

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-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). 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 mono-octylphenyl ether, 0.06% 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

Test type: Acute static

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 7.2. Test substance was added directly to the 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: 17 – 19 °C

Total suspended solids and pH on day of testing: 7.853 g/L (TSS), initial pH 6.7 and final pH 6.9. Initial TSS in 1000 mg/L concentration was 3.14 g/L, initial pH 6.7, final pH 6.8, and MLSS 3.34 g/L.

Element Basis: Respiration inhibition as determined by oxygen consumption.

Remarks: Values were 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 FC-206CE 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 H1480-2, 1990.

OTHER

Last changed: 6/26/00

LR H1480-2

Date 3/23/90

Analyst SS

ENVIRONMENTAL LABORATORY
ACTIVATED SLUDGE MIXED LIQUOR

Type Test Activated Sludge Respiration Inhibition Test OECD#209

Source Metrom Waste Treatment Plant, St. Paul, Mn.

Date Obtained 3/23/90 Date Used 3/23/90

Mixed Liquor Suspended Solids (MLSS) 7.853 g/L Adjustment g/L

pH initial 6.7, final 6.9

Comments _____

Test Substance

Sample Description EC2010CE Lot 502 (2/16/89)
highest concentration tested, 1000 mg/L

Sample pH initial 6.7, final 6.8 Adjustment _____

Comments MLSS of 1000 mg/L test soln; initial 3.14 g/L, final (3 Hr) 3.34 g/L

Reference Substance

Sample Description 3,5-Dichlorophenol
highest concentration tested, 10 mg/L

Sample pH initial 6.8, final 7.0 Adjustment _____

Comments Stock solution pH 7.2

Exposure Conditions

Test Duration 3 Hours

Test Temperature 17-19 °C

Comments _____

Lab Request No. H1480-2

Environmental Lab — Work Sheet

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Sample Description

Date: 3/23/90OECD ACTIVATED SLUDGE
RESPIRATION INHIBITION TEST #209Analyst: SA

Test Solutions Preparation

EC 2016 CE Lot 502 (2/16/89) Test substance appearance:
 clear yellow, foamy liquid, miscible with water.

Individual weights used.

Test Conc. mg/L	mg added per 500 ml	ml M ₀ 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 H1480-4.

* Slight amount of foam formed.

This test solution was duplicated. Used for measuring TSS.

LR H1480-2

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: EC 2010 CE Lot 502 (2/16/89)Source of Activated Sludge Mixed Liquor: Metro Waste Treatment Plant, St. Paul, MnDate Received: 3/23/90Date Used: 3/23/90Analyst: Rockelle Rabideau

30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	DISSOLVED OXYGEN CONCENTRATIONS mg/L											TEMP °C
	TIME-MINUTES											
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	
CONTROL I	7.4	6.5	5.8	5.3	4.7	4.2	3.7	3.1	2.6	2.1	1.6	18
320	6.0	4.6	4.0	3.4	2.8	2.3	1.7	1.3	0.8	4:45 0.5		17
560	3.0	1.7	1.1	1:59 0.5								18
1000	1.6	0:50 0.5										18
CONTROL II	5.8	5.2	4.5	4.0	3.5	2.9	2.5	1.9	1.4	0.9	3:27 0.5	18

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	DISSOLVED OXYGEN CONCENTRATIONS mg/L											TEMP °C
	0.5	1.0	1.5	2.0	2.5	TIME-MINUTES		4.0	4.5	5.0	5.5	
CONTROL I	6.5	6.1	5.6	4.9	4.4	3.8	3.2	2.4	2.0	1.5	1.0	19
320	6.3	5.7	5.2	4.6	4.1	3.5	3.0	2.4	1.9	1.3	0.9	18
560	5.9	5.3	4.7	4.2	3.6	3.1	2.5	2.0	1.4	1.0	3:27 0.5	18
1000	5.7	5.0	4.4	3.7	3.1	2.5	1.9	1.4	0.8	4:54 0.5		19
CONTROL II	5.8	4.9	4.4	3.8	3.3	2.7	2.4	1.7	1.2	0.5		18

pH of Activated Sludge Mixed Liquor:

Initial 6.7

Final 6.9

pH of highest concentration tested:

Initial 6.7

Final 6.8

Test Temperature: 17-19 °C

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTSLR H1480-2
Date 3/23/90
Analyst JSTest Substance PC 206-CE Lot 502 (2/16/89)

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	1.07164	1.1803	
320	1.0800	1.2400	— (2.5)
500	1.2243	1.3424	— (1)
1000	*		—
CONTROL II	1.1297	1.2387	

Percent Difference in Controls⁽³⁾ 4.8% 1.2095EC₅₀ (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	1.1939	1.2502	
320	1.0861	1.1909	2.0
500	1.0787	1.1828	2.7
1000	1.1699	1.2250	— (0.79)
CONTROL II	1.0767	1.1806	

Percent Difference in Controls⁽³⁾ 5.7% 1.2154EC₅₀ (95% Confidence Limits) mg/L _____

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

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

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

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., °Cθ = 1.047, x = T_{obs} - 20°C

C = Control

*Rate not calculable; oxygen depleted too rapidly, in less than one minute.

