

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-603EF. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number is not noted. The test sample is FC-603EF. Current information indicates it is a mixture of 1.30% PFOS, 10% diethylene glycol butyl ether, 54.15% water, 22.5% ethylene glycol, 2.6% propanesultone foamer, 5% Alkylether sulfate, 0.1% N-(3-Chloroallyl)hexaminium chloride, 0.9% Xanthan gum, 0.9% starch, 1.0% triethanolamine, and 0.05% tolyl triazole.

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

METHOD

Method: OECD Guideline 209

Test type: Acute static

GLP: No

Year Completed: 1996

Analytical monitoring: Dissolved oxygen, 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 Water 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.

Test vessels: Not given.

Number of concentrations: three plus 1 reference substance and blank control

Temperature: 19.4 – 20.6 °C

Total suspended solids and pH on day of testing: 2.96 g/L (TSS in final: 1.18 g/L), initial pH 7.3 and final pH 7.6.

Element Basis: Respiration inhibition as determined by oxygen consumption.

Remarks: Values corrected to 20°C for calculations.

RESULTS

Nominal concentrations: Blank control, 10, 100, and 1000 mg/L. The blank control and 1000 mg/L exposure concentration were evaluated in duplicate. Reference substance at 10 mg/L.

Element values: 30-minute EC₅₀ = >1000 mg/L
3-hour EC₅₀ = >1000 mg/L

Element values based on nominal concentrations.

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 3-hour EC₅₀ for activated sludge respiration inhibition was determined to be >1000 mg/L, the highest concentration tested.

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, sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in solution.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, Lab Request number P2581, 1996.

OTHER

Last changed: 6/26/00

ENVIRONMENTAL LABORATORY
ACTIVATED SLUDGE MIXED LIQUOR

LA P2581-1
Date 10-23-96
Analyst TKS
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Type Test DECO ACTIVATED SLUDGE RESPIRATION TEST #209

ACTIVATED SLUDGE MIXED LIQUOR

Date Obtained 10-23-96

Date Used 10-23-96

Mixed Liquor Suspended Solids (MLSS) 2.96 g/L Adjusted to — g/L

MLSS in final test solution 1.18 g/L

Initial pH 7.3 (1st test soln. prepared)

Final pH 7.6 (last soln. prepared)

Comments _____

TEST SUBSTANCE

Sample Description FC-603 EF

Initial pH of highest conc. tested (1000 mg/L) = 7.3

Adjusted to pH —

Comments _____

REFERENCE SUBSTANCE

Sample Description 3,5-DCP

Initial pH of stock solution (500 mg/L) = 7.2

Adjusted to pH —

Comments _____

EXPOSURE CONDITIONS

Test Duration 3 hours

Test Temperature Range: 19.4-20.6 °C

Comments _____

ENVIRONMENTAL LABORATORY

LR P2581
Pg 2098

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
TEST SOLUTIONS PREPARATION

Test Substance: FC-603EF

Sample Description: brown liquid

Analyst: TVS

Date: 10-23-96

Test Conc. mg/L	mg added per 500 ml	mL MQ(1) water per 500 ml	mL syn. sewage feed per 500 ml	mL microbial inoculum per 500 ml	Total Volume mL
Blank control	-	284	16	200	500
5	5	274	16	200	500
100	50	284	16	200	500
1000	500	284	16	200	500
1000	500	284	16	200	500
1000	500	284	16	200	500

* A 10ml aliquot was taken from a 50mg/100ml solution
10-24-96 TVS

Initial pH of highest conc tested: 7.2

(1) Millipore Milli-QR high purity water

ENVIRONMENTAL LABORATORY

LR P2581-1

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OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-603EFSource of Activated Sludge Mixed Liquor: Melro Waste Water Treatment Plant St. PaulDate Received: 10-23-96Date Used: 10-23-96Analyst: TKS

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	
BK CONTROL I	6.3	6.1	5.9	5.7	5.5	5.2	5.0	4.7	4.4	4.2	4.0	20.6
10	6.5	6.1	5.9	5.6	5.3	5.0	4.7	4.4	4.1	3.9	3.5	20.6
100	5.9	5.5	5.1	4.8	4.3	3.8	3.7	3.4	3.1	2.7	2.3	19.8
1000	2.9	2.5	2.2	1.9	1.5	1.3	0.9	0.6	0.3	0.1	0.0	20.6
1000	1.0	0.5	0.2	0.0	-	-	-	-	-	-	-	20.4
BK CONTROL II	7.1	6.8	6.7	6.4	6.3	6.0	5.9	5.5	5.3	5.1	4.8	20.3

