

TOXICITY TO AQUATIC PLANTS

TEST SUBSTANCE

Identity: A mixture containing perfluorooctanesulfonate; may also be referred to as PFOS, FC-95, or as a component of FC-206C and 81-2/8. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The test sample is FC-206C. Current information indicates it is a mixture of 0.73% PFOS, 76.09% water, 15% diethylene glycol butyl ether, 4% urea, 1.55% sodium octyl sulfate, 1.47% hydroxy foamer, 1.05% polyoxyethylene monooctylphenyl ether, 0.06% sodium lauryl sulfate, and 0.05% benzotriazole.

The following summary applies to a mixture with incompletely characterized concentration of impurities. Data may not accurately reflect the toxicity of the fluorochemical component of the test sample..

METHOD:

Method: USEPA – 600/9-78-018 and ASTM-E-35.21 Draft No. 1.

Type (test type): Acute Static

GLP: No

Year study performed: 1981

Species: *Selenastrum capricornutum*

Source: Originally obtained from USEPA-ERL, Corvallis, Oregon, maintained in culture medium at the 3M Environmental Laboratory, St. Paul, MN.

Element basis: Algal cell counts (cells/ml), cell dry weights.

Exposure period: 96 hours

Statistical Methods: Regression model.

Analytical monitoring: Not given.

Test Conditions:

Algal Nutrient Medium: Nutrient medium per U.S. EPA 600/9-78-018. 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 prior to use in assays.

Stock and test solution preparation: Stock solutions were prepared in the algal standard test medium, however no further description was provided.

Exposure vessels: 250 ml Erlenmeyers containing 50 ml test solution.

Agitation: Shaken continuously at 100 rpm.

Number of replicates: 3

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

Number of concentrations: five plus a blank control

Lighting: 400 foot-candles from continuous cool-white fluorescent lighting.

Water Chemistry:

pH range (0-96 hours): Not noted.

Test temperature range (0-96 hours): $23 \pm 2^\circ\text{C}$ (incubator)

RESULTS

Nominal concentrations: Bk control, 280, 420, 650, 1,000, and 1,550 mg/L

Element values:

96-hour EC₅₀ Cell-Dry weight: 345 (34 – 1,630) mg/L

96-hour EC₅₀ Algal Cell Count: 379 (106 – 1,137) mg/L

Element values are based on nominal concentrations.

Reversibility of Growth Inhibition: Aliquots of the 1,000 and 1,550 mg/L test solutions were diluted with algal medium and cultured for 10 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 EC₅₀ cell count value of 379 (106 – 1,137) mg/L and a 96-hour EC₅₀ based on cell dry weight of 345 (34 – 1,630) mg/L.

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, the study lacks information on purity of the test substance and it lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

Test was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, Lab Request number 6812S, 1981.

OTHER

Last changed: 6/26/00

TECHNICAL REPORT SUMMARY

Date
6/23/81

TO: TECHNICAL COMMUNICATIONS CENTER - 201-2CN

(Important - If report is printed on both sides of paper, send two copies to TCC.)

Division Environmental Engineering and Pollution Control (Envir. Lab)		Dept. Number 0535
Project DS-8-8P Comm. Chem. mte		Project Number 9970012600
Report Title Algal Assay: Bottle Test (AA:BT), Toxicity Testing FC-206C		Report Number 051
To E. A. Reiner - 21-2W		LR 6812 S
Author(s) M. T. Elnabarawy - 2-3E		Employee Number(s) 46981
Notebook Reference		No. of Pages Including Coversheet 5
SECURITY ☒ Open ☐ Closed		3M CHEMICAL REGISTRY ☐ Yes ☒ No

KEYWORDS:
(Select terms from 3M
Thesaurus. Suggest other
applicable terms.)

EE & PC Div.
(Env. Lab)

Algal
Assay
Bottle
Test
Toxicity

CURRENT OBJECTIVE:

To determine the algal growth responses (EC_{50} 's) following a 4-day exposure period to varying concentrations of FC-206C, followed by a 10-day recovery period.

REPORT ABSTRACT: (200-250 words) This abstract information is distributed by the Technical Communications Center to alert 3M'ers to Company R&D.

