

## ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

**Identity:** A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-203CE. (1-Octanesulfonic acid) (CAS # 2795-39-3).

**Remarks:** The 3M production lot number was 519. The test sample is FC-203CE. Current information indicates it is a mixture of 1.84% PFOS, 30% diethylene glycol butyl ether, 60.23% water, 2.56% Sultone foamer, 3.1% sodium octyl sulfate, 2.1% polyoxyethylene monoctylphenyl ether, 0.12% sodium lauryl sulfate, and 0.05% tolyltriazole.

***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 209

**GLP:** No

**Year Completed:** 1989

**Analytical monitoring:** Dissolved oxygen concentrations, pH, and temp.

**Statistical methods:** Results were determined by calculation of the % inhibition in respiration rate.

**Test organism source:** Activated sludge mixed liquor collected from the Metro Waste Treatment Plant, St. Paul, MN

**Test conditions:**

**Dilution water:** Millipore Milli-Q™ water

**Synthetic sewage:** per OECD method

**Reference and test solution preparation:** A stock solution of the reference substance, 3,5-dichlorophenol, was prepared by dissolving 500 mg in 10 mL 1N NaOH, diluted to 30 mL with Millipore Milli-Q™ water, then brought to the point of incipient precipitation with 1N H<sub>2</sub>SO<sub>4</sub> and diluted to 1L with Milli-Q™ water. The pH of the reference solution was measured to be 7.8. Test substance was added directly to test vessels.

**Test vessels:** Not given.

**Number of concentrations:** 5 plus 1 reference substance and 2 blank controls

**Temperature:** 19 – 21 °C

**Total suspended solids and pH on day of testing:** 4.1 g/L (TSS), initial pH 7.2 and final pH 7.5

**Element Basis:** Respiration inhibition as determined by oxygen consumption.

**Remarks:** Values corrected to 20 °C for calculations.

## RESULTS

**Nominal concentrations:** 2 Blank controls, reference substance, 100, 180, 320, 560, and 1000 mg/L

**Element values:** 30-minute  $EC_{50} = >1000$  mg/L  
3-hour  $EC_{50} = >1000$  mg/L

Element values based on nominal concentrations.

**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

The test substance 3-hour  $EC_{50}$  for activated sludge respiration inhibition was determined to be  $>1000$  mg/L.

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

## DATA QUALITY

**Reliability:** Klimisch ranking 2. Testing meets all 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 study was conducted by the 3M Company, Environmental Laboratory, St Paul, MN, Lab Request number G1358-1, 1989.

## OTHER

**Last changed:** 6/26/00

001108

LR G1358-1  
Date 6-9-89  
Analyst SAB

ENVIRONMENTAL LABORATORY  
ACTIVATED SLUDGE MIXED LIQUOR

Type Test O&CD Activated Sludge Respiration Inhibition #209

Source Metro Waste Treatment Plant St Paul, MN

Date Obtained 6-9-89 Date Used 6-9-89

Mixed Liquor Suspended Solids (MLSS) 4.1 g/L Adjustment None g/L

pH Initial = 7.2 Final = 7.5

Comments \_\_\_\_\_

Test Substance

Sample Description FC-203CE Lot 519

Sample pH Highest Conc. 1000mg/L = 7.2 Adjustment None

Comments \_\_\_\_\_

Reference Substance

Sample Description 3,5-Dichlorophenol

Sample pH Stock Soln. = 7.8 Adjustment None

Comments \_\_\_\_\_

Exposure Conditions

Test Duration 3-Hours

Test Temperature 19-21 °C

Comments Ran with LR G1357-2

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Request No. G1358-1

## Environmental Lab — Work Sheet

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Sample Description

FC-203CE Lot 519

OECD ACTIVATED SLUDGE  
RESPIRATION INHIBITION TEST #209Date: 6-9-89Analyst: SAB

## Test Solutions Preparation

Test Conc. mg/L	mg added per 500 ml	ml DI water per 500 ml	ml syn. sewage feed per 500 ml	ml microbial inoculum per 500 ml	Total volume ml
Blank Controls	-	284 +	16	+ 200	= 500
100	50 +	284 +	16	+ 200	= 500
180	90 +	284 +	16	+ 200	= 500
320	160 +	284 +	16	+ 200	= 500
560	280 +	284 +	16	+ 200	= 500
1000	500 +	284 +	16	+ 200	= 500

