TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

TEST SUBSTANCE

Identity: Perfluorooctylsulfonate, didecyldimethylammonium salt; may also be referred to as Fluoroalkyl ammonium derivative. [1-Decaminium, N-decyl-N,N-dimethyl-, salt with 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid (1:1), CAS # 251099-16-8]

Remarks: The 3M production lot number was Lot 1. The test sample is L-14394 referred to by the test laboratory as P3025. The sample was labeled F-11615, Lot 1. The test sample is a mixture of the test substance in water (approximately 30-40% test substance, 60-70% water, and 0-5% of residual perfluorochemicals). All values reported relate to this mixture. The test sample appears to be a 2-phase dispersion (clear liquid with opaque solid) which rapidly separates after agitation. No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: OECD 201
Test: Static acute
GLP: No
Date exposure completed: 1/11/97 (study repeated on 1/13/97; second study exposure completed 1/17/97)
Species: *Selenastrum capricornutum*
Source: Originally from The American Type Culture Collection (ATCC), Strain 22662.
Analytical monitoring: Nominal concentrations. PH, conductivity, and temperature were monitored.
Element basis: Growth rate and number of cells/mL.
Exposure period: 96-hours
Start date: 1/7/97
End date: 1/11/97
Test organisms laboratory culture: Algae cultures were growing in OECD-recommended culture medium at AScI Corporation, Duluth, MN for 4 days before test initiation.
Test Conditions:

Test temperature range: 25.6 – 26.0°C

Dilution water source: The algal medium was prepared to OECD recommended concentrations by spiking deionized water with nutrient stocks (including Na_2EDTA). The medium was sterilized via 0.22 µm filtration prior to use in the test. The pH of the synthetic algal medium at test initiation was 7.89.

Stock and test solution preparation: Water accommodated fractions. Test solutions were prepared for each test concentration by mass addition of test substance in 500 mL of algal medium. The test substance was thoroughly homogenized before each mass was determined. The solutions were vigorously stirred for 20-hours (vortex 1/2 – 1/3 solution depth). The upper layer of the aqueous phase was pipetted from the vessels after the solutions had settled for 1-hour. The test solution in the highest two exposure concentrations, 24 and 40 mg/L were a cloudy milkish color, while the 14.4 mg/L and 5.2 mg/L concentrations were clear, and the 8.6 mg/L concentration was slightly cloudy. A white precipitate was also observed on the bottom of the flasks at all loading concentrations during WAF preparation.

Exposure vessels: Sterile 250 mL borosilicate Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

Agitation: Shaken continuously at 100 rpm

Number of replicates: Six for control, three per test substance loading

Initial algal cell loading: 1.0×10^4 cells/mL

Number of concentrations: Five plus a negative control

Water chemistry:

pH range (0 – 96 hours)

7.89 – 9.84 (control exposure)

8.13 – 9.32 (40 mg/L exposure)

Conductivity range (0 – 96 hours)

131 - 150 µmhos/cm (control exposure)

134 - 156 µmhos/cm (40 mg/L exposure)

Test temperature range (0 – 96 hours)

25.6 – 26.0°C

Light levels: (0 – 96 hours)

730– 750 ft-c from continuous cool-white fluorescent lighting

Remarks: Inconsistent growth inhibition at increasing exposure concentrations was seen, particularly at the 8.6 mg/L exposure concentration. As a result, the 48 and 72-hour EL50 based on cell growth were less than the lowest exposure concentration (5.2 mg/L), while the 48, 72, and 96-hour EL50 based on growth rate were greater than the highest exposure concentration (40 mg/L). The NOEL (cell density) was also lower than the lowest exposure concentration.

RESULTS

Nominal concentrations: Bk control, 5.2, 8.6, 14.4, 24, 40 mg/L.
Element value:
24-hour EL_{50} (cell density) = 15.8 (13.2 – 18.8) mg/L
24-hour E_rL_{50} (growth rate) = 15.9 (14.4-17.6) mg/L
48-hour EL_{50} (cell density) = <5.2 mg/L (CI not calculable)
48-hour E_rL_{50} (growth rate) = >40 mg/L (CI not calculable)
72-hour EL_{50} (cell density) = <5.2 mg/L (CI not calculable)
72-hour E_rL_{50} (growth rate) = >40 mg/L (CI not calculable)
96-hour EL_{50} (cell density) = 20.8 (16.4 – 26.4) mg/L
96-hour E_rL_{50} (growth rate) = >40 mg/L (CI not calculable)
96-hour NOEL (cell density): 1.9 (1.6 – 2.5) mg/L*
96-hour NOEC (growth rate): 18.9 (14.6 – 22.1) mg/L*
*NOEL values are approximations using the IC_{10} as the endpoint.

All element values based on nominal concentrations.

Statistical methods: Cell densities, growth rates and percent inhibition values were calculated using an Excel spreadsheet. The Trimmed Spearman-Karber method and the Inhibition Concentration (ICp) approach (Version 2.01, USEPA-ERL, Duluth, MN) were used for EL and NOEL determinations.