The algal acute 4-day EC_{50} values obtained for freshwater green algae (Selenastrum Capricornutum) following exposure to FC-206C in triplicate experiments were 345 and 379 mg/l. These values represent the algal growth response as measured by cell-dry weight and algal cell-count, respectively.

No test material concentration was defined as the no-effect level.

The algal biomass monitoring measurements following a 10-day recovery period indicated insignificant algicidal effect at concentrations tested. Since the algal cells have recovered and resumed logarithmic growth when resuspended in fresh medium, the 4-day median growth response (EC_{50}) is interpreted as an inhibitory effect.

CONCLUSIONS

In these triplicated 4-day EC₅₀ algal assays, the calculated values for freshwater green algae (Selenastrum Capricornutum) were 345 and 379 mg/l. These values represent the algal growth response as measured by cell-dry weight and algal cell count, respectively.

No test material concentration was defined as the no-effect level.

The algal biomass monitoring measurements following a 10-day recovery period indicated little or no algicidal effects at concentrations tested. Since the algal cells have recovered and resumed logarithmic growth when resuspended in fresh medium, the 4-day median growth response (EC₅₀) is interpreted as an inhibitory effect.

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 aquatic primary producers, plants, and phytoplankton.

Toxic effects are tested on growing algal cultures that undergo cell-division during the test. Algal growth response include: a) no effect, b) stimulatory, c) inhibitory, d) algistatic, or algicidal.

The test substance is a clear amber liquid, soluble in water at ambient room temperature. These bioassays performed on this material evaluated its potential algal toxicity and the data generated form the basis of this report.

MATERIAL AND EXPERIMENTAL DESIGN

These algal assays consisted of a 4-day exposure period followed by a 10-day recovery period. The test protocol utilized for this study was modified after those described by USEPA - 600/9-78-018 and ASTM-E-35.21 Draft No. 1. (1), (2).

Test Species:

A culture of the freshwater green algae (Chlorophyceae), Selenastrum Capricornutum of the order chlorococcales was obtained from USEPA - ERL, Corv., Oregon (February 13, 1981). The test algae are non-motile unicellular cells and having the appearance of a new moon. The algal culture was stored in the dark at 40°C until use.

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 7-10 day old stock culture will insure a sufficient amount of viable algal cells in the exponential growth phase. The initial algal cell count in the stock culture was determined with the aid of a hemocytometer (370,000 cells/ml).

Algal Culture Medium:

Standard culture and test medium was prepared as outlined in Attachment #1. This algal culture medium provided all nutrients essential for algal growth and also served as the diluent for all algal operations including the preparation of stock solutions. The pH of this synthetic algal medium was adjusted to 7.5 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 were used as flask closures, allowing for free gas exchange.

Range-Finding Test (Exploratory):

The exploratory test consisted of determining the 4-day algal biomass in single flasks containing standard culture medium plus the test material at concentrations covering several orders of magnitude: 0.1, 1.0, 10, 100 and 1,000 mg/l.

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 test consisted of two culture stages: a 4-day exposure period followed by a 10-day recovery period. The purpose of the exposure (contact) period was to verify inhibitory effects. The recovery (subculture) stage provided information on algistatic/algicidal effects.

The following FC-206C logarithmic concentration: 280, 420, 650, 1,000 and 1,550 mg/l were used to initiate the 4-day contact period. This dose range of dilutions was selected to bracket 4-day EC_{50} values predicted from preliminary exploratory testing. Three flasks containing 100% fresh algal culture medium plus algal cells comprised the nontreated controls. The recovery stage was initiated by

subculturing (0.5 ml/50 ml) from each of the three replicates of the two highest test concentrations into flasks containing fresh algal medium. These two treatments induced inhibitory effects greater than 80% during the contact period.

Fresh stock solutions of the test substance were prepared in the algal standard test medium. See Attachment #3.