Initial pH highest conc. tested, 1000 mg/L; = 7.2

Control I shared with LR G1357-2 as Control II

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-203CE Lot 519  
 Source of Activated Sludge Mixed Liquor: Metro Waste Treatment Plant St Paul, MN  
 Date Received: 6-9-89 Date Used: 6-9-89  
 Analyst: Susan A. Boach

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30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	DISSOLVED OXYGEN CONCENTRATIONS mg/L											TEMP C°
	TIME-MINUTES											
	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
CONTROL I	7.8	7.6	7.0	6.5	6.0	5.5	5.0	4.5	4.0	3.5	3.0	20
100	6.5	6.2	5.5	5.0	4.4	3.9	3.3	2.8	2.3	1.8	1.3	20
180	7.3	7.0	6.3	5.7	5.0	4.4	3.7	3.1	2.5	1.9	1.3	20
320	6.3	5.9	5.1	4.4	3.7	2.9	2.2	1.5	0.8	<del>0.5</del> 3.25		20
560	6.2	5.8	4.9	4.0	3.0	2.1	1.2	<del>0.5</del> 1.45				20
1000	6.8	6.4	5.6	4.8	4.0	3.1	2.3	1.5	0.7	<del>0.5</del> 8.20		19
CONTROL II	7.2	7.0	6.5	5.9	5.4	4.9	4.3	3.8	3.3	2.7	2.2	21

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	DISSOLVED OXYGEN CONCENTRATIONS mg/L											TEMP C°
	TIME-MINUTES											
	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
CONTROL I	7.8	7.6	7.0	6.5	6.0	5.4	4.9	4.3	3.9	3.3	2.8	21
100	7.1	6.8	6.3	5.8	5.2	4.7	4.2	3.7	3.2	2.7	2.2	20
180	8.9	8.8	8.5	8.1	7.7	7.3	6.9	6.4	6.1	5.7	5.3	19
320	8.6	8.5	8.2	7.8	7.4	7.1	6.7	6.3	5.9	5.5	5.1	19
560	8.4	8.3	7.9	7.6	7.2	6.8	6.4	6.0	5.6	5.2	4.7	20
1000	7.6	7.4	6.8	6.3	5.8	5.2	4.7	4.2	3.6	3.1	2.6	21
CONTROL II	7.3	7.1	6.6	6.0	5.4	4.9	4.3	3.8	3.2	2.7	2.1	21

pH of Activated Sludge Mixed Liquor: Initial 7.2 Final 7.5  
 pH of highest concentration tested: Initial 7.5 Final 7.0  
 Test Temperature: 19-21°C

Test Substance FC-203CF Lot 519

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30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected <sup>(1)</sup> to 20°C	% Inhibition in Respiration Rate <sup>(2)</sup>
CONTROL I	0.5055	0.5055	-
100	0.5388	0.5388	-
180	0.6333	0.6333	-
320	0.7249	0.7249	-
560	0.9248	0.9248	-
1000	0.8137	0.8519	-
CONTROL II	0.5345	0.5105	-

Percent Difference in Controls<sup>(3)</sup> 1.0%  
EC<sub>50</sub> (95% Confidence Limits) mg/L > 1000 mg/L

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected <sup>(1)</sup> to 20°C	% Inhibition in Respiration Rate <sup>(2)</sup>
CONTROL I	0.5315	0.5076	-
100	0.5127	0.5127	1.3
180	0.3964	0.4150	20
320	0.3800	0.3979	23
560	0.3958	0.3958	24
1000	0.5327	0.5088	2.0
CONTROL II	0.5558	0.5309	-

Percent Difference in Controls<sup>(3)</sup> 4.5%  
EC<sub>50</sub> (95% Confidence Limits) mg/L > 1000 mg/L

(1) Temp. Correction =  $\frac{R_{T,obs}}{\theta^x}$  (mg O<sub>2</sub>/L-min.)

(3) % Difference =  $\frac{|RC_1 - RC_2|}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

(2) % Inhibition =  $\frac{\left(\frac{RC_1 + RC_2}{2}\right) - R_s}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

The two control respiration rates should be within 15% of each other

See pg 1 for test Soln. prep.

