TOXICITY TO AQUATIC PLANTS

TEST SUBSTANCE

Identity: N-ethylperfluorooctane sulfonamidoethanol; may also be referred to as N-EtFOSE Alcohol or FM-3422. (1-Octanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-(2-hydroxyethyl)-, CAS # 1691-99-2)

Remarks: Material is an off-white, waxy solid of uncharacterized purity. The limited water solubility of this material prohibited a definitive determination of the aquatic toxicity.

METHOD

Method Followed: Modified (modeled) after those described by USEPA – 600/9-78-018; ASTM-E-35.23 Draft No. 2; OECD; A.G. Payne.
Type (test type): Acute Static bioassay
GLP: No
Year study performed: 1981
Species/Strain/Supplier: Freshwater green algae, *Selenastrum capricornutum.* Obtained from USEPA – ERL, Corv., Oregon (July 14, 1981).
Analytical monitoring: Algal cell counts (cells/ml) and temperature.
Exposure period: 4, 7, 10, and 14 days
Statistical Methods: EC_{50} values and 95% confidence limits were calculated utilizing a linear regression model.

Test Condition Remarks:

Inoculum: Algae from a 7-day-old stock culture were used as inoculum to give a starting optimum inoculum level of green algae $1x10^4$ cells/ml. The use of a 7 to 10-day-old stock culture insured the presence of a sufficient number of viable algal cells in the exponential growth phase. The initial algal cell count in the stock culture was determined using a hemocytometer (338,000 cells/ml).

Algal Nutrient Medium: Sterile synthetic algal nutrient medium. 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 adjusted to 7.5±0.1 prior to use in assays.

Culture Flasks: Sterile culture flasks consisted of 250 ml Erlenmeyers containing 50 ml synthetic algal nutrient medium.

Full-Scale Test (Definitive):

All definitive algal assays were carried out in triplicate using 250 ml Erlenmeyer flasks containing 50 ml of test solution. The definitive assay consisted of four exposure periods: 4, 7, 10, and 14 days.

The following N-EtFOSE Alcohol, Lot 716, logarithmic concentrations: 180, 320, 560, 1000, and 1800 mg/L were used to initiate the exposure study (individual weights). Three flasks containing 100% fresh algal nutrient medium plus algal cells comprised the nontreated controls.

0.5 ml Ethanol / 1 liter algal nutrient medium was used as a carrier solvent.

Test Conditions:

All algal operations were carried out under aseptic conditions in order to avoid contamination with bacteria and other algae. Algal cultures were maintained in an environmental chamber under the following standard growth conditions:

Temperature: 23±2°C (70-77°F)
Fluorescent illumination: 400 ft. candles ± 10%
Free gas exchange: Continuous Platform Shaking, 100 ± 10 rpm.

RESULTS

Testing ended with 4, 7, 10, and 14-day calculated EC_{50} values greater than the solubility limit of the test material. EC_{50} calculated values were greater than 1800 mg/L (based on reduction in cell count).

CONCLUSIONS

Calculated EC_{50} values indicate this material has insignificant toxicity to algae.

DATA QUALITY

Reliability: Klimisch ranking = 3. This study lacks information on purity of the test substance and actual measurements of the amount of test substance in solution. Testing was conducted at approximately 5 orders of magnitude above the solubility limit of the test substance.

REFERENCES

3M Technical Report Summary, Multi-Phase Algal Assay Test Method, New Methods Development – N-EtFOSE Alcohol, Lot 716, Report Number 008, Project Number 9970030000, M. T. Elnabarawy, December 30, 1981.

OTHER

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

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TECHNICAL REPORT SUMMARY

Date: 12/30/81

TO: TECHNICAL COMMUNICATIONS CENTER – 201-2CN

(Important – If report is printed on both sides of paper, send two copies to TCC.) See "Guidelines for Completion of Technical Report Summary" available from Information Liaison or TCC (3-5545).