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	
BK CONTROL I	7.4	7.3	7.0	6.8	6.6	6.4	6.1	5.9	5.7	5.4	5.1	20.1
10	7.4	7.1	6.7	6.6	6.3	6.0	5.7	5.4	5.2	5.0	4.6	20.1
100	7.6	7.3	7.0	6.8	6.5	6.3	6.0	5.8	5.6	5.4	5.1	19.4
1000	5.0	4.7	4.4	4.1	3.8	3.6	3.3	3.0	2.7	2.5	2.2	20.0
1000	2.5	2.1	1.9	1.6	1.2	0.9	0.6	0.4	0.3	0.1	-	20.6
BK CONTROL II	7.1	7.4	7.2	7.0	6.7	6.5	6.3	6.0	5.8	5.6	5.4	20.1

pH of activated sludge mixed liquor:

Initial: 7.3

Final: 7.6

pH of highest concentration tested:

Initial: 7.1

Final: 7.0

Test temperature: 19.4 = 20.6

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST - 209

Lr: P2581
Date: 10/23/96

CALCULATION OF RESULTS

Test Substance: EC-603EE

Analyst: Trudy Stiefenhofer

30-Minute Exposure

Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp, °C	R (1)	Ra (2)	% Inh. (3)
BK1	6.3	6.1	5.9	5.7	5.5	5.2	5.0	4.7	4.4	4.2	4.0	20.6	0.4836	0.4639	
10	6.5	6.1	5.9	5.6	5.3	5.0	4.7	4.4	4.1	3.9	3.5	20.6	0.5806	0.5569	
100	6.5	6.1	5.9	5.6	5.3	5.0	4.7	4.4	4.1	3.9	3.5	19.8	0.5806	0.5887	
1000	2.9	2.5	2.2	1.9	1.5	1.3	0.9	0.6	0.3	0.1	0.0	20.6	0.6167	0.5915	
1000	1.0	0.5										20.4			
BK2	7.1	6.8	6.7	6.4	6.3	6.0	5.9	5.5	5.3	5.1	4.8	20.3	0.4509	0.4416	

% diff in controls (4): 4.9

EC50 (95%CI) = > 1000 mg/L

RaCm (5): 0.4527

3-Hour Exposure

Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp, °C	R (1)	Ra (2)	% Inh. (3)
BK1	7.4	7.3	7.0	6.8	6.6	6.4	6.1	5.9	5.7	5.4	5.1	20.1	0.4715	0.4682	
10	7.4	7.1	6.7	6.6	6.3	6.0	5.7	5.4	5.2	5.0	4.6	20.1	0.5382	0.5345	
100	7.6	7.3	7.0	6.8	6.5	6.3	6.0	5.8	5.6	5.4	5.1	19.4	0.4776	0.4979	
1000	5.0	4.7	4.4	4.1	3.8	3.6	3.3	3.0	2.7	2.5	2.2	20.0	0.5515	0.5515	
1000	2.5	2.1	1.9	1.6	1.2	0.6						20.6	0.7400	0.7098	
BK2	7.7	7.4	7.2	7.0	6.7	6.5	6.3	6.0	5.8	5.6	5.4	20.1	0.4545	0.4514	

% diff in controls (4): 3.7

EC50 (95%CI) = > 1000 mg/L

RaCm (5): 0.4598

(1) R = Oxygen uptake rate (mg O₂/L-min)

(2) Ra = R adjusted to 20°C = [R/θ Ex]

(3) % Inhibition = [(RaCm) - RaS / (RaCm)] 100

(4) % difference in controls: [(RaBk1 - RaBk2) / RaCm] 100 (Two controls must be within 15% of each other.)

(5) Mean adjusted R, controls = (RaBk1 + RaBk2) / 2

θ = 1.072

Bk = blank control, S = sample

x = Tobs - 20°C

RaCm = mean adjusted R, controls

Tobs = sample temp

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST # 209
TEST SOLUTIONS PREPARATION

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Test Substance: 3,5-Dichloropheno1

Sample Description: Aldrich (red label) Lot # 00410KZ

Analyst: Tks

Date: 10-23-96

Stock Solution

Preparation: 500mg/L; each 10ml = 0.5mg

D. dissolved 500mg 3,5-DCP in 10ml 1.0N NaOH

D. diluted to approx 30ml with mill.-Q water

Added 1.0N H₂SO₄ under stirring until incipient ppt

D. diluted to 1.0L with mill.-Q water

pH stock solution = 6.6

Test Conc. mg/L	mL added per 500 mL	mL MQ(1) water per 500 mL	mL syn. sewage feed per 500 mL	mL microbial inoculum per 500 mL	Total Volume mL
BLK CONTROL	—	284	16	200	500
10	10	274	16	200	500

