

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

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

Remarks: The 3M production lot number was not noted. The test sample is FC-203FC. Current information indicates it is a mixture of 0.85% PFOS, 17% diethylene glycol butyl ether, 74.46% water, 2.34% acrylic foamer, 1.7% alkyl ether sulfate, 2.55% sodium octyl sulfate, 0.12% sodium nitrite, 0.09% sodium molybdate, 0.85% triethanolamine, and 0.04% tolyltriazole.

The following summary applies to 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: Static acute

GLP: No

Year Completed: 1997

Analytical monitoring: Dissolved oxygen concentrations, temperature, and pH.

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

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

Test condition:

Dilution water: Carbon-filtered well water (3M well #2, St. Paul, MN)

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 7.7. Test substance was added directly to the test vessels.

Test vessels: Not given.

Number of concentrations: five plus blank control

Temperature: 19.1 – 20.3°C

Total suspended solids and pH on day of testing: 4.53 g/L (TSS in final: 1.81 g/L), initial pH 8.0 and final pH 8.5

Element Basis: Respiration inhibition as determined by oxygen consumption.

Remarks: Values corrected to 20°C for calculations.

RESULTS

Nominal concentrations: Blank control, 10, 50, 100, 500, and 1000 mg/L. The blank control and 1000 mg/L were duplicated.

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 FC-203FC 3-hour EC₅₀ for activated sludge respiration inhibition was determined to be >1000 mg/L. This concentration inhibited respiration by ~13%.

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

DATA QUALITY

Reliability: Klimisch ranking = 2. This study 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 R1335, 1997.

OTHER

Last changed: 6/26/00

ENVIRONMENTAL LABORATORY
ACTIVATED SLUDGE MIXED LIQUOR

LA R1335
Date 3-24-97
Analyst TKS
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Type Test OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #2

ACTIVATED SLUDGE MIXED LIQUOR

Date Obtained 3-24-97

Date Used 3-24-97

Mixed Liquor Suspended Solids (MLSS) 4.53 g/L Adjusted to — g/L

MLSS in final test solution 1.81 g/L

Initial pH 8.0 (1st test soln. prepared)

Final pH 8.5 (last soln. prepared)

Comments _____

TEST SUBSTANCE

Sample Description FC-203FC AFFF

Initial pH of highest conc. tested (1000mg/L) = 7.5 Adjusted to pH —

Comments _____

REFERENCE SUBSTANCE

Sample Description 3,5-Dichlorophenol

Initial pH of stock solution (500 mg/L) = 7.7 Adjusted to pH —

Comments _____

EXPOSURE CONDITIONS

Test Duration 3 hours

Test Temperature Range: 19.1-20.3 °C

Comments _____

ENVIRONMENTAL LABORATORY

LR R1335

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OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
TEST SOLUTIONS PREPARATIONTest Substance: FC-203FC AFFFSample Description: golden liquidAnalyst: TKSDate: 3-24-97

Test Conc. mg/L	mg added per 500 ml	well 3-24-97 mL MC(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
10	5	284	16	200	500
50	25	284	16	200	500
100	50	284	16	200	500
500	250	284	16	200	500
1000	500	284	16	200	500
1000	500	284	16	200	500

Inoculated pH of highest conc tested = 7.5

- (1) Millipore Milli-Q[®] high purity water
3-24-97 TKS

ENVIRONMENTAL LABORATORY

LR R1385

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OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-203FC AFFFSource of Activated Sludge Mixed Liquor: Metro Waste Water Treatment Plant St. PaulDate Received: 3-24-97Date Used: 3-24-97Mw, ~~SS~~Analyst: 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	7.8	7.6	7.4	7.1	6.8	6.3	5.9	5.4	5.1	4.7	4.2	19.6
10	7.1	6.8	6.3	5.9	5.5	5.1	4.7	4.2	3.7	3.2	2.8	20.2
50	7.6	7.4	6.8	6.3	5.9	5.4	5.0	4.6	4.1	3.7	3.3	20.0
100	7.4	7.0	6.6	6.2	5.8	5.3	4.9	4.5	4.1	3.7	3.2	20.0
500	6.8	6.3	5.9	5.4	4.8	4.4	3.8	3.3	2.9	2.4	1.9	19.9
1000	4.1	3.7	3.0	2.3	1.7	1.1	0.6	—	—	—	—	20.2
1000	5.1	4.4	3.8	3.2	2.7	2.1	1.7	1.1	0.5	—	—	19.9
BK CONTROL II	6.6	6.1	5.9	5.3	4.9	4.4	4.0	3.7	3.2	2.8	2.4	20.2

