

TOXICITY TO AQUATIC PLANTS

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 201

Type: Acute Static

GLP: No

Year study performed: 1997

Species: *Selenastrum capricornutum*

Source: Originally obtained from ATCC, Rockville, MD, maintained in culture medium at ASci-DETD, Duluth, MN

Element basis: Algal cell counts (cells/ml), specific growth rates.

Exposure period: 96 hours

Statistical Methods: Statistical analyses were performed using the Trimmed Spearman-Kärber method.

Analytical monitoring: pH, conductivity, and temperature

Test Conditions:

Algal Nutrient Medium: Nutrient medium per OECD. 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 8.0.

Stock and test solution preparation: A 2000 mg/L primary stock solution was prepared by dissolving 700 mg in 350 mL algal medium, followed by mechanical stirring. Each test concentration was made by volume addition of the stock to algal medium.

Exposure vessels: 250 ml Erlenmeyer flasks containing 100 ml test solution, sealed with foam stoppers.

Agitation: 100 rpm.

Number of replicates: 3 for exposure concentrations, 6 for blank control.

Initial cell loading: 1.0×10^4 cells/mL (1.3×10^4 cells/mL actual).

Number of concentrations: five plus a blank control

Lighting: Continuous lighting at 740 ft-candles using cool-white fluorescent lamps.

Water Chemistry:

pH range (0-96 hours):

8.00 – 9.90 (control exposure)

4.72 – 7.90 (1000 mg/L exposure)

Conductivity range (0-96 hours):

151 – 186 μ mhos/cm (control exposure)

159 – 170 μ mhos/cm (1000 mg/L exposure)

Test temperature range (0-96 hours):

24.8 – 25.8 °C (incubator)

RESULTS

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

Algal Growth EC₅₀ Values

Exposure (Contact) Hours	Cell-Count mg/L (95% C.I.)	Average Specific Growth Rate mg/L (95% C.I.)
24	233 (159 – 342)	160 (150 – 170)
48	<130 (Not Calculable)	251 (204 – 309)
72	<130 (Not Calculable)	572 (434 – 756)
96	<130 (Not Calculable)	644 (528 – 834)

Element values: 96-hour EC₅₀ = <130 mg/L (cell count)

96-hour E_rC₅₀ = 664 (528 – 834) mg/L (growth rate)

96-hour NOEC = 23 mg/L (cell count)

96-hour NOEC = 96 mg/L (growth rate)

Element values are based on nominal concentrations.

Reversibility of Growth Inhibition: Aliquots of the 1000 mg/L test solution were diluted with algal medium and cultured for an additional period. 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

FC-3003 exhibits a 96-hour EC₅₀ cell count value of <130 mg/L and a 96-hour E_rC₅₀ growth rate value of 664 (528 – 834) mg/L.

The 96-hour No Observed Effect Concentration (NOEC) is 23 mg/L for cell count and 96 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. 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

GROWTH INHIBITION OF R1331 FOR
GREEN ALGA (*Selenastrum capricornutum*)

DATA STANDARD

OECD GUIDELINE 201

STUDY COMPLETED

April 3, 1997

TESTING FACILITY

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

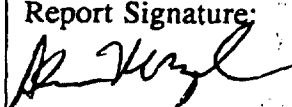
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STUDY IDENTIFICATION NUMBERS

ASCI Study ID# 5010-068

3M Company Lab Request# R1331

1.0 GENERAL INFORMATION

Study Title	Growth Inhibition of R1331 for Green Alga (<i>Selenastrum capricornutum</i>).	
Data Standard	OECD Guideline 201.	
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	Todd McGuire	
Project Director	Joe Amato	
Testing Facility Director	Donald Mount	
Definitive Test Dates	March 25-29, 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 4-d median effect concentration (EC_{50}) and the 4-d no observable effect concentration (NOEC) of the test substance, R1331, 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: R1331, clear amber-colored liquid, specific gravity = 1.02 (water = 1), stable, and completely soluble in water. Test concentrations: 0 (algal medium control), 130, 216, 360, 600, and 1000 mg/L. A stock solution was prepared by mass addition to algal medium followed by mechanical stirring. Each test concentration was made by volume addition of the stock to algal medium.
Test Organisms	Source: ATCC 22662 Inoculum preparation: Algal stock was subcultured in OECD recommended 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 1.0×10^4 cells per ml (1.3×10^4 actual concentration).
Algal Medium	Preparation: Deionized water was spiked with nutrient stocks (including Na_2EDTA) to 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 8.00. Sterilized via $0.22 \mu m$ filtration prior to use in the test.
Exposure Chambers	Type: Sterilized via autoclave 250-ml borosilicate-glass Erlenmeyer flasks sealed with foam stoppers. Use in test: 100 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.

Incubation	<i>Duration:</i> 4 days. <i>Daily photoperiod:</i> Continuous at 740 ft-candles using cool-white fluorescent lamps. Test chambers held on shaker table set at 100 rpm. <i>Temperature:</i> $25.0 \pm 2^{\circ}\text{C}$.
Observations	<i>Biological:</i> Daily cell counts using direct microscopic enumeration via Improved Neubauer hemacytometer. <i>Water chemistry:</i> (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). Statistical analyses performed using Trimmed Spearman-Kärber Method (EC_{50}'s) and ICp analysis.

