TOXICITY TO AQUATIC PLANTS

TEST SUBSTANCE

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:

Method: OECD Guideline 201
Type: Acute static
GLP: No
Year completed: 1996
Species: *Selenastrum capricornutum*
Source: In-house culture. Originally from ATCC, Rockville, MD
Element basis: Algal cell counts (cells/mL) and average specific growth rates.
Exposure period: 96 hours
Statistical Methods: IC_{50} values determined by the Trimmed Spearman-Karber Method and Toxstat software package, Version 3.5.
Analytical monitoring: pH and temperature.

Test Conditions:

Algal Nutrient Medium: Per OECD method. This nutrient medium provided all mineral nutrients essential for algal growth and also served as the diluent for all algal operations. The pH of this synthetic algal medium was 9.06.

Stock and test solution preparation: A 1000 mg/L primary stock solution was prepared by diluting 150 mg of test substance in 150 mL of standard algal nutrient medium. Aliquots were then added directly to dilution water in test vessels to create test solutions.

Exposure vessels: 125 mL borosilicate-glass Erlenmeyer flasks containing 50 mL of test solution and capped with foam stoppers.

Agitation: Shaken continuously at 100 rpm.
Number of replicates: 3 for exposure concentrations, 6 for blank control.
Initial cell loading: 1.0×10^4 cells/mL (1.2×10^4 actual)
Number of concentrations: five plus a blank control
Lighting: Continuous 400 ft-candles using cool-white fluorescent lamps.
Water Chemistry:
pH range (0-96 hours): 7.48 – 9.06
Test temperature range (0-96 hours): 24.2 – 25.8 °C

RESULTS

Nominal concentrations: Blank control, 31.25, 62.5, 125, 250, and 500 mg/L

Algal Growth Response EC_{50} values

Exposure (Contact) Hours	Cell-Count mg/L (95% C.I.)	Average Specific Growth Rate mg/L (95% C.I.)
24	139 (117 - 165)	171 (146 - 199)
48	153 (124 - 189)	290 (254 - 330)
72	188 (173 - 204)	275 (252 - 300)
96	141 (123 - 162)	294 (265 - 326)

Element values are based on nominal concentrations.

Reversibility of Growth Inhibition: Aliquots of the 500 mg/L test solution were diluted with algal medium and cultured for 7 days. Based on growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

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 substance exhibits a 96-hour EC_{50} cell count value of 141 (123 – 162 mg/L) and a 96-hour E_rC_{50} growth rate value of 294 (265 – 326) mg/L.

The 96-hour No Observed Effect Concentration (NOEC) is 31.25 mg/L for cell count and 125 mg/L for growth rate. This test substance was determined to be algistatic.

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

DATA QUALITY

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 the solution.

REFERENCES

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

OTHER

Last changed: 6/26/00.

STUDY TITLE

GROWTH INHIBITION OF FC-603EF, LIGHT WATER(TM) ATC 3%, AR-AFFF TO GREEN ALGA (*Selenastrum capricornutum*)

DATA STANDARD

OECD GUIDELINE 201

STUDY COMPLETED

November 22, 1996

TESTING FACILITY

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

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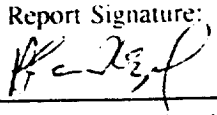
STUDY IDENTIFICATION NUMBERS

ASci Study ID# 5010-068

3M Company Lab Request# P2581

1.0 GENERAL INFORMATION

Study Title	Growth Inhibition of FC-603EF, Light Water (TM) ATC 3%, AR-AFFF to Green Alga (*Selenastrum capricornutum*).	
Data Standard	OECD Guideline 201.	
Sponsor	3M Environmental Technology and 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 Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Lab Request#	P2581.	
Testing Facility	ASci Corporation/ASci-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	
Principal Investigator	Todd McGuire	
Project Director	Joe Amato	
Testing Facility Director	Donald Mount	
Definitive Test Dates	November 4-8, 1996.	
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 4-d median effect concentration (EC_{50}), the 4-d no observable effect concentration (NOEC), and algicidal/algistatic effect of the test substance, FC-603EF, Light Water(TM) ATC 3%, AR-AFFF for green alga (*Selenastrum capricornutum*) under static test conditions.

This study was conducted according to OECD Guideline 201 (OECD 1993).

3.0 TEST METHODS

Test Substance	*Properties*: Amber-colored liquid, specific gravity ca. 1.06 (water = 1), stable, and completely soluble in water. *Test concentrations*: 0 (algal medium control), 31.25, 62.5, 125, 250, and 500 mg/L.
Test Organisms	*Source*: ATCC 22662 *Inoculum preparation*: Algal stock was subcultured in OECD medium for 4 days before test initiation. *Use in test*: Stock culture was diluted and spiked into test solutions to obtain an initial cell density of 1.0 X 10^4 cells per ml (1.2 X 10^4 actual concentration).
Algal Medium	*Preparation*: Deionized water was spiked with nutrient stocks (including Na_2EDTA) to within OECD recommended concentrations. The most recent chemical analysis for deionized water is in Raw Data Package. *Characteristics*: At test time, the medium had a pH of 9.06. Sterilized via autoclave prior to use in the test.