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	7.6	7.3	6.9	6.5	6.1	5.7	5.3	4.9	4.5	4.0	3.6	20.2
10	6.6	6.2	5.9	5.5	5.1	4.7	4.2	3.7	3.3	2.9	2.5	20.2
50	6.8	6.5	6.2	5.8	5.4	5.0	4.6	4.2	3.8	3.4	2.9	19.9
100	7.1	6.7	6.3	5.9	5.5	5.1	4.7	4.2	3.9	3.5	3.1	19.8
500	7.5	7.3	6.9	6.6	6.3	5.8	5.5	5.1	4.8	4.4	4.0	19.7
1000	7.9	7.6	7.3	6.9	6.5	6.3	5.9	5.4	5.1	4.7	4.5	19.3
1000	8.2	7.9	7.6	7.3	7.0	6.8	6.3	6.0	5.7	5.4	5.1	19.7
BK CONTROL II	8.1	7.6	7.4	7.0	6.6	6.2	5.8	5.4	5.0	4.6	4.2	19.6

pH of activated sludge mixed liquor:

Initial 8.0Final 8.5

pH of highest concentration tested:

Initial 7.7Final 8.5Test temperature: 19.3 - 20.2

CALCULATION OF RESULTS

OECD Activated Sludge
Respiration Inhibition Test #209Lr: B1335
Date: 3/24/97

Test Substance: FC-203FC AFFF

Analyst: Trudy Stiefenhoefer

30-Minute Exposure

mg/L	Dissolved Oxygen Concentrations, mg/L											Temp, °C	R (1)	Ra (2)	% Inh. (3)
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5				
BK1	7.8	7.6	7.4	7.1	6.8	6.3	5.9	5.4	5.1	4.7	4.2	19.6	0.7770	0.7989	
10	7.1	6.8	6.3	5.9	5.5	5.1	4.7	4.2	3.7	3.2	2.8	20.2	0.8848	0.8726	○
50	7.6	7.4	6.8	6.3	5.9	5.4	5.0	4.6	4.1	3.7	3.3	20.0	0.8958	0.8958	○
100	7.4	7.0	6.6	6.2	5.8	5.3	4.9	4.5	4.1	3.7	3.2	20.0	0.8400	0.8400	○
500	6.8	6.3	5.9	5.4	4.8	4.4	3.8	3.3	2.9	2.4	1.9	19.9	0.9903	0.9972	○
1000	4.1	3.7	3.0	2.3	1.7	1.1	0.6					20.2	1.2457	1.2285	○
1000	5.1	4.4	3.8	3.2	2.7	2.1	1.7	1.1	0.5			19.9	1.0929	1.1005	○
BK2	6.6	6.1	5.9	5.3	4.9	4.4	4.0	3.7	3.2	2.8	2.4	20.2	0.8424	0.8308	

% diff in controls (4): 3.9

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

RaCm (5): 0.8148

3-Hour Exposure

mg/l	Dissolved Oxygen Concentrations, mg/L											Temp, °C	R (1)	Ra (2)	% Inh. (3)
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5				
BK1	7.6	7.3	6.9	6.5	6.1	5.7	5.3	4.9	4.5	4.0	3.6	20.2	0.8194	0.8081	
10	6.6	6.2	5.9	5.5	5.1	4.7	4.2	3.7	3.3	2.9	2.5	20.2	0.8485	0.8368	○
50	6.8	6.5	6.2	5.8	5.4	5.0	4.6	4.2	3.8	3.4	2.9	19.9	0.8000	0.8056	○
100	7.1	6.7	6.3	5.9	5.5	5.1	4.7	4.2	3.9	3.5	3.1	19.8	0.8036	0.8149	○
500	7.5	7.3	6.9	6.6	6.3	5.8	5.5	5.1	4.8	4.4	4.0	19.7	0.7285	0.7438	7.5
1000	7.9	7.6	7.3	6.9	6.5	6.3	5.9	5.4	5.1	4.7	4.5	19.3	0.7127	0.7483	6.9
1000	8.2	7.9	7.6	7.3	7.0	6.8	6.3	6.0	5.7	5.4	5.1	19.7	0.6315	0.6448	19.8
BK2	8.1	7.6	7.4	7.0	6.6	6.2	5.8	5.4	5.0	4.6	4.2	19.6	0.7782	0.8001	

% diff in controls (4): 1.0

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

RaCm (5): 0.8041

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

(2) Ra = R adjusted to 20°C = [R/x].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

x = 1.072

Bk = blank control, S = sample

x = Tobs - 20°C

RaCm = mean adjusted R, controls

Tobs = sample temp

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ENVIRONMENTAL LABORATORY

LR R1335
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OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST # 209 TEST SOLUTIONS PREPARATION

Test Substance: 3,5- Dichlorophenol

Sample Description: Aldrich (red label) lot # 09615 KG DQ

Analyst: TKS

Date: 3-24-97

Stock Solution

Preparation: 500mg/L, each 1.0ml = 0.5mg

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

Diluted to approx 30ml with milli-Q water

Added 8.0ml 1.0N H₂SO₄ to incipient ppt.

