

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 (2/21/89). 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 monooctylphenyl 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: 1990

Analytical monitoring: Dissolved oxygen concentrations, pH and temperature.

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 condition:

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 10mL 1N NaOH, diluted to 30mL with Millipore Milli-Q™ water, then brought to the point of incipient precipitation with 1N H₂SO₄ and diluted to 1L with Milli-Q™ water. The pH of the reference solution was measured to be 7.4. Test substance was added directly to test vessels and was apparently miscible with water.

Test vessels: Not given

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

Temperature: 20 – 22 °C

Total suspended solids and pH on day of testing: 5.033 g/L and initial pH 6.6 and final pH 6.9. Initial TSS of 1000 mg/L concentration was 2.020 g/L, pH 6.6 and final TSS of 1000 mg/L concentration was 2.200 g/L, pH 6.8.

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, 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 H1480-1, 1990.

OTHER

Last changed: 6/26/00

Analyst AS

ENVIRONMENTAL LABORATORY
ACTIVATED SLUDGE MIXED LIQUOR

Type Test Activated Sludge Respiration Inhibition Test OECD#209

Source Metro Waste Treatment Plant, St. Paul, Mn

Date Obtained 3/19/90 Date Used 3/19/90

Mixed Liquor Suspended Solids (MLSS) 5.035 g/L Adjustment g/L

PH initial 10.6, final 10.9

Comments

Test Substance

Sample Description EC203CE Lot 519 (2/21/89)

highest concentration tested
1000 mg/L
Sample pH initial 10.2, final 10.8 Adjustment

Comments MLSS of 1000mg/L + test soln; initial 2.02g/L, final (3Hr) 2.2g/L

Reference Substance

Sample Description 3,5-Dichlorophenol

highest concentration tested, 10 mg/L
Sample pH initial 6.7, final 6.8 Adjustment

Comments Stock soln. pH 7.4

Exposure Conditions

Test Duration 3 Hours

Test Temperature 20-22, °C

Comments

Lab Request No. H1480-1

Environmental Lab — Work Sheet

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Sample Description

Date: 3/19/90

OECD ACTIVATED SLUDGE
RESPIRATION INHIBITION TEST #209

Analyst: 3

Test Solutions Preparation

FC 203 CE Lot 519 (2/21/89). Test substance appearance: clear yellow, foamy liquid, miscible with water.

Individual weights used.

Test Conc. mg/L	mg added per 500 ml	ml MQ water per 500 ml	ml syn. sewage feed per 500 ml	ml microbial inoculum per 500 ml	Total volume ml
320	160 +	284 +	16	+ 200	= 500
560	280 +	284 +	16	+ 200	= 500
1000*	500 +	284 +	16	+ 200	= 500
Bk Control I	0 +	284 +	16	+ 200	= 500

Shared Bk Control II with LR H1480-3.

* Very foamy. Large amount of foam formed.

This test solution was duplicated. Used for measuring TSS.

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: MC-203CE Lot 519 (2/21/89)

Source of Activated Sludge Mixed Liquor: Metro Waste Treatment Plant, St. Paul, MN

Date Received: 3/19/90

Date Used: 3/19/90

Analyst: Rochelle Robideau

30 - MINUTE EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES											TEMP °C
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	
CONTROL I	7.1	6.3	5.9	5.4	5.0	4.6	4.1	3.7	3.3	2.9	2.5	21
320	4.9	4.0	3.4	2.9	2.3	1.8	1.3	0.8	4.17 0.5			20
5100	3.5	2.4	1.6	1.0	2.26 0.5							20
1000	3.0	1.8	1.2	0.7	2.07 0.5							20
CONTROL II	5.8	5.3	4.9	4.4	4.1	3.6	3.2	2.8	2.4	2.0	1.6	21

