

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-201AF. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was not noted. The test sample is FC-201AF. Current information indicates it is a mixture of 3% PFOS, 18.59% diethylene glycol butyl ether, 37.81% water, 14% ethylene glycol, 8.25% Acrylic foamer, 9% sodium octyl sulfate, 0.3% sodium lauryl sulfate, 3% triethanolamine, 6% sulfonated alkyl ether salts, 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 Guideline 209

Test type: Static acute

GLP: No

Year Completed: 1995

Analytical monitoring: Dissolved oxygen, 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: 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 7.3. Test substance was added directly to the test vessels.

Test vessels: Not given.

Number of concentrations: one plus blank control, in duplicate and 1 reference substance

Temperature: 19.3 – 20.7°C.

Total suspended solids and pH on day of testing: 4.2 g/L (TSS in final: 1.7 g/L), initial pH 6.0 and final pH 6.5.

Element Basis: Respiration inhibition as determined by oxygen consumption.

Remarks: Uptake rates corrected to 20°C for calculations.

RESULTS

Nominal concentrations: Blank controls and 1000 mg/L in duplicate plus one 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 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 EC₅₀ 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. 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 the solution.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, Lab Request number N2964, 1995.

OTHER

Last changed: 6/26/00

ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE MIXED LIQUOR

LR N2964
Date 9/14/95
Analyst GLS
Page 1 of 7Type Test OECD Activated Sludge Respiration Inhibition Test #209ACTIVATED SLUDGE MIXED LIQUORDate Obtained 9/14/95 Date Used 9/14/95Mixed Liquor Suspended Solids (MLSS) 4.2 g/L Adjusted to — g/LMLSS in final test solution 1.7 g/LInitial pH 6.0 (1st test soln. prepared) Final pH 6.5 (last soln. prepared)Comments After letting sludge settle out, decanted ~2L off the top layer
then mixed remaining sludge.TEST SUBSTANCESample Description EC-201 AFInitial pH of highest conc. tested (1000 mg/L) = 7.1 Adjusted to pH —Comments —REFERENCE SUBSTANCESample Description 3,5-DichlorophenolInitial pH of stock solution (10 mg/L) = 7.3 Adjusted to pH —Comments —EXPOSURE CONDITIONSTest Duration 3 hrsTest Temperature Range: 19.3 to 20.7 °CComments —

LR N2964

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
TEST SOLUTIONS PREPARATION

Test Substance: FC-201 AF

Sample Description: Amber-brown liquid

Analyst: GWS

Date: 9/14/95

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
<u>Blank Control</u>	<u>—</u>	<u>294</u>	<u>16</u>	<u>200</u>	<u>500</u>
<u>1000</u>	<u>500</u>	<u>294</u>	<u>16</u>	<u>200</u>	<u>500</u>

Tested pH at 1000 mg/L, pH = 7.1

(1) Millipore Milli-QR high purity water

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LR N2964

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-201AFSource of Activated Sludge Mixed Liquor: Metro/Waste Water Treatment Plant St Paul, MNDate Received: 9/14/95Date Used: 9/14/95Analyst: GLJ

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	7.0	6.6	6.3	6.0	5.6	5.3	5.0	4.7	4.4	4.1	3.7	19.9
1000	4.2	3.4	2.8	2.2	1.5	0.9						20.7
DUP	4.0	3.3	2.8	2.2	1.5	1.0						20.6
BK CONTROL II	8.0	7.6	7.4	7.2	6.9	6.6	6.3	6.1	5.8	5.4	5.0	19.8

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	7.7	7.4	7.2	6.9	6.7	6.4	6.1	5.8	5.5	5.3	4.9	19.4
1000	5.1	4.5	4.0	3.6	3.0	2.6	2.0	1.6	1.0	0.5		20.3
DUP	6.3	5.8	5.4	4.9	4.4	3.9	3.4	3.0	2.5	2.0	1.5	19.9
BK CONTROL II	8.3	8.0	7.8	7.5	7.2	7.0	6.7	6.4	6.1	5.8	5.5	19.3

pH of activated sludge mixed liquor:

Initial 6.0Final 6.5

pH of highest concentration tested:

Initial 6.7Final 6.8Test temperature: 19.3, 20.7

ENVIRONMENTAL LABORATORY
OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #200
CALCULATION OF RESULTS

Lr: N2964
Date: 9/14/95

Test Substance: EC-201AF

Analyst: GWJ

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	7.0	6.6	6.3	6.0	5.6	5.3	5.0	4.7	4.4	4.1	3.7	19.9	0.6364	0.6408	
1000.0	4.2	3.4	2.8	2.2	1.5	0.9						20.7	1.2600	1.2001	-97
Dup.	4.0	3.3	2.8	2.2	1.5	1.0						20.6	1.1800	1.1318	-86
BK2	8.0	7.6	7.4	7.2	6.9	6.6	6.3	6.1	5.8	5.4	5.0	19.8	0.5709	0.5789	