Exposure Chambers	*Type*: Sterilized 125-ml borosilicate-glass Erlenmeyer flasks sealed with foam stoppers. *Use in test*: 50 ml of algal medium or test solution/chamber. Six control replicates and three replicates per test substance concentration were employed. During the test, kept stoppered, except when experimental observations were made. Solutions were not aerated during the exposures.
Incubation	*Duration*: 4 days. *Daily photoperiod*: Continuous at 400 ft-candles using cool-white fluorescent lamps. Test chambers held on shaker table set at 100 rpm. *Temperature*: 25.0 ± 2°C.
Observations	*Biological*: Daily cell counts using direct microscopic enumeration via Improved Neubauer hemocytometer. *Water chemistry*: (1) At test initiation and termination- pH, conductivity, and temperature, (2) daily- temperature. All determinations were made according to APHA methods (1995).
Endpoint Calculations	1-, 2-, 3-, and 4-d EC_{50}'s, and 4-d NOEC based on both cell counts and average specific growth rates (μ). Algicidal/algistatic determination was made post toxicity test termination.

4.0 RESULTS

Test Substance Toxicity (Pertinent data are in Tables 1, 2, and 3)	*Cell Count 1-d* EC_{50}: 139 mg/L (117-165). *μ 1-d* EC_{50}: 171 mg/L (146-199). *Cell Count 2-d* EC_{50}: 153 mg/L (124-189). *μ 2-d* EC_{50}: 290 mg/L (254-330). *Cell Count 3-d* EC_{50}: 188 mg/L (173-204). *μ 3-d* EC_{50}: 275 mg/L (252-300). *Cell Count 4-d* EC_{50}: 141 mg/L (123-162). *μ 4-d* EC_{50}: 294 mg/L (265-326). *Cell Count 4-d NOEC*: 31.25 mg/L. *μ 4-d NOEC*: 125 mg/L. Effect of test substance was determined to be algistatic based on recovery of cells exposed to 500 mg/L concentration.
Test Conditions	*Temperature (°C)*: 24.2-25.8. *Light intensity ft-cdls*: 400. *pH*: 7.48-9.06. *Specific conductivity (μmhos/cm)*: 137-152.

5.0 TEST QA/QC

QA/QC Criteria	Data
Algal counts in control must increase by at least a factor of 16 during the first 3-d of the exposure.	During the first 3-d of the test control algal counts increased by a factor of approximately 39.
Test duration must be 4-d.	The test duration was 4-d.

6.0 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). 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-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ. Sci. Technol. 11(7): 714-719; Correction 12 (4): 417 (1978).

West, Inc. 1996. Toxstat Software Package Version 3.5.

TABLE 1. EC_{50}'S, 95% Confidence Intervals, and 4-d NOEC'S from Algal Growth Inhibition Test with FC-603EF Light Water(TM) ATC 3%, AR-AFFF

Endpoint	Data*		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Cell Count	1-d EC_{50}	139	117-165
	2-d EC_{50}	153	124-189
	3-d EC_{50}	188	173-204
	4-d EC_{50}	141	123-162
	4-d NOEC	31.25	Not Applicable

Endpoint	Data*		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Average Specific Growth Rate (μ)	1-d EC_{50}	171	146-199
	2-d EC_{50}	290	254-330
	3-d EC_{50}	275	252-300
	4-d EC_{50}	294	265-326
	4-d NOEC	125	Not Applicable

* Values Based on Nominal Test Concentrations

TABLE 2. Cells per ml and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to FC-603EF Light Water (TM), ATC 3%, AR-AFFF

