

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-206CE. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was 502. The test sample is FC-206CE (2/16/89). It's purity was not sufficiently characterized, though current information indicates it is a mixture of 0.92% PFOS, 15% diethylene glycol butyl ether, 76.09% water, 4% urea, 1.28% Sultone foamer, 1.55% sodium octyl sulfate, 1.05% polyoxyethylene monooctylphenyl ether, 0.06% sodium lauryl sulfate, and 0.05% tolytriazole.

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

METHOD:

Method: OECD 201 and ISO/DIS 8692

Type: Acute Static

GLP: No

Year study performed: 1989

Species: *Selenastrum capricornutum*

Source: Originally obtained from ATCC, Rockville, MD, maintained in culture medium at 3M Environmental Laboratory, St. Paul, MN.

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

Exposure period: 96 hours

Statistical Methods: Not given.

Analytical monitoring: pH and temperature.

Test Conditions:

Algal Nutrient Medium: Nutrient medium per OECD 201 and ISO/DIS 8692. 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 7.6 adjusted to 8.3. One hundred mL of this pH-adjusted nutrient concentrate solution was added to 900 mL of Millipore Milli-Q™ water. Final pH 8.3.

Stock and test solution preparation: A 1000 mg/L stock solution was prepared by diluting 1000 mg of test substance in 900 mL of Millipore Milli-Q™ water then adding 100 mL of the pH-adjusted nutrient concentrate solution. Final pH of this stock solution was 7.9. Aliquots were then added directly to dilution water in test vessels to create test solutions.

Exposure vessels: 250 mL Erlenmeyer flasks containing 100 mL test solution and capped with stainless steel closures.

Agitation: Shaken continuously at ~100 rpm.

Number of replicates: 3 for blank controls and 2 for test material treatments

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):

8.3 – 9.4 (control exposure)

6.8 – 7.9 (1000 mg/L exposure)

Test temperature (0-96 hours):

24 °C (incubator)

RESULTS

Nominal concentrations: Bk control, 100, 180, 320, 560, and 1000 mg/L

Algal Growth Response at 96 Hours

Nominal Conc., mg/L	Avg. Growth Rate	% Change in Growth Rate
Bk. Control	1.3853	—
100	1.3461	-2.8
180	1.2933	-6.6
320	1.0908	-21
560	1.0378	-25
1000	0.8193	-41

Element values are based on nominal concentrations.

Reversibility of Growth Inhibition: Not given

Remarks: Testing was conducted on a mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical proportion alone.

CONCLUSIONS

FC-206CE exhibits a 96-hour percent change in growth rate compared to the control of -41% at the 1000 mg/L treatment.

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 actual measurements 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 G1358-2, 1989.

OTHER

Last changed: 6/26/00

ENVIRONMENTAL LABORATORY
STANDARD NUTRIENT MEDIUM FOR FRESHWATER ALGAE

91358
5/3/89
2092/1057

NUTRIENT	CONCENTRATION IN STOCK SOLUTION	FINAL CONCENTRATION IN TEST SOLUTION
<u>STOCK SOLUTION 1: MACRONUTRIENTS</u>		
NH_4Cl	1.5 g/L	15 mg/L
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	1.2 g/L	12 mg/L
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1.8 g/L	18 mg/L
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.5 g/L	15 mg/L
KH_2PO_4	0.16 g/L	1.6 mg/L

STOCK SOLUTION 2: Fe-EDTA

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	80 mg/L	80 ug/L
$\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$	100 mg/L	100 ug/L

STOCK SOLUTION 3: TRACE ELEMENTS

H_3BO_3	185 mg/L	185 ug/L
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	415 mg/L	415 ug/L
ZnCl_2	3 mg/L	3 ug/L
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	1.5 mg/L	1.5 ug/L
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	0.01 mg/L	0.01 ug/L
$\text{Na}_2\text{MgO}_4 \cdot 2\text{H}_2\text{O}$	7 mg/L	7 ug/L

STOCK SOLUTION 4: NaHCO_3

NaHCO_3	50 g/L	50 mg/L
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Sterilize stock solutions 1-4 by membrane filtration
(mean pore diameter 0.2 μm).