Where:  
R = Oxygen uptake rate (mg O<sub>2</sub>/L-min.)  
T<sub>obs</sub> = Sample temp., C°  
θ = 1.047, x = T<sub>obs</sub> - 20°C  
C = Control

Environmental Lab — Work Sheet

Sample Description  
2,5-Dichlorophenol  
Reference Substance  
Aldrich Red Label  
Lot D7-060-0

OECD ACTIVATED SLUDGE  
RESPIRATION INHIBITION TEST #209

Date: 6-9-89

Analyst: SAB

Stock and Test Solutions Preparation

STOCK SOLUTION: 500 mg 3,5-DCP / 1.0 L      Each 1.0 ml = 0.5 mg

- Dissolved 500 mg 3,5-DCP in 10 ml 1.0N NaOH
- Diluted to 30 ml with Milli-Q water
- Added, under stirring, 1.0N H<sub>2</sub>SO<sub>4</sub> dropwise until incipient ppt.
- Diluted to 1.0 Liter with Milli-Q water

Stock Solution pH = 7.8

Test Conc. mg/L	ml stock soln per 500 ml	ml DI water per 500 ml	ml syn. sewage feed per 500 ml	ml microbial inoculum per 500 ml	Total volume ml
Blank controls	-	284	+ 16	+ 200	= 500
10	10	+ 274	+ 16	+ 200	= 500

Controls Shared with LR 61358-1

ENVIRONMENTAL LABORATORY  
OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209  
RAW DATA

Test Substance: 3,5-Dichlorophenol Reference Substance  
 Source of Activated Sludge Mixed Liquor: Metro Waste Treatment Plant St Paul, MN  
 Date Received: 6-9-89 Date Used: 6-9-89  
 Analyst: Aaron A. Beach

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30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	DISSOLVED OXYGEN CONCENTRATIONS mg/L											TEMP C°
	TIME-MINUTES											
	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
CONTROL I	7.8	7.6	7.0	6.5	6.0	5.5	5.0	4.5	4.0	3.5	3.0	20
10	8.0	7.9	7.6	7.3	7.0	6.7	6.4	6.0	5.7	5.4	5.1	21
CONTROL II	7.2	7.0	6.5	5.9	5.4	4.9	4.3	3.8	3.3	2.7	2.2	21

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	DISSOLVED OXYGEN CONCENTRATIONS mg/L											TEMP C°
	TIME-MINUTES											
	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
CONTROL I	7.8 7.5	7.6 7.5	7.0	6.5	6.0	5.4	4.9	4.3	3.9	3.3	2.8	21
10	9.3	9.3	9.1	9.0	8.8	8.6	8.5	8.3	8.1	8.0	7.8	18
CONTROL II	7.3	7.1	6.6	6.0	5.4	4.9	4.3	3.8	3.2	2.7	2.1	21

pH of Activated Sludge Mixed Liquor: Initial 7.2 Final 7.5  
 pH of highest concentration tested: Initial 7.5 Final 7.9  
 Test Temperature: 18-21°C

Test Substance 3,5-Dichlorophenol Reference Substance

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30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected <sup>(1)</sup> to 20°C	% Inhibition in Respiration Rate <sup>(2)</sup>
CONTROL I	0.5055	0.5055	-
10	0.3145	0.3004	41
CONTROL II	0.5345	0.5105	-

Percent Difference in Controls<sup>(3)</sup> 1.0%  
EC<sub>50</sub> (95% Confidence Limits) mg/L 10 mg/L induced 41% inhibition

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected <sup>(1)</sup> to 20°C	% Inhibition in Respiration Rate <sup>(2)</sup>
CONTROL I	0.5315	0.5076	-
10	0.1655	0.1814	65
CONTROL II	0.5558	0.5309	-

Percent Difference in Controls<sup>(3)</sup> 4.5%  
EC<sub>50</sub> (95% Confidence Limits) mg/L 10 mg/L induced 65% inhibition

(1) Temp. Correction =  $\frac{R_{T,obs}}{\theta^x}$  (mg O<sub>2</sub>/L-min.)

(3) % Difference =  $\frac{|RC_1 - RC_2|}{\frac{RC_1 + RC_2}{2}} \times 100$

(2) % Inhibition =  $\frac{\left(\frac{RC_1 + RC_2}{2}\right) - R_S}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

The two control respiration rates should be within 15% of each other

Where:  
R = Oxygen uptake rate (mg O<sub>2</sub>/L-min.)  
T<sub>obs</sub> = Sample temp., C°  
θ = 1.047, x = T<sub>obs</sub> - 20°C  
C = Control

See pg 4 for test soln. prep.

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