Diluted to 1.0L with milli-Q water

Stock sol pH = 7.7

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
<u>BU CONTROL</u>	<u>-</u>	<u>284</u>	<u>16</u>	<u>200</u>	<u>500</u>
<u>10</u>	<u>10</u>	<u>274</u>	<u>16</u>	<u>200</u>	<u>500</u>

Initial pH highest conc tested = 7.7

(1) Millipore Milli-Q^R high purity water

ENVIRONMENTAL LABORATORY

LR R1335

Pg 6 of 8

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. P.Date Received: 3-24-97Date Used: 3-24-97Analyst: 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												
	7.8	7.6	7.4	7.1	6.8	6.3	5.9	5.4	5.1	4.7	4.2	19.6
10	7.3	7.1	6.9	6.6	6.2	5.9	5.6	5.3	5.1	4.8	4.5	20.3
BK CONTROL II												
	6.6	6.1	5.9	5.3	4.9	4.4	4.0	3.7	3.2	2.8	2.4	20.2

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												
	7.6	7.3	6.9	6.5	6.1	5.7	5.3	4.9	4.5	4.0	3.6	20.2
10	8.9	9.0	9.0	8.8	8.5	8.4	8.3	8.1	7.9	7.7	7.6	19.1
BK CONTROL II												
	8.1	7.6	7.4	7.0	6.6	6.2	5.8	5.4	5.0	4.6	4.2	19.6

pH of activated sludge mixed liquor:

Initial 8.0Final 8.5

pH of highest concentration tested:

Initial 8.0Final 8.9Test temperature: 19.1 - 20.3

CALCULATION OF RESULTS

OECD Activated Sludge
Respiration Inhibition Test #209Lr: R1335
Date: 3/24/97

Test Substance: 3,5-Dichlorophenol

Analyst: Trudy Stiefenhofer

30-Minute Exposure

mg/L	Dissolved Oxygen Concentrations, mg/L											Temp. °C	R (1)	Ra (2)	% Inh. (3)
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5				
BK1	7.8	7.6	7.4	7.1	6.8	6.3	5.9	5.4	5.1	4.7	4.2	19.6	0.7770	0.7989	
10	7.3	7.1	6.9	6.6	6.2	5.9	5.6	5.3	5.1	4.8	4.5	20.3	0.5891	0.5769	29.2
BK2	6.6	6.1	5.9	5.3	4.9	4.4	4.0	3.7	3.2	2.8	2.4	20.2	0.8424	0.8308	

% diff in controls (4): 3.9

RaCm (5): 0.8148

3-Hour Exposure

mg/L	Dissolved Oxygen Concentrations, mg/L											Temp. °C	R (1)	Ra (2)	% Inh. (3)
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5				
BK1	7.6	7.3	6.9	6.5	6.1	5.7	5.3	4.9	4.5	4.0	3.6	20.2	0.8194	0.8081	
10	8.9	9.0	9.0	8.8	8.5	8.4	8.3	8.1	7.9	7.7	7.6	19.1	0.3333	0.3549	55.9
BK2	8.1	7.6	7.4	7.0	6.6	6.2	5.8	5.4	5.0	4.6	4.2	19.6	0.7782	0.8001	

% diff in controls (4): 1.0

RaCm (5): 0.8041

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

(2) Ra= R adjusted to 20°C= [R/x.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

s= 1.072

RaCm= mean adjusted R, controls

Bk= blank control, S= sample

Tobs= sample temp

x= Tobs-20°C

898048
3-24-97
TKS
K1335

Chain of Custody Record

3M Environmental Laboratory

Form 35176 - PWO

04278

3M Requester
3M Project Lead
3M Project Number
3M Department Number
Lab Request/Template Number

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) Debra L Favre		Contractor Face		Number Containers of each						Total Containers of Each Item	☐ Samples Split See Attachment	Comments
Sample Signature Debra L Favre		Date 3/24/97		Preservatives								
Item #	Sample Description	Date	Time	Matrix	None	HNO ₃	H ₂ SO ₄	NaOH	VOC			
1	Mixed liquor	3/24/97	0915							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	Debra L Favre/3M	0912	3/24/97	Self	Linda Stuyvenberg	0913	3-24-97