SOLUTION 5: NUTRIENT CONCENTRATE

(Prepare freshly before each test.)

Add 100 mL of STOCK SOLUTION 1;
10 mL of STOCK SOLUTION 2;
10 mL of STOCK SOLUTION 3;
and 10 mL of STOCK SOLUTION 4.

Make up to 1,000 mL with Millipore Milli-Q water.

Adjust the pH to 8.3 ± 0.2 . pH 7.7 $\rightarrow$ adj to 8.3

SOLUTION 6: STANDARD ALGAL NUTRIENT MEDIUM Diluent & Controls

Add 100 mL of the pH-adjusted Nutrient Concentrate Solution
to 900 mL of Milli-Q water.

REFERENCES:

- (1) OECD 1984. Algal Growth Inhibition Test, 201. In: Guidelines for Testing of Chemicals. Organization for Economic Cooperation and Development, Paris, France.
- (2) ISO 1987. Water Quality - Algal Growth Inhibition Test, ISO/DIS 8692. International Organization for Standardization, Geneva, Switzerland.

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14558-2
5/3/89
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ENVIRONMENTAL LABORATORY
STANDARD NUTRIENT MEDIUM FOR FRESHWATER ALGAE

NUTRIENT	CONCENTRATION IN STOCK SOLUTION	FINAL CONCENTRATION IN TEST SOLUTION
<u>STOCK SOLUTION 1: MACRONUTRIENTS</u>		
NH ₄ Cl	1.5 g/L	15 mg/L
MgCl ₂ ·6H ₂ O	1.2 g/L	12 mg/L
CaCl ₂ ·2H ₂ O	1.8 g/L	18 mg/L
MgSO ₄ ·7H ₂ O	1.5 g/L	15 mg/L
KH ₂ PO ₄	0.16 g/L	1.6 mg/L

STOCK SOLUTION 2: Fe-EDTA

FeCl ₃ ·6H ₂ O	80 mg/L	80 ug/L
Na ₂ EDTA·2H ₂ O	100 mg/L	100 ug/L

STOCK SOLUTION 3: TRACE ELEMENTS

H ₃ BO ₃	185 mg/L	185 ug/L
MnCl ₂ ·4H ₂ O	415 mg/L	415 ug/L
ZnCl ₂	3 mg/L	3 ug/L
CoCl ₂ ·6H ₂ O	1.5 mg/L	1.5 ug/L
CuCl ₂ ·2H ₂ O	0.01 mg/L	0.01 ug/L
Na ₂ MoO ₄ ·2H ₂ O	7 mg/L	7 ug/L

STOCK SOLUTION 4: NaHCO₃

NaHCO ₃	50 g/L	50 mg/L
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Sterilize stock solutions 1-4 by membrane filtration
(mean pore diameter 0.2 um).

SOLUTION 5: NUTRIENT CONCENTRATE
(Prepare freshly before each test.)

Add 100 mL of STOCK SOLUTION 1;
10 mL of STOCK SOLUTION 2;
10 mL of STOCK SOLUTION 3;
and 10 mL of STOCK SOLUTION 4.

Make up to 1,000 mL with Milli-Q water.
Adjust the pH to 8.3 ± 0.2.

SOLUTION 6: STANDARD ALGAL NUTRIENT MEDIUM FC206CE Stock 1000 ug/L

Add 100 mL of the pH-adjusted Nutrient Concentrate Solution
to 900 mL of Milli-Q (containing 1000 ug FC206CE) water.

REFERENCES:

- (1) OECD 1984. Algal Growth Inhibition Test, 201. In: Guidelines for Testing of Chemicals. Organization for Economic Cooperation and Development, Paris, France.
- (2) ISO 1987. Water Quality - Algal Growth Inhibition Test. ISO/DIS 18692. International Organization for Standardization, Geneva, Switzerland.