Control response: satisfactory

Biological observations after 96-hours:

Nominal Loading Test Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Growth Rate
Blank Control	6,930,000	-	-
5.2	4,330,000	-38	-7
8.6	5,720,000	-17	-3
14.4	4,220,000	-39	-8
24	3,020,000	-56	-13
40	2,500,000	-64	-16

Observations: Algal cell counts in each test vessel were determined by means of direct microscopic counts with an Improved Newbauer hemacytometer.

CONCLUSIONS

The test sample 96-hour EL_{50} interval for *Selenastrum capricornutum* was determined using two calculation methods. By cell density, it was 20.8 mg/L with a 95% confidence interval of 16.4 – 26.4 mg/L, and by growth rate >40 mg/L (CI not calculable). The 96-hour NOEL was determined to be 1.9 mg/L using cell density and 18.9 mg/L using growth rate.

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

DATA QUALITY

Reliability: Klimisch ranking 3. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized. Accurate EL_{50}'s could not be calculated at each observation period due to the inconsistent dose-response relationship and low inhibition observed at the exposure concentration loadings. Additionally, data is for a mixture and toxicity cannot be positively attributed to didecyldimethylammonium Perfluorooctylsulfonate salt alone.

Report quality: Two definitive studies were conducted on this test substance at the same laboratory, in the same time period. A careful examination of the raw data revealed several errors during report preparation. These are:

1. The "study completed" dates printed on the report covers (January 29, 1997 and February 21, 1997) are switched with each other. The sections under "1.0 General Information" for "Definitive Test Dates" are correct for each report, however.
2. The discussion under section "3.0 Test Methods" for "Test loadings" are switched. However, the test concentrations listed in this section are correct in each report.
3. The algicidal/algistatic determination test was only conducted after the second study. The discussion under "4.0 Results" in the report with the cover reading January 29, 1997 should not have been included. No evidence was seen in the raw data that the algicidal/algistatic determination was conducted during the first study. The raw data from the second study revealed that the test substance was, in fact, algicidal.

REFERENCES

This study was conducted at ASci Corporation, Environmental Testing Division, Duluth, MN at the request of the 3M Company.

OTHER

Last changed: 5/24/00

STUDY TITLE

GROWTH INHIBITION OF _P3025_ TO
GREEN ALGA (*Selenastrum capricornutum*)

DATA STANDARD

OECD GUIDELINE 201

STUDY COMPLETED

February 21, 1997

TESTING FACILITY

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

Tel. No. (218) 722-4040
Fax No. (218) 722-2592

STUDY IDENTIFICATION NUMBERS

ASci Study ID# 5010-068

3M Company Lab Request# P3025

1.0 GENERAL INFORMATION

Study Title	Growth Inhibition of P303K to Green Alga (*Selenastrum capricornutum*).	
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#	P3025.	
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	January 7-11, 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 loading (EL_{50}) and the 4-d no observable effect loading (NOEL) of the test substance, P3025, for green alga (*Selenastrum capricornutum*) under static test conditions.

This study was conducted according to OECD Guideline 201 (OECD 1993). Test solution preparation was water-accommodated fraction (WAF) per Girling & Whale 1994.

3.0 TEST METHODS

Test Substance	*Properties*: P3025, two phase liquid, specific gravity ca. 1.2 (water = 1), stable, and insoluble in water. *Test loadings*: 0 (algal medium control), 5.2, 8.6, 14.4, 24, and 40 mg/L. Test loading range was determined from range-finding test results, and results of previous definitive tests in which inappropriate concentration ranges were used. Each test loading was prepared by mass addition of test substance to 500-ml aliquots of algal medium. Test substance was thoroughly homogenized before each mass was determined. Each solution was vigorously stirred (vortex 1/2 of flasks' depth) for 20 hours then allowed to settle for one hour to obtain WAF's. During WAF preparation, a white precipitate was observed at all loadings, except the 14.4 mg/L loading. A second flask loaded at 14.4 mg/L was also set up, and this second solution produced the precipitate. Both 14.4 mg/L loadings were tested, and both produced essentially the same results. The presence or lack of precipitation during WAF preparation did not appear to affect the test substance toxicity. Test solutions were obtained from flasks by pipetting off upper layer aqueous phase.

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 (1.0×10^4 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 7.89. Sterilized via 0.22 filtration prior to use in the test.
Exposure Chambers	*Type*: Sterilized 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 loading were employed. During the test, kept stoppered, except when experimental observations were made.
Incubation	*Duration*: 4 days. *Daily photoperiod*: Continuous at 740 ft-candles using cool-white fluorescent lamps. Test chambers held on shaker table set at 100 rpm. *Temperature*: $25.0 \pm 2°C$.
Observations	*Biological*: Daily cell counts using direct microscopic enumeration via Improved Neubauer hemacytometer. *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 EL_{50}'s, and 4-d NOEL based on both cell counts and average specific growth rates (μ).