INITIAL PH of highest conc. tested = 7.2

(1) Millipore Milli-Q[®] high purity water

ENVIRONMENTAL LABORATORY

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OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: 3,5-DichlorophenolSource of Activated Sludge Mixed Liquor: Metro Waste Water Treatment Plant, St. Paul, MDate Received: 10-23-96Date Used: 10-23-96Analyst: TKS

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	
BK CONTROL I	6.3	6.1	5.9	5.7	5.5	5.2	5.0	4.7	4.4	4.2	4.0	20.6
10	7.7	7.4	7.3	7.1	6.9	6.8	6.6	6.5	6.3	6.1	5.9	20.3
BK CONTROL II	7.1	6.8	6.7	6.4	6.3	6.0	5.9	5.5	5.3	5.1	4.8	20.3

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	
BK CONTROL I	7.4	7.3	7.0	6.8	6.6	6.4	6.1	5.9	5.7	5.4	5.1	20.1
10	8.5	8.4	8.2	8.1	8.0	7.9	7.8	7.7	7.6	7.5	7.4	20.2
BK CONTROL II	7.7	7.4	7.2	7.0	6.7	6.5	6.3	6.0	5.8	5.6	5.4	20.1

pH of activated sludge mixed liquor:

Initial: 7.3Final: 7.6

pH of highest concentration tested:

Initial: 7.2Final: 7.6Test temperature: 20.1-20.6

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST 209

Lr: P2581-1

Date: 10/23/96

CALCULATION OF RESULTS

Test Substance: 3,5-Dichlorophenol

Analyst: Trudy Stiefenhofer

30-Minute Exposure

Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp, °C	R (1)	Ra (2)	% Inh. (3)
BK1	6.3	6.1	5.9	5.7	5.5	5.2	5.0	4.7	4.4	4.2	4.0	20.6	0.4836	0.4639	
10	7.7	7.4	7.3	7.1	6.9	6.8	6.6	6.5	6.3	6.1	5.9	20.3	0.3309	0.3241	28.4
BK2	7.1	6.8	6.7	6.4	6.3	6.0	5.9	5.5	5.3	5.1	4.8	20.3	0.4509	0.4416	

% diff in controls (4): 4.9

RaCm (5): 0.4527

3-Hour Exposure

Dissolved Oxygen Concentrations, mg/L

mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	Temp, °C	R (1)	Ra (2)	% Inh. (3)
BK1	7.4	7.3	7.0	6.8	6.6	6.4	6.1	5.9	5.7	5.4	5.1	20.1	0.4715	0.4682	
10	8.5	8.4	8.2	8.1	8.0	7.9	7.8	7.7	7.6	7.5	7.4	20.2	0.2109	0.2080	54.8
BK2	7.7	7.4	7.2	7.0	6.7	6.5	6.3	6.0	5.8	5.6	5.4	20.1	0.4545	0.4514	

% diff in controls (4): 3.7

RaCm (5): 0.4598

(1) R= Oxygen uptake rate (mg O₂/L-min)

(2) Ra= R adjusted to 20°C= [R/o Ex]

(3) % Inhibition= [(RaCm)-RaS/(RaCm)]100

(4) % difference in controls: [(RaBk1-RaBk2)/RaCm]100 (Two controls must be within 15% of each other.)

(5) Mean adjusted R, controls= [RaBk1+RaBk2]/2

o= 1.072

Bk= blank control, S= sample

x= Tobs-20°C

RaCm= mean adjusted R, controls

Tobs= sample temp

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Chain of Custody Record

3M Environmental Laboratory

04187

Form 35178 - PWO

3M Facility 3m main plant	3M Project Title Mixed Liquor	3M Project Number
Address	3M Facility Contact	3M Department Number
City, State, Zip Code	3M Telephone Number	Lab Request/Template Number

Sampled By (Print) John Plachka		Contractor Pace		Number Containers of each		Total Containers of Each Item	☐ Samples Split See Attachments	Comments		
Sampler Signature <i>[Signature]</i>		Date 10/23/96		Preservatives						
Item #	Sample Description	Date	Time	Matrix	None	HNO ₃	H ₂ SO ₄	NaOH	VOC	Sub
1	Mixed Liquor 1023	10/23/96	9:15	water						1
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										

Item #	Relinquished By/Affiliation	Time	Date	Shipped Via	Received By/Affiliation	Time	Date
1	<i>[Signature]</i> Pace	9:43	10/23/96	Pace	<i>[Signature]</i>	09:44	10/23/96

Original - Accompanying Samples

Last Page - Originator

See Reverse Side For Instructions

10/23/96 TKS P2581