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ENVIRONMENTAL LABORATORY

STANDARD NUTRIENT MEDIUM FOR FRESHWATER ALGAE

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NUTRIENT	CONCENTRATION IN STOCK SOLUTION	FINAL CONCENTRATION IN TEST SOLUTION
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STOCK SOLUTION 1: MACRONUTRIENTS

NH_4Cl	1.5 g/L	15 mg/L
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	1.2 g/L	12 mg/L
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1.8 g/L	18 mg/L
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.5 g/L	15 mg/L
KH_2PO_4	0.16 g/L	1.6 mg/L

STOCK SOLUTION 2: Fe-EDTA

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	80 mg/L	80 ug/L
$\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$	100 mg/L	100 ug/L

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H_3BO_3	185 mg/L	185 ug/L
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	415 mg/L	415 ug/L
ZnCl_2	3 mg/L	3 ug/L
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	1.5 mg/L	1.5 ug/L
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	0.01 mg/L	0.01 ug/L
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	7 mg/L	7 ug/L

STOCK SOLUTION 4: NaHCO_3

NaHCO_3	50 g/L	50 mg/L
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Sterilize stock solutions 1-4 by membrane filtration (mean pore diameter 0.2 um).

SOLUTION 5: NUTRIENT CONCENTRATE
(Prepare freshly before each test.)

Add 100 mL of STOCK SOLUTION 1;
10 mL of STOCK SOLUTION 2;
10 mL of STOCK SOLUTION 3;
and 10 mL of STOCK SOLUTION 4.

Make up to 1,000 mL with Milli-Q water.
Adjust the pH to 8.3 ± 0.2 .

SOLUTION 6: STANDARD ALGAL NUTRIENT MEDIUM = 2000x Stock Soln (1000 mg/L)

Add 100 mL of the pH-adjusted Nutrient Concentrate Solution to 900 mL of Milli-Q (containing 100 mg of FeCl₃) water.
pH 8.1

REFERENCES:

- (1) OECD, 1984. Algal Growth Inhibition Test, 201. In: Guidelines for Testing of Chemicals. Organization for Economic Cooperation and Development, Paris, France.
- (2) ISO, 1987. Water Quality - Algal Growth Inhibition Test, ISO/DIS 8692. International Organization for Standardization, Geneva, Switzerland.

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ENVIRONMENTAL LABORATORY
FRESHWATER ALGAE GROWTH INHIBITION TEST
ALGAL CELL COUNT, No./mL
HEMOCYTOMETER METHOD*

LR 913582
Page 40 of 4

TEST DAY 4 (9/6/88) TEST TYPE AA:BT DATE 5/4/89
MATERIAL TESTED: FC206 CE (2/16/87) LOT 502
ALGAL SPECIES: Green Algae, Selenastrum capricornutum
ANALYST: Rachelle F. Hildeau

TREATMENT mg/L	TOTAL OF 25 FIELDS COUNTED	No./mL	MEAN $\pm$ SD	pH
BK Control				
I	270	2,400,000		
II	268	2,680,000		
FC206 CE				
1-1	2826	2,820,000		
2-1	219	2,190,000		
10-1-2	228	2,280,000		
2-2	224	2,240,000		
100-1-3	175	1,750,000		
2-3	170	1,700,000		
1000-1-4	16	160,000		
2-4	16	160,000		

Test Temperature 24°C

*0.1 mm deep Hemocytometer; area of field 1 x 1 and 0.1 mm deep.

Test methodology conforms with the following two test protocols:

- (1) OECD #201 "Algal Growth Inhibition Test"
- (2) US-EPA #EC-8 "Algal Acute Toxicity Test"

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ENVIRONMENTAL LABORATORY
FRESHWATER ALGAE GROWTH INHIBITION TEST
ALGAL CELL COUNT, No./mL
HEMOCYTOMETER METHOD^a

LR 41358-2
App 5047

TEST DAY 4 (9/10/89) TEST TYPE AA:ST DATE 5/28/89
MATERIAL TESTED: FC206CE (2/11/89) LOT 502
ALGAL SPECIES: Green Algae, Selenastrum capricornutum
ANALYST: Kyckelle Fabian