4.0 RESULTS

Test Substance Toxicity (Pertinent data are in Tables 1, 2, and 3)	*Cell Count 1-d* EL_{50}: 15.8 mg/L (13.2-18.8). μ *1-d* EL_{50}: 15.9 mg/L (14.4-17.6). *Cell Count 2-d* EL_{50}: <5.2 mg/L. μ *2-d* EL_{50}: >40 mg/L. *Cell Count 3-d* EL_{50}: <5.2 mg/L. μ *3-d* EL_{50}: >40 mg/L. *Cell Count 4-d* EL_{50}: 20.8 mg/L (16.4-26.4). μ *4-d* EL_{50}: >40 mg/L. *Cell Count 4-d NOEL*: 1.9 mg/L (1.6-2.5). μ *4-d NOEL*: 18.9 mg/L (14.6-22.1). NOEC values are approximations using the IC_{10} as the endpoint. Effect of test substance was determined to be algicidal based on the results of a post-test determination where cells previously exposed to a 400 mg/L loading failed to increase in number after 9 days. These definitive test results were not applied to determine the EL_{50}'s as the final cell counts for all loadings resulted in greater than 50% inhibition.
Test Conditions	*Temperature (°C)*: 25.6-26.0. *Light intensity ft-cdls*: 730-750. *pH*: 7.84-10.26. *Specific conductivity (µmhos/cm)*: 131-160.

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 388.
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.

Girling, A.E., G.F. Whale, and D.M.M. Adema. 1994. A Guideline Supplement for Determining the Aquatic Toxicity of Poorly Water-Soluble Complex Mixtures using Water-Accommodated Fractions. Chemosphere, Vol. 29, No. 12, pp. 2645-2649.

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).

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. EL_{50}'S, 95% Confidence Intervals, and 4-d NOEL'S from Algal Growth Inhibition Test with P3025

Endpoint	Data[a]		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Cell Count	1-d EL_{50}	15.8	13.2-18.8
	2-d EL_{50}	<5.2	Not Calculable
	3-d EL_{50}	<5.2	Not Calculable
	4-d EL_{50}	20.8	16.4-26.4
	4-d NOEL	1.9	1.6-2.5

Endpoint	Data[a]		95% Confidence Interval (mg/L)
	Time	(mg/L)	
Average Specific Growth Rate (μ)	1-d EL_{50}	15.9	14.4-17.6
	2-d EL_{50}	>40	Not Calculable
	3-d EL_{50}	>40	Not Calculable
	4-d EL_{50}	>40	Not Calculable
	4-d NOEL	18.9	14.6-22.1

[a] IC_{10} Values Determined by ICp Program Used to Approximate NOEC's

TABLE 2. Cells per ml and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to P3025

Test Loading (mg/L)	Replicate	Cells/ml (10^4)			
		1 d	2 d	3 d	4 d
Control	A	6	52	396	649
	B	5	55	378	638
	C	3	53	420	759
	D	5	51	354	682
	E	4	46	372	792
	F	4	54	408	638
	Mean	4.5 (-)	51.8 (-)	388 (-)	693 (-)
5.2	A	1	15	105	396
	B	1	19	145	440
	C	4	22	95	462
	Mean	2.0 (-56)	18.7 (-64)	115 (-70)	433 (-38)
8.6	A	2	25	245	506
	B	3	23	240	572
	C	2	25	245	638
	Mean	2.3 (-48)	24.3 (-53)	243 (-37)	572 (-17)
14.4	A	4	17	92	363
	B	2	17	111	418
	C	3	15	94	484
	Mean	3.0 (-33)	16.3 (-68)	99 (-74)	422 (-39)
24	A	1	9	55	276
	B	2	11	52	300
	C	1	11	53	330
	Mean	1.3 (-70)	10.3 (-80)	53 (-86)	302 (-56)
40	A	0	8	46	204
	B	2	10	59	240
	C	1	10	66	306
	Mean	1.0 (-78)	9.3 (-82)	57 (-85)	250 (-64)

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

* 0-d Mean Algal Count = 1.0 X 10^4

TABLE 3. Average Specific Growth Rates (μ) and Percentage Difference from Mean Control Value for *Selenastrum capricornutum* Exposed to P3025

Test Loading (mg/L)	Replicate	Average Specific Growth Rate (μ)[a]			
		1 d	2 d	3 d	4 d
Control	Mean	1.504	1.974	1.987	1.635
5.2	Mean	0.693 (-54)	1.464 (-26)	1.582 (-20)	1.518 (-7)
8.6	Mean	0.833 (-45)	1.595 (-19)	1.831 (-8)	1.587 (-3)
14.4	Mean	1.099 (-27)	1.396 (-29)	1.532 (-23)	1.511 (-8)
24	Mean	0.262 (-83)	1.166 (-41)	1.325 (-33)	1.428 (-13)
40	Mean	0 (-100)	1.115 (-44)	1.348 (-32)	1.380 (-16)

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

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

* 0-d Mean Algal Count = 1.0×10^4