Division: Environmental Laboratory (EE & PC)	Dept. Number: 0535
Project: New Methods Development - FM-3422, Lot 716	Project Number: 9970030000
Report Title: Multi-Phase Algal Assay Test Method	Report Number: 008
To: D. Bacon/R. Bohon/E. Reiner/A. Welter	
Author(s): M. T. Elnabarawy	Employee Number(s): 46981
Notebook Reference:	No. of Pages Including Coversheet:

SECURITY ▶ ☐ Open (Company Confidential) ☐ Closed (Special Authorization) | 3M CHEMICAL REGISTRY ▶ New Chemicals Reported ☐ Yes ☒ No

KEYWORDS: Include Lab Code

EE & PC Div. (Env. Lab)

CURRENT OBJECTIVE:

1. To evaluate the algal growth response of freshwater green algae "Selenastrum capricornutum" over several generations as they may be affected by exposure to the fluorochemical FM-3422, Lot 716.

2. To develop an algal test protocol based on variable exposure or recovery periods to the test compound.

REPORT ABSTRACT: This abstract information is distributed by the Technical Communications Center to alert 3M'ers to Company R&D. It is Company confidential material.

In these triplicated four-phase assays, the initial effects on organisms following varied exposures to FM-3422 were assessed over several generations. Test results gave an indication of the possible effects on algal populations. Algal biomass was assessed quantitatively in terms of algal cell-count (No./ml), and the median growth response EC_{50} (mg/l) was calculated

Information Liaison Initials:

CONCLUSIONS

1) Materials possessing an EC_{50} greater than 1,000 mg/l are considered to be insignificantly phytotoxic to algae and aquatic plant species.

2) Results indicated that extended exposures (e.g., >7 days) induced no greater reduction in algal cell counts.

SUMMARY

A four-phase algal assay was developed to evaluate algal growth response of the freshwater unicellular green algae "Selenastrum capricornutum" over several generations as they may be affected by exposure to the fluorochemical, FM-3422. This multiphase algal assay evaluated algal growth responses during four consecutive exposure periods: 4, 7, 10, and 14 days. Algal growth response was measured in terms of an increase in biomass as algal cell-count (No./ml). Cell-count results used for the calculation of EC_{50}'s (mg/l) were averages of triplicated sets of test culture flasks.

In these triplicated multiphase algal assays, the calculated EC_{50} values (mg/l) for freshwater green algae "Selenastrum capricornutum" are presented in Table 1. These values represent the median growth response (EC_{50} mg/l) following exposure to the test material for 4, 7, 10, and 14 days.

INTRODUCTION

In testing the possible effects of chemical substances on the aquatic environment, unicellular algae are recommended as a model system for evaluating the influence of chemicals on algal growth (aquatic primary producers), aquatic plants, and phytoplankton.

Toxic effects are tested on growing algal cultures that undergo cell-division during the test. These growth responses may be: a) stimulatory, b) inhibitory and/or algicidal.

MATERIAL AND EXPERIMENTAL DESIGN

The test substance (FM-3422, Lot 716) is an off-white solid, with limited solubility in water at ambient room temperature. The bioassays performed on this material evaluated its potential algal toxicity and the data generated form the basis of this report.

The test protocol utilized for this study was modified after those described by USEPA - 600/9-78-018; ASTM-E-35.23 Draft No. 2; OECD; A. G. Payne. (1), (2), (3), (4).

Test Species:

A bacteria-free culture of the freshwater planktonic green algae (Chlorophyceae), Selenastrum capricornutum of the order chlorococcales was obtained from USEPA - ERL, Corv., Oregon (July 14, 1981). The test algae are non-motile unicellular cells, having the appearance of a new moon. The algal culture was stored in the dark at 4°C.