TREATMENT	TOTAL OF 25 FIELDS COUNTED	No./mL	MEAN $\pm$ SD	pH
Blank Control I	254	2,540,000		9.3
II	256	2,560,000		9.4
III	255	2,550,000		9.3
FC206CE				
100mg/L 1-1	222	2,220,000		
2-1	214	2,140,000		8.5
180mg/L 1-2	179	1,790,000		
2-2	174	1,740,000		8.0
320mg/L 1-3	79	790,000		
2-3	78	780,000		7.1
560mg/L 1-4	64	640,000		
2-4	63	630,000		7.1
1000mg/L 1-5	27	270,000		
2-5	26	260,000		7.0

Test Temperature 24°C

^a 0.1 mm deep Hemocytometer; area of field 1 x 1 and 0.1 mm deep.

Test methodology conforms with the following two test protocols:

- (1) OECD #201 "Algal Growth Inhibition Test"
- (2) US-EPA #EC-8 "Algal Acute Toxicity Test"

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Lab Request No. 41358-2

Environmental Lab - Work Sheet

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Sample Description

ALGAL ASSAY: BOTTLE TEST (AA:BT)
COMPARISON OF GROWTH RATESDate: 5/4/89MATERIAL TESTED: FC2016 CE (2/16/89) LOT 502Analyst: 73ALGAL SPECIES: Freshwater Green Algae, Selenastrum capricornutum

TREATMENT	CELL COUNT 4-DAY	GROWTH RATE ⁽¹⁾ 4-DAY	% CHANGE ⁽²⁾ 4-DAY
Blank Control	2.70×10^6	1.39962	
II	2.168×10^6	1.3947	1.3987
1mg/l, 1-1	2.82×10^6	1.41052	1.4092 + 0.75
2-1	2.79×10^6	1.4078	
10mg/l, 1-2	2.28×10^6	1.35732	1.3551 - 3.1
2-2	2.24×10^6	1.3529	
100mg/l, 1-3	1.75×10^6	1.29122	1.2876 - 7.9
2-3	1.70×10^6	1.2839	
1000mg/l, 1-4	1.16×10^5	0.169312	0.16931 - 50
2-4	1.16×10^5	0.16931	

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(1) Specific Growth Rate (u) = $\frac{\ln N_t - \ln N_0}{t}$ (2) Percent Change in Growth Rate = $\frac{(\text{Control} - \text{Sample})}{\text{Control}} \times 100$

Environmental Lab - Work Sheet

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Sample Description

ALGAL ASSAY: BOTTLE TEST (AA:BT)
COMPARISON OF GROWTH RATESDate: 5/28/89Analyst: 83MATERIAL TESTED: MC2016 CE (2/16/89) LOT 502ALGAL SPECIES: Freshwater Green Algae, Selenastrum capricornutum

TREATMENT	CELL COUNT 4-DAY	GROWTH RATE ⁽¹⁾ 4-DAY	% CHANGE ⁽²⁾ 4-DAY
Bk Control I	2.54×10^6	1.3843	1.3853
II	2.56×10^6	1.38103	
III	2.55×10^6	1.3853	
100mg/L 1-1	2.22×10^6	1.3507	1.3461 -2.8
2-1	2.14×10^6	1.3415	
180mg/L 1-2	1.79×10^6	1.2918	1.2933 -6.6
2-2	1.74×10^6	1.2898	
320mg/L 1-3	7.9×10^5	1.0924	1.0908 -2.1
2-3	7.8×10^5	1.0892	
560mg/L 1-4	6.4×10^5	1.0397	1.0378 -2.5
2-4	6.3×10^5	1.0358	
1000mg/L 1-5	2.7×10^5	0.8240	0.8193 -4.1
2-5	2.6×10^5	0.8145	

$$(1) \text{ Specific Growth Rate } (u) = \frac{\ln N_1 - \ln N_0}{t}$$

$$(2) \text{ Percent Change in Growth Rate } = \frac{(\text{Control} - \text{Sample})}{\text{Control}} \times 100$$

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