3 - HOUR EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES											TEMP °C
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	
CONTROL I	5.9	5.6	5.2	4.8	4.5	4.1	3.7	3.2	2.8	2.4	2.0	21
320	6.1	5.7	5.3	4.9	4.6	4.1	3.8	3.4	3.0	2.6	2.2	20
5100	5.9	5.4	5.0	4.6	4.2	3.8	3.4	3.1	2.7	2.3	1.9	20
1000	6.1	5.6	5.3	4.9	4.5	4.2	3.8	3.4	3.0	2.6	2.3	20
CONTROL II	5.9	5.5	5.1	4.6	4.2	3.8	3.3	2.9	2.5	2.1	1.6	22

pH of Activated Sludge Mixed Liquor: Initial 6.6 Final 6.9
pH of highest concentration tested: Initial 6.6 Final 6.8
Test Temperature: 20-22°C

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTSLR H/480-1
Date 3/19/90
Analyst BBB
page 3 of 3Test Substance MC-203CE Lot. 519 (2/21/89)

30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected ⁽¹⁾ to 20°C	% Inhibition in Respiration Rate ⁽²⁾
CONTROL I	0.8497	0.8116	
320	1.0583	1.0583	— - 3d.5
560	1.3178	1.3178	— - 65
1000	1.1611	1.1611	— - 45
CONTROL II	0.8230	0.7861	

Percent Difference in Controls⁽³⁾ 3.2%EC₅₀ (95% Confidence Limits) mg/L _____

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected ⁽¹⁾ to 20°C	% Inhibition in Respiration Rate ⁽²⁾
CONTROL I	0.8036	0.7675	
320	0.7733	0.7733	0.4
560	0.7709	0.7709	0.7
1000	0.7491	0.7491	3.5
CONTROL II	0.8606	0.7852	

Percent Difference in Controls⁽³⁾ 2.3%EC₅₀ (95% Confidence Limits) mg/L _____

$$(1) \text{ Temp. Correction} = \frac{R_{T, \text{obs}}}{\theta^x} \quad (\text{mg O}_2/\text{L-min.})$$

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

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

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

Where:

R = Oxygen uptake rate (mg O₂/L-min.)T_{obs} = Sample temp., °Cθ = 1.047, x = T_{obs} - 20°C

C = Control

TSS Solids [TS] [TSS] [TDS]
 3M Environmental Laboratory - Raw Data Sheet

No. Tests
No. Hours
Analyst and Date Analyzed
OK Check

Method Reference: Std. Methods (17th ed.) pp. 2-75 to 2-77, Sec. 2540 D.
 (14th ed.) pp. 89-98, sec. 208

Calculation: $\text{mg/l Residue} = \frac{\text{mg residue} \times 1000}{\text{ml sample}}$

Metro Waste Treatment Plant, St. Paul, Mn
 Mixed Liquor (obtained & used 3/19/90)
 For LRH1480-1, 3, 6 & 3, 5-Dcp

Sample Description	Reported Result mg/l	ml Sample	Gross Weight after 105 °C	Pan + Filter Crucible Weight (g)	Residue Weight (g)	Residue mg/l	Comments
Initial Sludge Metro Waste Treatment Plant 1 St. Paul, Mn.		5	1.1435	1.1183	0.0252	5040	} $\bar{X} = 5033 \pm 12 \text{ mg/L}$
2		5	1.1405	1.1153	0.0252	5040	
3		5	1.1404	1.1153	0.0251	5020	
Initial in FC203CE 1000 mg/L test solution		5	1.1234	1.1132	0.0102	2040	} $\bar{X} = 2020 \pm 20 \text{ mg/L}$
4		5	1.1308	1.1207	0.0101	2020	
5		5	1.1397	1.1297	0.0100	2000	
Initial in FC203CE 1000 mg/L with 505214 200 mg/L test solution		5	1.1235	1.1136	0.0099	1980	} $\bar{X} = 2027 \pm 42 \text{ mg/L}$
8		5	1.1230	1.1128	0.0102	2040	
9		5	1.1271	1.1168	0.0103	2060	
5Hr in FC203CE 1000 mg/L test solution		5	1.1357	1.1247	0.0110	2200	} $\bar{X} = 2200 \pm 20 \text{ mg/L}$
11		5	1.1257	1.1148	0.0109	2180	
12		5	1.1186	1.1075	0.0111	2220	
5Hr in FC203CE 1000 mg/L with 505214 200 mg/L test solution		5	1.1255	1.1145	0.0110	2200	} $\bar{X} = 2233 \pm 12 \text{ mg/L}$ 3/19/90-23
14		5	1.1225	1.1114	0.0111	2220	
15		5	1.1259	1.1148	0.0111	2220	