Inoculum:

Algae from a 7-day-old stock culture were used as inoculum to give a starting optimum inoculum level of green algae Selenastrum capricornutum 1×10^4 cells/ml. The use of a 7 to 10-day-old stock culture insured the presence of a sufficient number of viable algal cells in the exponential growth phase. The initial algal cell count in the stock culture was determined using a hemocytometer (338,000 cells/ml).

Algal Nutrient Medium:

Mineral (inorganic) standard nutrient medium for culturing and testing algae was prepared as outlined in Attachment #1. 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 adjusted to 7.5±.1 prior to use in assays.

Culture Flasks:

Each 250 ml Erlenmeyer containing 50 ml of test solution comprised a test flask. Culture flasks and all other glassware were specially prepared as described in Attachment #2. Autoclaved foam plugs used as flask closures, permitted free gaseous exchange to occur.

Full-Scale Test (Definitive):

All definitive algal assays were carried out in triplicate using 250 ml Erlenmeyer flasks containing 50 ml of test solution. The definitive assay consisted of four simultaneous exposure tests: 4, 7, 10 and 14 days.

The following FM-3422, Lot 716 logarithmic concentrations: 180, 320, 560, 1,000 and 1,800 mg/l were used to initiate the exposure study. Three flasks containing 100% fresh algal nutrient medium plus algal cells comprised the nontreated controls. Procedure and steps for preparation of algal test flasks are outlined in Attachment #3.

Test Conditions:

All algal operations were carried out under aseptic conditions, in order to avoid contamination with bacteria and other algae. Algal cultures were maintained in an environmental chamber under the following standard growth conditions:

Temperature - 23 ± 2°C (70-77°F)

Fluorescent illumination - 400 ft.C. ± 10%

Free gas exchange - Continuous Platform Shaking 100 ± 10 rpm.

Algal Biomass Monitoring:

The algal growth response was appraised quantitatively by using algal cell counts (cells/ml). Procedures for algal biomass measurements are briefly described in Attachment #4.

EC_{50} values and 95% confidence limits were calculated utilizing 3M SIXCUR, a TRAC System for reqression models of experimental data.

TEST RESULTS

The results of biomass measurements of the green algae Selenastrum capricornutum used in these studies are self-explanatory and are detailed in the attached data sheets. Calculated EC_{50} (mg/l) values indicating algal growth response to FM-3422, Lot 716, following exposure periods of 4, 7, 10, and 14 days are presented in Table 1. EC_{50} calculated values were greater than 1,800 mg/l, indicating insignificant toxic effects to algae.

The data reported herein are based on studies developed and performed by M. T. Elnabarawy and R. R. Robideau. Accompanying data sheets comprise original data.

It should be noted that all the reported EC values were calculated on the basis of the initial concentrations of FM-3422, Lot 716, in test solutions at the beginning of the bioassay.

REFERENCES

(1) Miller, W. E., J. C. Greene, and T. Shiroyama. 1978. The Selenastrum Capricornutum Printz Algal Assay Bottle Test: Experimental design, application, and data interpretation. U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-600/9-78-018. 125 p.

(2) ASTM-E-35.23. 1981. Proposed Standard Practice For Conducting Toxicity Tests with Freshwater and Saltwater Algae. Draft No. 2.

(3) OECD Guidelines for Testing of Chemicals (1981) Section 2, Effects on Biotic Systems, Test 201 "Algae, Growth Inhibition Test," Adopted May 12, 1981.

(4) Payne, A. G. and R. H. Hall, 1979. A Method for Measuring Algal Toxicity and Its Application to the Safety Assessment of New Chemicals. ASTM STP #667, p. 171-180.

TABLE 1

Algal Growth Response to
FM-3422, Lot 716
EC_{50}'s (mg/l)(1)
Based on Reducation In Cell Count(2)

Exposure (Contact) Days	
4	>1,800
7	>1,800
10	>1,800
14	>1,800

(1) Method of EC_{50} calculation: 3M SIXCUR, a TRAC system for regression models of experimental data.

(2) Algal cell-count (No./ml) measured in triplicated sets of culture flasks.