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: 3/23/90Analyst: SA

Stock and Test Solutions Preparation

3,5-Dichlorophenol (Reference Substance for LR H1480-2,4,5)

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

-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 7.2.

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

Shared Bk Controls with LR H1480-2,4,5.

ENVIRONMENTAL LABORATORY

LR H1480
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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: 3/23/90Date Used: 3/23/90Analyst: Rockelle R. Bidreau

30 - MINUTE EXPOSURE

Test Substance Concentration (mg/L)	DISSOLVED OXYGEN CONCENTRATIONS mg/L											TEMP °C
	TIME-MINUTES											
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	
CONTROL I	7.4	6.5	5.8	5.3	4.7	4.2	3.7	3.1	2.6	2.1	1.6	18
10	7.5	6.9	6.6	6.2	5.8	5.4	5.0	4.6	4.2	3.8	3.4	18
CONTROL II	5.8	5.2	4.5	4.0	3.5	2.9	2.5	1.9	1.4	0.9	5:27 0.5	18

3 - HOUR EXPOSURE

Test Substance Concentration (mg/L)	DISSOLVED OXYGEN CONCENTRATIONS mg/L											TEMP °C
	0.5	1.0	1.5	2.0	2.5	TIME-MINUTES		4.0	4.5	5.0	5.5	
CONTROL I	6.5	6.1	5.6	4.9	4.4	3.8	3.2	2.4	2.0	1.5	1.0	19
10	7.7	7.6	7.3	7.0	6.8	6.6	6.3	6.0	5.8	5.5	5.2	19
CONTROL II	5.8	4.9	4.4	3.8	3.3	2.7	2.4	1.7	1.2	0.5		18

pH of Activated Sludge Mixed Liquor:

Initial 6.7

Final 6.9

pH of highest concentration tested:

Initial 6.8

Final 7.0

Test Temperature: 18-19°C

ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209 CALCULATION OF RESULTS

LR H1480

Date 3/23/90

Analyst SSA

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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 I	1.07164	1.1803	
10	0.7891	0.8682	2.8
CONTROL II	1.1297	1.2387	

Percent Difference in Controls⁽³⁾ 4.8%

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	1.1939	1.2502	
10	0.5200	0.5445	55
CONTROL II	1.07164	1.1806	

Percent Difference in Controls⁽³⁾ 5.7%

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

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

Method Reference: Std. Methods (17th ed) pp. 2-75 to 2-77, Sec. 2540 D.
 (14th ed) pp. 89-98, sec. 200- Metro Waste Treatment Plant, St. Paul, Mn
 Calculation: $\text{mg/l Residue} = \frac{\text{mg residue} \times 1000}{\text{ml sample}}$ Mixed liquor (obtained & used 3/23/90)
 For LR H1480-2, 4, 5 & 3, 5-DOP

No. Tests

No. Hours

Analyst and Date Analyzed

QA Check

Sample Description	Reported Result mg/l	ml Sample	Gross Weight after 103 °C	Pan + Filter - Crucible Weight (g)	Residue Weight (g)	Residue mg/l	Comments
Initial Sludge Metro Waste Treatment Plant 1 St. Paul, Mn.		5	1.1549	1.1156	0.0393	7860	} $\bar{X} = 7853 \pm 12 \text{ mg/L}$
2		5	1.1589	1.1197	0.0392	7840	
3		5	1.1645	1.1252	0.0393	7860	
Initial in FC206CE 1000 mg/L test solution		5	1.1418	1.1263	0.0155	3100	} $\bar{X} = 3140 \pm 40 \text{ mg/L}$
4		5	1.1365	1.1208	0.0157	3140	
5		5	1.1365	1.1206	0.0159	3180	
Initial in FC206CE 1000 mg/L with WTS214 200 mg/L test solution		5	1.1352	1.1194	0.0158	3160	} $\bar{X} = 3143 \pm 12 \text{ mg/L}$
6		5	1.1334	1.1175	0.0159	3180	
7		5	1.1341	1.1184	0.0157	3140	
Initial in FC206CE 1000 mg/L test solution		5	1.1338	1.1171	0.0167	3340	} $\bar{X} = 3340 \pm 40 \text{ mg/L}$
8		5	1.1384	1.1219	0.0165	3300	
9		5	1.1355	1.1186	0.0169	3380	
Initial in FC206CE 1000 mg/L with WTS214 200 mg/L test solution		5	1.1322	1.1154	0.0168	3360	} $\bar{X} = 3380 \pm 20 \text{ mg/L}$
10		5	1.1358	1.1189	0.0169	3380	
11		5	1.1380	1.1210	0.0170	3400	
12							
13							
14							
15							