Lab Request No. H1480

Environmental Lab — Work Sheet

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Sample Description
 3,5-Dichlorophenol, 97%
 Reference Substance
 Aldrich Red Label
 Milwaukee, Wis.
 Lot D7,060-0

OECD ACTIVATED SLUDGE
 RESPIRATION INHIBITION TEST #209

Date: 3/19/90Analyst: SS

Stock and Test Solutions Preparation

3,5-Dichlorophenol (Reference Substance for LR H1480-1,3,6)

STOCK SOLUTION: 500mg 3,5-DCP/L. Each 1mL = 0.5mg

- Dissolved 500mg 3,5-DCP in 10mL 1.0N NaOH

- Diluted to 30mL with Milli-Q water

- Added 1.0N H_2SO_4 dropwise while stirring, until incipient precipitation.

- Diluted to one liter with Milli-Q water.

Stock solution pH 7.4.

Test Conc. mg/L	ml stock soln per 500 ml	ml MQ water per 500 ml	ml syn. sewage feed per 500 ml	ml microbial inoculum per 500 ml	Total volume ml
10	10	+ 274	+ 16	+ 200	= 500
Bk Control III:	0	+ 284	+ 16	+ 200	= 500

Shared Bk Control II with H1480-b.

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: 3/19/90 Date Used: 3/19/90
Analyst: Rockelle Rabideau

30 - MINUTE EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES											TEMP °C
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	
CONTROL II	5.8	5.3	4.9	4.4	4.1	3.6	3.2	2.8	2.4	2.0	1.6	21
10	6.9	6.7	6.5	6.3	6.0	5.8	5.5	5.2	5.0	4.7	4.4	21
CONTROL III	6.0	5.6	5.2	4.8	4.3	4.0	3.6	3.1	2.7	2.3	1.8	21

3 - HOUR EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS mg/L

Test Substance Concentration (mg/L)	TIME-MINUTES											TEMP °C
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	
CONTROL II	5.9	5.5	5.1	4.6	4.2	3.8	3.3	2.9	2.5	2.1	1.6	22
10	7.2	7.1	6.9	6.7	6.6	6.4	6.3	6.1	5.9	5.7	5.5	22
CONTROL III	5.5	5.0	4.6	4.1	3.6	3.2	2.6	2.2	1.8	1.4	1.0	22

pH of Activated Sludge Mixed Liquor: Initial 6.6
pH of highest concentration tested: Initial 6.7
Test Temperature: 21- 22°C

Final 6.9
Final 6.8

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTS

LR H/480
Date 3/19/90
Analyst SSS
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Test Substance 3,5-Dichlorophenol (Reference Substance)

30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected ⁽¹⁾ to 20°C	% Inhibition in Respiration Rate ⁽²⁾
CONTROL II	0.8230	0.7861	
10	0.5152	0.4921	38
CONTROL III	0.8364	0.7989	

Percent Difference in Controls⁽³⁾ 1.6%

EC₅₀ (95% Confidence Limits) mg/L _____

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	Oxygen Uptake Rate R	R Corrected ⁽¹⁾ to 20°C	% Inhibition in Respiration Rate ⁽²⁾
CONTROL II	0.81606	0.7852	
10	0.3442	0.3141	161
CONTROL III	0.9055	0.8262	

Percent Difference in Controls⁽³⁾ 5.1%

EC₅₀ (95% Confidence Limits) mg/L _____

(1) Temp. Correction = $\frac{R_{T,obs}}{\theta^x}$ (mg O₂/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

Where:

R = Oxygen uptake rate (mg O₂/L-min.)

T_{obs} = Sample temp., °C

θ = 1.047, x = T_{obs} - 20°C

C = Control