ATTACHMENT 1

NUTRIENT MEDIUM FOR FRESHWATER ALGAE

A. MACRONUTRIENTS STOCK SOLUTIONS (CONCENTRATED)
(Prepared separately with deionized water.)

1) 25.500 gm $NaNO_3$ in 1 liter DI water.

2) 1.044 gm K_2HPO_4 in 1 liter DI water.

3) 12.159 gm $MgCl_2 \cdot 6H_2O$ in 1 liter DI water.

4) 14.700 gm $MgSO_4 \cdot 7H_2O$ in 1 liter DI water.

5) 4.410 gm $CaCl_2 \cdot 2H_2O$ in 1 liter DI water.

6) 15.000 gm $NaHCO_3$ in liter DI water.

B. MICRONUTRIENTS STOCK SOLUTION (CONCENTRATED)
(Combined in a single one-liter stock mix.)

1) 185.5 mg H_3BO_3

2) 415.6 mg $MnCl_2 \cdot 4H_2O$

3) 3.27 mg $ZnCl_2$

4) 1.43 mg $CoCl_2 \cdot 6H_2O$

5) 0.01 mg $CuCl_2$

6) 7.26 mg $Na_2MoO_4 \cdot 2H_2O$

7) 96 mg $FeCl_3$

8) 300 mg $Na_2EDTA \cdot 2H_2O$

PREPARATION OF SYNTHETIC ALGAL NUTRIENT MEDIUM

- Add one ml of each macronutrient stock solution plus one ml micronutrients stock mix per 1 liter of deionized water;
- sonicate and then filter through 0.22 µm membrane;
- adjust pH to 7.5 ± 0.1;
- store in the dark at 4°C.

ATTACHMENT 2

PREPARATION OF ALGAL CULTURE FLASKS

All flasks used in maintaining and testing algae were made of borosilicate glass (KIMAX).

- flasks were brushed inside with a stiff bristle brush;
- washed with non-phosphate detergent (MICRO) and rinsed 3 times with tap water;
- rinsed with a 10% HCl solution;
- rinsed 3 times with tap water and 3 times with D.I. water;
- dried in an oven at 70°C for 2 hours (placed inverted);
- and autoclaved with foam plugs inserted at 121°C for 20 minutes.

ATTACHMENT 3

PREPARATION OF ALGAL TEST FLASKS
(IN CHRONOLOGICAL ORDER)

FM3422 LOT 716

ALGAL FLASKS	CONTENTS						
	ALGAL MEDIUM (ml) **	+	TEST MATERIAL (ml) or (mg/50ml)*	+	ALGAL INOCULUM (ml)	=	TOTAL (ml)
CONTROL	48.5	+	——	+	1.5	=	50
1 180 mg/l	48.5	+	9	+	1.5	=	50
2 320 mg/l	48.5	+	16	+	1.5	=	50
3 560 mg/l	48.5	+	28	+	1.5	=	50
4 1000 mg/l	48.5	+	50	+	1.5	=	50
5 1800 mg/l	48.5	+	90	+	1.5	=	50
6		+		+		=	50
7		+		+		=	50

1) Determine initial algal cell count in the stock culture.

2) Adjust pH of algal nutrient medium to 7.5 + 0.1 ml.

3) Make up stock solutions with algal nutrient medium as desired.

A) Control flasks preparations:

1) Add inoculum (volume predetermined); initial cell loading of 1×10^4 cells/ml;

2) Add algal nutrient medium to bring volume to 50ml; swirl flasks, and place autoclaved foam plugs.

B) Test flasks:

1) Add algal nutrient medium;

2) Add test material (dissolved in algal medium if required);

3) Swirl flasks;

4) Add algal inoculum;

5) Swirl flasks, and place autoclaved foam plugs.

