

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-3003. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was not given. The test sample is FC-3003, identified by the laboratory as R1331. Current information indicates it is a mixture of 1% PFOS, 15% diethylene glycol butyl ether, 79.39% water, 2% Sultone foamer, 1.5% sodium octyl sulfate, 1% sodium decyl sulfate, 0.06% 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: OECD Guideline 203.

Type: Acute static

GLP: No

Year completed: 1997

Species: *Pimephales promelas*

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

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

Exposure period: 96-hours

Statistical methods: The LC₅₀ 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

Length and weight: Average length = 16.6 mm
Average weight = 0.036 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: 48 mg/L as CaCO₃

Alkalinity: 44 mg/L as CaCO₃

Conductivity: 119 µmhos/cm

pH: 8.08

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 weight/volume basis.

Concentrations dosing rate: Once.

Stability of the test chemical solutions: Not noted.

Exposure vessels: 4 L 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):

7.68 – 8.08 (control exposure)

7.60 – 7.92 (1000 mg/L exposure)

Temperature range (0-96 hours): 20.5 – 20.7 °C

Conductivity range (0-96 hours):

119 – 121 µmhos/cm (control exposure)

133 – 136 µmhos/cm (1000 mg/L exposure)

Dissolved oxygen range (0-96 hours):

6.9 – 8.7 mg/L (control exposure)

5.1 – 8.6 mg/L (1000 mg/L exposure)

RESULTS

Nominal concentrations: Blank control, 130, 216, 360, 600, and 1000 mg/L

Element values: 96-hour LC₅₀ = 472 (376 – 593) mg/L

96-hour NOEC = 216 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

The test sample 96-hour LC₅₀ for fathead minnow was determined to be 472 mg/L with a 95% confidence interval of 376 to 593 mg/L. The 96-hour No Observed Effect Concentration (NOEC) is 216 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.

REFERENCES

This test was conducted by AScI Corporation, Duluth Environmental Testing Division, Duluth, MN at the request of the 3M Company, Lab Request number R1331, 1997.

OTHER

Last changed: 6/26/00

STUDY TITLE

ACUTE TOXICITY OF R1331 TO
FATHEAD MINNOW (*Pimephales promelas*)

DATA STANDARD

OECD GUIDELINE 203

STUDY COMPLETED

June 26, 1997

TESTING FACILITY

ASCI Corporation/ASCI-Duluth
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# R1331

1.0 GENERAL INFORMATION

Study Title	Acute Toxicity of R1331 to Fathead Minnow (<i>Pimephales promelas</i>).	
Data Standard	OECD Guideline 203.	
Sponsor	3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 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. (612) 778-7452.	
Sponsor's Lab Request#	R1331.	
Testing Facility	ASCI Corporation/ASCI-Duluth 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	June 17-21, 1997.	
Data Validation and Report Review	Alan Mozol	Report Signature: 
Retention of Raw Data and Final Report	Two years from the date study is completed.	
Location of Raw Data and Final Report	ASCI Corporation/ASCI-Duluth Environmental 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% effect concentrations (LC_{10} and LC_{50}), and the 96-hour no observable effect concentration (NOEC) of the test substance, R1331, for fathead minnow (*Pimephales promelas*) under static test conditions.

This study was conducted according to the ASCI study plan retained by the Sponsor and OECD Guideline 203 (OECD 1993).

3.0 TEST METHODS

Test Substance	Properties: R1331, amber liquid, specific gravity ca. 1.0 (water = 1), stable, and completely soluble in water. Test concentrations: 0 (dilution water control), 130, 216, 360, 600, and 1000 mg/L. Test solutions prepared by mass addition of test substance to dilution water followed by vigorous stirring.
Test Organisms	Source: Healthy, juvenile fathead minnows (<i>Pimephales promelas</i>) obtained from ASCI-DETD in-house culture. Holding/acclimation: The fish used in the tests were obtained from ASCI's in-house culture. The fish used in the study were hatched between May 17 and 23, 1997. For the 48 hours preceding test initiation mortality was 0%, and the fish were not fed for 24 hours before test initiation. The fish mean length was 16.6 ± 2.5 mm, and mean wet weight was 36 ± 19 mg. Loading rate was calculated to be 120 mg of fish per L test solution. Use in test: Used 10 organisms/replicate and 2 replicates/test concentration.
Dilution Water	Source: Dechlorinated City of Duluth, MN, tap water. The most recent chemical analysis is in Raw Data Package. Characteristics: At test time, the water had a hardness of 48 mg/L (as $CaCO_3$) and a pH of 8.08. Aerated for 24 h prior to use in the test.