Test Conc. (mg/L)	Replicate	Cells/ml (10^4)			
		1 d	2 d	3 d	4 d
Control	A	8.5 (-)	24 (-)	49 (-)	60 (-)
	B	8.0 (-)	25 (-)	48 (-)	62 (-)
	C[a]	- (-)	- (-)	- (-)	- (-)
	D	5.5 (-)	28 (-)	39 (-)	52 (-)
	E	6.0 (-)	37 (-)	55 (-)	91 (-)
	F	9.5 (-)	27 (-)	43 (-)	79 (-)
	Mean	7.50 (-)	27.7 (-)	46.4 (-)	68.5 (-)
31.25	A	8.5 (13)[b]	17 (-39)	52 (12)	65 (-5)
	B	7.0 (-7)	19 (-33)	46 (-2)	60 (-13)
	C	8.0 (7)	22 (-21)	41 (-13)	56 (-18)
	Mean	7.83 (4)	19.2 (-31)	46.0 (-1)	60.2 (-12)
62.5	A	10 (33)	19 (-31)	45 (-4)	49 (-29)
	B	6.5 (-13)	17 (-40)	45 (-4)	52 (-24)
	C	5.5 (-27)	22 (-22)	44 (-5)	54 (-21)
	Mean	7.33 (-2)	19.0 (-31)	44.3 (-4)	51.7 (-25)
125	A	3.5 (-53)	20 (-30)	43 (-8)	49 (-29)
	B	1.0 (-87)	18 (-37)	39 (-16)	46 (-34)
	C	4.0 (-47)	15 (-48)	38 (-18)	46 (-34)
	Mean	2.83 (-62)	17.2 (-38)	39.8 (-14)	46.7 (-32)
250	A	3.0 (-60)	8.5 (-69)	12 (-75)	14 (-80)
	B	2.5 (-67)	5.5 (-80)	10 (-78)	20 (-72)
	C	2.5 (-67)	10 (-64)	9.5 (-80)	14 (-80)
	Mean	2.67 (-64)	8.00 (-71)	10.3 (-78)	15.7 (-77)
500	A	2.0 (-73)	2.5 (-91)	2.0 (-96)	2.0 (-97)
	B	0 (-100)	1.5 (-95)	1.5 (-97)	0.5 (-99)
	C	3.0 (-60)	3.0 (-89)	1.5 (-97)	4.0 (-94)
	Mean	1.67 (-78)	2.33 (-92)	1.67 (-96)	2.17 (-97)

[a] Control Replicate C Values Omitted as an Outlier

[b] Parenthetic Values = % Difference from Control Mean Value

* 0-d Mean Algal Count = 1.2×10^4

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Sponsor Study ID# P2581

TABLE 3. Average Specific Growth Rates (μ) and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to FC-603EF Light Water(TM) AR-AFFF

Test Conc. (mg/L)	Replicate	Average Specific Growth Rate (μ)*			
		1 d	2 d	3 d	4 d
Control	A	1.958 (-)	1.487 (-)	1.233 (-)	0.978 (-)
	B	1.897 (-)	1.508 (-)	1.230 (-)	0.984 (-)
	C[b]	- (-)	- (-)	- (-)	- (-)
	D	1.522 (-)	1.566 (-)	1.156 (-)	0.940 (-)
	E	1.609 (-)	1.707 (-)	1.272 (-)	1.082 (-)
	F	2.069 (-)	1.547 (-)	1.189 (-)	1.045 (-)
	Mean	1.833 (-)	1.570 (-)	1.218 (-)	1.011 (-)
31.25	A	1.958 (7)[c]	1.325 (-16)	1.256 (3)	0.998 (-1)
	B	1.764 (-4)	1.368 (-13)	1.212 (-1)	0.976 (-3)
	C	1.897 (4)	1.454 (-7)	1.173 (-4)	0.961 (-5)
	Mean	1.876 (2)	1.385 (-12)	1.215 (-0)	0.979 (-3)
62.5	A	2.120 (16)	1.381 (-12)	1.204 (-2)	0.927 (-8)
	B	1.689 (-8)	1.311 (-17)	1.204 (-2)	0.942 (-7)
	C	1.522 (-17)	1.443 (-8)	1.201 (-2)	0.952 (-6)
	Mean	1.810 (-1)	1.381 (-12)	1.203 (-2)	0.941 (-7)
125	A	1.070 (-42)	1.394 (-11)	1.189 (-2)	0.927 (-8)
	B	0.182 (-100)	1.340 (-15)	1.160 (-5)	0.909 (-10)
	C	1.204 (-34)	1.246 (-21)	1.152 (-5)	0.909 (-10)
	Mean	0.859 (-53)	1.330 (-15)	1.167 (-4)	0.915 (-9)
250	A	0.916 (-50)	0.979 (-38)	0.753 (-38)	0.614 (-39)
	B	0.734 (-60)	0.761 (-52)	0.707 (-42)	0.697 (-31)
	C	0.734 (-60)	1.060 (-32)	0.690 (-43)	0.605 (-40)
	Mean	0.799 (-56)	0.949 (-40)	0.718 (-41)	0.642 (-36)
500	A	0.511 (-72)	0.367 (-77)	0.170 (-86)	0.128 (-87)
	B	0 (-100)	0.112 (-93)	0.074 (-94)	-0.219 (-100)
	C	0.916 (-50)	0.458 (-71)	0.074 (-94)	0.301 (-70)
	Mean	0.329 (-82)	0.332 (-79)	0.110 (-91)	0.148 (-85)

* $\mu = \frac{\ln \text{Cells per ml } t_n - \ln \text{Cells/ml } t_0}{t_n - t_0}$

[b] Control Replicate C Values Omitted as an Outlier

[c] Parenthetic Values = % Difference from Control Mean Value

* 0-d Mean Algal Count = 1.2×10^4

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