4.0 RESULTS

Test Substance Toxicity (Pertinent data are in Tables 1, 2, and 3)	<i>Cell Count 1-d EC_{50}:</i> 233 mg/L (159-342). μ 1-d EC_{50}: 160 mg/L (150-170). <i>Cell Count 2-d EC_{50}:</i> < 130 mg/L. μ 2-d EC_{50}: 251 mg/L (204-309). <i>Cell Count 3-d EC_{50}:</i> < 130 mg/L. μ 3-d EC_{50}: 572 mg/L (434-756). <i>Cell Count 4-d EC_{50}:</i> < 130 mg/L. μ 4-d EC_{50}: 664 mg/L (528-834). <i>Cell Count 4-d NOEC (IC10):</i> 23 mg/L (22-24). μ 4-d NOEC (IC10): 96 mg/L (88-105). Effect was determined to be algistatic based on production of cells exposed during the definitive test to the 1000 mg/L test substance concentration.
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Test Conditions	<i>Temperature (°C): 24.8-25.8.</i> <i>Light intensity ft-cdls: 740.</i> <i>pH: 4.72-9.90.</i> <i>Specific conductivity (μmhos/cm): 129-186.</i>
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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 157.
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-Kärber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ. Sci. Technol. 11(7): 714-719; Correction 12 (4): 417 (1978).
- US EPA. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0). Environmental Research Laboratory, Duluth, Minnesota.

TABLE 1. EC₅₀'S, 95% Confidence Intervals, and 4-d NOEC'S from Algal Growth Inhibition Test with R1331

Endpoint	Data ^a		95 % Confidence Interval (mg/L)
	Time	(mg/L)	
Cell Count	1-d EC ₅₀	233	159-342
	2-d EC ₅₀	< 130	Not Calculable
	3-d EC ₅₀	< 130	Not Calculable
	4-d EC ₅₀	< 130	Not Calculable
	4-d NOEC (IC ₁₀)	23	22-24

Endpoint	Data ^a		95 % Confidence Interval (mg/L)
	Time	(mg/L)	
Average Specific Growth Rate (μ)	1-d EC ₅₀	160	150-170
	2-d EC ₅₀	251	204-309
	3-d EC ₅₀	572	434-756
	4-d EC ₅₀	664	528-834
	4-d NOEC (IC ₁₀)	96	88-105

^a Values Based on Nominal Test Concentrations

TABLE 2. Cells per ml and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to R1331

Test Conc. (mg/L)	Replicate	Cells/ml (10^4)			
		1 d	2 d	3 d	4 d
Control	A	4	35	200	726
	B	2	35	215	660
	C	3	35	200	627
	D	5	40	195	616
	E	3	31	205	704
	F	5	30	210	638
	Mean	3.7	34.3	204	662
130	A	2	13	69	310
	B	4	17	61	270
	C	2	14	64	275
	Mean	2.7 (-28)	14.7 (-57)	64.7 (-68)	285 (-57)
216	A	2	5	39	195
	B	1	6	49	171
	C	1	5	40	165
	Mean	1.3 (-64)	5.3 (-84)	42.7 (-79)	177 (-73)
360	A	3	4	23	75
	B	1	6	25	78
	C	2	5	32	93
	Mean	2.0 (-46)	5.0 (-85)	26.7 (-87)	82.0 (-88)
600	A	1	3	18	41
	B	1	4	14	39
	C	2	5	14	33
	Mean	1.3 (-64)	4.0 (-88)	15.3 (-92)	37.7 (-94)
1000	A	2	4	10	12
	B	1	4	6	13
	C	2	2	10	14
	Mean	1.7 (-55)	3.3 (-90)	8.7 (-96)	13.0 (-98)

* Parenthetic Values = % Difference from Control Mean Value

* 0-d Mean Algal Count = 1.3×10^4

TABLE 3. Average Specific Growth Rates (μ) and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to R1331

Test Conc. (mg/L)	Average Specific Growth Rate (μ) ^a			
	1 d	2 d	3 d	4 d
Control	1.046	1.636	1.686	1.558
130	0.731 (-30)	1.213 (-26)	1.302 (-23)	1.348 (-13)
216	0 (-100)	0.703 (-57)	1.164 (-31)	1.228 (-21)
360	0.431 (-59)	0.674 (-59)	1.007 (-40)	1.036 (-34)
600	0 (-100)	0.562 (-66)	0.822 (-51)	0.842 (-46)
1000	0.268 (-74)	0.466 (-72)	0.634 (-62)	0.576 (-63)

^a $\mu = \frac{\ln \text{Cells per ml } t_2 - \ln \text{Cells/ml } t_0}{t_2 - t_0}$

^b Parenthetic Values = % Difference from Control Mean Value

* 0-d Mean Algal Count = 1.3×10^4