</i> Healthy, less than 24-h old <i>Daphnia magna</i> neonates obtained from ASCI stock cultures maintained in water of test dilution water quality.</p> <p><i>Use in test:</i> Gravid adults isolated in dilution water 24 h before the test initiation. Used 5 organisms/replicate and 4 replicates/test concentration. Handled with a smooth bore pipet with an inside diameter of 6 mm. Did not feed during the test.</p>           |
| Dilution Water    | <p><i>Source:</i> Dechlorinated City of Duluth, MN, tap water. The most recent chemical analysis is in Raw Data Package.</p> <p><i>Characteristics:</i> At test time, the water had a hardness of 45 mg/L (as <math>CaCO_3</math>) and a pH of 7.92. Aerated for 24 h prior to using in the test.</p>                                                                                                                                                |
| Exposure Chambers | <p><i>Type:</i> 250-ml borosilicate glass beakers.</p> <p><i>Use in test:</i> 200 ml of dilution water or test solution/chamber. During the test, kept covered, except when experimental observations were made.</p>                                                                                                                                                                                                                                 |

|                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Incubation            | <p><i>Duration:</i> 48 h.</p> <p><i>Daily photoperiod:</i> 16 h light and 8 h dark, maintained using cool-white fluorescent bulbs.</p> <p><i>Temperature:</i> 21 °C.</p>                                                                                                                                                                                                                                                                                                                                                |
| Observations          | <p><i>Biological:</i> Daily- gently agitated the exposure chambers and then counted immobilized and dead test organisms (immobilized organisms were those that did not swim within 15 seconds after exposure chambers were gently agitated). Did not remove any daphnids from the exposure chambers at 24 h.</p> <p><i>Water chemistry:</i> (1) At test initiation- total hardness and alkalinity, (2) daily- temperature, pH, DO, and conductivity. All determinations were made according to APHA methods (1995).</p> |
| Endpoint Calculations | <p><i>EC<sub>50</sub>:</i> Trimmed Spearman-Kärber method.</p> <p><i>NOEC:</i> Steel's Many-One Rank Test (TOXSTAT statistical software, Version 3.1, University of Wyoming).</p>                                                                                                                                                                                                                                                                                                                                       |

### 3.0 RESULTS

|                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Test Substance Toxicity (Pertinent data are in Table I) | <p>24-h <math>EC_{50}</math>: &gt;1000 mg/L (Not Calculable).</p> <p>48-h <math>EC_{50}</math>: 479 mg/L (378-607).</p> <p>48-h NOEC: 250 mg/L.</p>                                                                                                                                                                                                                                                                         |
| Ranges of Water Chemistry Parameters                    | <p>Total hardness (as <math>CaCO_3</math> mg/L): 45.</p> <p>Alkalinity (as <math>CaCO_3</math> mg/L): 43-51.</p> <p>Temperature (<math>^{\circ}C</math>): 20.1-22.4 <math>^{\circ}C</math>. Range exceeded by +0.4 <math>^{\circ}C</math>. Exceedance likely had no bearing on toxicity measurement.</p> <p>DO (mg/L): 7.4-9.0.</p> <p>pH: 7.69-8.13.</p> <p>Specific conductivity (<math>\mu mhos/cm</math>): 132-191.</p> |

### 4.0 TEST QA/QC

| QA/QC Criteria                                                                                                        | Data                                                                                           |
|-----------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Test organism dead, immobilized or trapped in control exposure must not be more than 10%.                             | 0% dead, immobilized or trapped.                                                               |
| 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 83% of the air saturation value at 21.0 $^{\circ}C$ . |
| Test duration must be 48 h.                                                                                           | The test duration was 48 h.                                                                    |
| Reference substance toxicity test (this test is optional).                                                            | Current control chart for <i>D. magna</i> acute testing is included in the raw data package.   |



## 5.0 REFERENCES

American Public Health Association (APHA). 1995. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, D.C.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Kärber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ. Sci. Technol. 11(7):714-719; Correction 12(4):417 (1978).

Organization for Economic Cooperation and Development (OECD). 1993. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, Washington, D.C.

University of Wyoming. 1989. Toxstat Version 3.1. Fish Physiology and Toxicology Laboratory, Department of Zoology and Physiology. University of Wyoming, Laramie, WY.

**TABLE 1. ACUTE TOXICITY OF FC-603EF LIGHT WATER(TM) ATC 3% AR-AFFF TO  
D. MAGNA**

| Test Substance<br>Concentration<br>(mg/L) | Cumulative Number and Percent of Dead and Immobilized Organisms* |         |
|-------------------------------------------|------------------------------------------------------------------|---------|
|                                           | 24 h                                                             | 48 h    |
| 0                                         | 0 (0)                                                            | 0 (0)   |
| 62.5                                      | 0 (0)                                                            | 0 (0)   |
| 125                                       | 0 (0)                                                            | 0 (0)   |
| 250                                       | 0 (0)                                                            | 0 (0)   |
| 500                                       | 3 (15)                                                           | 12 (60) |
| 1000                                      | 5 (25)                                                           | 17 (85) |

\* Percentages are in parenthesis.

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

| EC <sub>50</sub> and NOEC | Data (mg/L) | 95% Confidence Interval |
|---------------------------|-------------|-------------------------|
| 24-h EC <sub>50</sub>     | > 1000      | Not Calculable          |
| 48-h EC <sub>50</sub>     | 479         | 378-607                 |
| 48-h NOEC                 | 250         | Not Applicable          |