Exposure Chambers	<i>Type:</i> 4-L glass jars. <i>Use in test:</i> 3.0 L of dilution water or test solution/chamber. During the test, kept covered, except when experimental observations were made. Solutions were not aerated during the exposures.
Incubation	<i>Duration:</i> 96 hours. <i>Daily photoperiod:</i> 16 h light and 8 h dark, maintained using cool white fluorescent lamps. <i>Temperature:</i> 21.0° ± 1°C.
Observations	<i>Biological:</i> Daily- Test organisms with abnormal behavior and signs of stress, and dead test organisms. <i>Water chemistry:</i> (1) At test initiation- total hardness and alkalinity, (2) daily- temperature, DO, conductivity, and pH. All determinations were made according to APHA methods (1995).
Endpoint Calculations	<i>LC₁₀:</i> Inhibition Concentration (ICp) Method. <i>LC₅₀'s:</i> Trimmed Spearman-Kärber Method. <i>NOEC:</i> Fisher's Exact Test (TOXSTAT statistical software, Version 3.5, West Inc., and University of Wyoming).

3.0 RESULTS

Test Substance Toxicity (Pertinent data are in Table 1)	24-h LC_{50}: > 1000 mg/L (Not Calculable). 48-h LC_{50}: > 1000 mg/L (Not Calculable). 72-h LC_{50}: > 1000 mg/L (Not Calculable). 96-h LC_{10}: 173 mg/L (Not Calculable). 96-h LC_{50}: 472 mg/L (376-593). 96-h NOEC: 216 mg/L.
Ranges of Water Chemistry Parameters	Total hardness (as $CaCO_3$ mg/L): 44-48. Alkalinity (as $CaCO_3$ mg/L): 42-44. Temperature ($^{\circ}C$): 20.5-20.7. DO (mg/L): 5.1-8.7. pH: 7.60-8.08. Specific conductivity (μmhos/cm): 118-136.

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 57% of the air saturation value at 21.0°C. The lowest value was measured at the 72-hour test interval for the 1000 mg/L concentration. At the 96-hour interval the DO was 64% of saturation. The reduced DO at the high test concentration did not appear to invalidate the test as significant lethality and acceptable DO's occurred at the 600 mg/L test concentration.
Test duration must be 96 h.	The test duration was 96 h.
Reference Substance Toxicity Test (this test is optional).	Control chart for sodium chloride reference toxicity testing is in 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.

Organization for Economic Cooperation and Development (OECD). 1981/1993. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, 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).

USEPA. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach. Version 2.0.

West Inc., and University of Wyoming. Toxstat Software Package. Version 3.5. Cheyenne, Wyoming.

TABLE 1. ACUTE TOXICITY OF R1331 TO FATHEAD MINNOWS (*P. promelas*)

Test Substance Concentration (mg/L)	Cumulative Number of Dead Organisms and Percent Mortality ^a			
	24 h	48 h	72 h	96 h
0 (control)	0 (0)	0 (0)	0 (0)	0 (0)
130	0 (0)	0 (0)	0 (0)	0 (0)
216	0 (0)	0 (0)	1 (5)	4 (20)
360	0 (0)	0 (0)	1 (5)	9 (45)
600	0 (0)	0 (0)	1 (5)	9 (45)
1000	3 (15)	3 (15)	9 (45)	18 (90)

^a Percentages are in parenthesis.

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

LC's and NOEC	Data (mg/L) ^a	95% Confidence Interval
24-h LC ₅₀	> 1000	Not calculable
48-h LC ₅₀	> 1000	Not calculable
72-h LC ₅₀	> 1000	Not calculable
96-h LC ₁₀	173	Not reliable
96-h LC ₅₀	472	376-593
96-h NOEC	216	Not applicable

^a Values based on nominal test concentrations.