* INDIVIDUAL WEIGHTS PLACED DIRECTLY INTO CULTURE FLASKS.
** 0.5ml ETHANOL/1 LITER ALGAL NUTRIENT MEDIUM, USED AS CARRIER SOLVENT.
- HEMACYTOMETER COUNTS 33, 34, 36, 41, 25; 33.8 338,000 CELLS/ml.
- ALGAL INOCULUM VOLUME PER FLASK $\frac{(1 \times 10^4 \text{ cells/ml} \times 50\text{ml})}{338{,}000} = 1.48 = 1.5\text{ml}/50\text{ml}$ FT

ATTACHMENT 3

PREPARATION OF ALGAL TEST FLASKS
(IN CHRONOLOGICAL ORDER)

FM3422, LOT 7116

ALGAL FLASKS	CONTENTS						
	ALGAL MEDIUM (ml)	+	TEST MATERIAL (ml) or (mg/50ml)*	+	ALGAL INOCULUM (ml)	=	TOTAL (ml)
CONTROL	48.5	+	—	+	1.5	=	50
1		+		+		=	50
2		+		+		=	50
3 560 mg/l	48.5	+	28	+	1.5	=	50
4		+		+		=	50
5		+		+		=	50
6		+		+		=	50
7		+		+		=	50

1) Determine initial algal cell count in the stock culture.

2) Adjust pH of algal nutrient medium to 7.5 + 0.1 ml.

3) Make up stock solutions with algal nutrient medium as desired.

A) Control flasks preparations:

1) Add inoculum (volume predetermined); initial cell loading of 1×10^4 cells/ml;

2) Add algal nutrient medium to bring volume to 50ml; swirl flasks, and place autoclaved foam plugs.

B) Test flasks:

1) Add algal nutrient medium;

2) Add test material (dissolved in algal medium if required);

3) Swirl flasks;

4) Add algal inoculum;

5) Swirl flasks, and place autoclaved foam plugs.

* INDIVIDUAL WEIGHTS PLACED DIRECTLY INTO CULTURE FLASKS.

- HEMACYTOMETER COUNTS 33, 34, 36, 41, 25; 33.8 338000 cells/ml
- ALGAL INOCULUM VOLUME PER FLASK $\frac{(1\times10^4 \text{ cells/ml})(50\text{ml})}{338{,}000} = 1.48 = 1.5\text{ml}/50\text{ml FLAS}$

ATTACHMENT 4

ALGAL BIOMASS MONITORING

A. GRAVIMETRIC CELL DRY WEIGHT (1)

The filter recommended is Millipore type BD with an 0.6 micrometer pore size.
The method is as follows:

- Dry filters for two hours at 70°C in an oven;
- Cool filters in a desiccator containing desiccant, for at least two hours before weighing;
- Filter a suitable measured aliquot of the culture under a vacuum or pressure not to exceed 8psi;
- Rinse the filter funnel with D.I. water;
- Dry the filter to constant weight at 70°C, cool in a desiccator for two hours and weigh.

Basic instrument used: Analytical Balance; Mettler ME 30.

B. SPECTROPHOTOMETRIC DETERMINATION OF CHLOROPHYLL a (2)

Basic instrument used: Stectrophotometer; Bausch & Lomb, Spectronic 20.

C. IMPROVED NEUBAUER 0.1 mm DEEP HEMOCYTOMETER COUNTING CHAMBER AND OPTICAL MICROMETER (Used to measure diameter of algal MCV). (3)

(1). Miller, W. E., J. C. Greene, and T. Shiroyama. 1978. Selenastrum capricornutum Printz Algal Assay Bottle Test: Experimental Design, Application, and Data Interpretation Protocol. Ecol. Res. Series EPA-600/9-78-018. Corvallis, Oregon. pp. 27.

(2). APHA-AWWA-WPCF (1975). "Standard Methods for the Examination of Water and Wastewater," (M. Franson, manag. ed.), 14th edition, pp. 1030-1031.

(3). Brite-Line (R), American Optical Corporation, Buffalo, New York.