Test Conditions:

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

*Temperature - $23 \pm 2^{\circ}\text{C}$ ($70-77^{\circ}\text{F}$)

*Fluorescent illumination - 400 ft.C. $\pm 10\%$

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

Algal Biomass Monitoring:

The algal growth response was measured by algal cell-dry weight (mg/l) and by cell count (cells/ml). Both growth measurements were made with algal cultures after the 4-day exposure period and only cell-count was made with the subcultures after a 10-day recovery period. Both procedures of biomass measurements are briefly described in Attachment #4.

EC₅₀ values and 95% confidence limits were calculated utilizing 3M SIXCUR, a TRAC System for regression 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 data sheets attached. The results of the definitive test are summarized in Table 1.

Table 1

Definitive Test - EC₅₀ values, mg/l with Selenastrum
Capricornutum

Type Test	Algal Biomass Monitoring	
	Cell-Dry Weight mg/l	Cell Count No./1 ml
4-Day Exposure Period	345(34-1630)*	379(106-1137)*

*95% Confidence limits.

The data reported herein are based on studies performed by M. T. Elnabarawy. Accompanying data sheets comprise original data. A copy of this report is filed in the Environmental Laboratory Archive System, Building 2-3E.

It should be noted that the reported EC₅₀ values were evaluated on the basis of the calculated initial concentrations of FC-206C in test solutions.

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-35.21. 1980. Proposed Standard Practice For Conducting Toxicity Tests with Freshwater and Saltwater Algae. Draft No. 1.

ENVIRONMENTAL LABORATORY
ALGAL ASSAY: BOTTLE TEST (AA:BT)
TOXICITY TEST DATA SHEET

LR 68125

Type Test 4 Day Exposure

Material Tested FC-206 C Lot _____ Physical Description Clear Amber Liquid

Algal Species: Green Algae, Selenastrum capricornutum

US EPA Lab

Source Corvallis, Oregon Date Rec'd 2-18-81 Age of Inoculum 7 days

Test Container: 250 ml Erlenmeyer flasks with foam plugs

Sample-to-Volume Ratio: 10 to 250 Initial Cell Loading: 10000 cells/ml

Initial pH of Synthetic Algal Medium: 7.5 ± 0.1

Test Conditions: Temperature - 23 ± 2°C (70-77°F)
Fluorescent Illumination - 400 ft.c. ± 10 %
Free Gas Exchange - Continuous platform-shaking at 100 ± 10 rpm.

Date Started 6-1-81 Date Completed 6-15-81 Investigator M.T. Elhabraw

ALGAL CELL DRY WEIGHT, MEAN VALUE in mg/l (1)
TYPE TEST

TREATMENT	EXPOSURE: 4-DAY CONTACT PERIOD	RECOVERY: 10-DAY SUBCULTURE PERIOD
CONTROL	6.5	
280	5.0	
420	1.3	
650	1.0	
1000	.78	
1550	.56	
EC50 (2)		
ALGAL GROWTH EFFECTS	NO EFFECTS/STIMULATORY/INHIBITORY	ALGISTATIC/ALGICIDAL

ALGAL CELL COUNT, MEAN VALUE NO./ml (1)
TYPE TEST

TREATMENT	EXPOSURE: 4-DAY CONTACT PERIOD	RECOVERY: 10-DAY SUBCULTURE PERIOD
CONTROL	400,000	240,000
280	320,000	
420	120,000	
650	90,000	
1,000	60,000	150,000
1,550	40,000	140,000
EC50 (2)		
ALGAL GROWTH EFFECTS	NO EFFECTS/STIMULATORY/INHIBITORY	ALGISTATIC/ALGICIDAL

(1) Based on mean values of each triplicate sets of flasks.

(2) Method of calculations:

ENVIRONMENTAL LABORATORY
ALGAL ASSAY: BOTTLE TEST (AA:BT)
RECOVERY STAGE DATA SUMMARY
ALGAL CELL COUNT, cells/ml (MEAN VALUES)

LR 68125

FC 206 C

TREATMENT	INITIAL ALGAL CELL LOADING (1)	FINAL ALGAL CELL COUNT (2)
CONTROL	4,000	240,000
1 1000	600	150,000
2 1550	400	140,000
3		
4		
5		
REF. TOX.		

(1). Based on 0.5ml/50ml subculture made from 4-day exposure cultures.

(2). 10-day recovery cultures.