% diff in controls (4): 10.2

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

RaCm (5): 0.6099

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	7.7	7.4	7.2	6.9	6.7	6.4	6.1	5.8	5.5	5.3	4.9	19.4	0.5552	0.5788	
1000.0	5.1	4.5	4.0	3.6	3.0	2.6	2.0	1.6	1.0	0.5		20.3	1.0000	0.9794	-68
Dup.	6.3	5.8	5.4	4.9	4.4	3.9	3.4	3.0	2.5	2.0	1.5	19.9	0.9600	0.9667	-66
BK2	8.3	8.0	7.8	7.5	7.2	7.0	6.7	6.4	6.1	5.8	5.5	19.3	0.5600	0.5879	

% diff in controls (4): 1.6

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

RaCm (5): 0.5834

- (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
RaCm= mean adjusted R, controls
Bk= blank control, S= sample
Tobs= sample temp
x= Tobs-20°C

1994/08/17

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LR N2965/N2964

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209 TEST SOLUTIONS PREPARATION

Test Substance: 3,5-Dichlorophenol Reference Substance

Sample Description: Aldrich Chemical Co. Red Label Lot # 00410KZ

Analyst: G.W.J.

Date: 9/14/95

Stock Solution

Preparation: 0.5g/L; 1 mL = 0.5mg

Weighed out 0.5g 3,5-DCP. Then added to 10 mL of 1N NaOH and diluted up to ~30 mL with Milli-Q H₂O, while stirring, until dissolved. Then added ~8 mL of 1N H₂SO₄ while stirring, until the point of incipient precipitation. Then finally diluted to the mark of 1L with Milli-Q H₂O.

Initial pH = 7.3

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
Blank Control	—	284	16	200	500
10	10	274	16	200	500

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

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LR N2964/N2965

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: 3,5-Dichlorophenol Reference Substance
 Source of Activated Sludge Mixed Liquor: Metcalf Water Treatment Plant, St. Paul, MN
 Date Received: 9/14/95 Date Used: 9/14/95
 Analyst: GUS

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
EK CONTROL I	7.0	6.6	6.3	6.0	5.6	5.3	5.0	4.7	4.4	4.1	3.7	19.9
10	8.0	7.8	7.7	7.5	7.2	7.0	6.8	6.6	6.4	6.1	5.9	20.3
EK CONTROL II	8.0	7.6	7.4	7.2	6.9	6.6	6.3	6.1	5.8	5.4	5.0	19.8

3-HOUR EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS, mg/L

Test Substance Concentration (mg/L)	0.5	1.0	1.5	2.0	TIME - MINUTES			4.0	4.5	5.0	5.5	Temp °C
					2.5	3.0	3.5					
EK CONTROL I	7.7	7.4	7.2	6.9	6.7	6.4	6.1	5.8	5.5	5.3	4.9	19.4
10	8.3	8.2	8.1	8.0	7.9	7.7	7.6	7.4	7.3	7.1	7.0	20.4
EK CONTROL II	8.3	8.0	7.8	7.5	7.2	7.0	6.7	6.4	6.1	5.8	5.5	19.3

pH of activated sludge mixed liquor: Initial 6.0 Final 6.5
 pH of highest concentration tested: Initial 6.5 Final 7.0
 Test temperature: 19.3 + 20.4

ENVIRONMENTAL LABORATORY
OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTS

L.R N2965/N2964
Date: 9/14/95

Test Substance: 3,5-DICHLOROPHENOL

Analyst: GWJ

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	7.0	6.6	6.3	6.0	5.6	5.3	5.0	4.7	4.4	4.1	3.7	19.9	0.6364	0.6408	
10	8.0	7.8	7.7	7.5	7.2	7.0	6.8	6.6	6.4	6.1	5.9	20.3	0.4339	0.4250	30
BK2	8.0	7.6	7.4	7.2	6.9	6.6	6.3	6.1	5.8	5.4	5.0	19.8	0.5709	0.5789	

% diff in controls (4): 10.2

RaCm (5): 0.6099

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	7.7	7.4	7.2	6.9	6.7	6.4	6.1	5.8	5.5	5.3	4.9	19.4	0.5552	0.5788	
10	8.3	8.2	8.1	8.0	7.9	7.7	7.6	7.4	7.3	7.1	7.0	20.4	0.2776	0.2700	54
BK2	8.3	8.0	7.8	7.5	7.2	7.0	6.7	6.4	6.1	5.8	5.5	19.3	0.5600	0.5879	

% diff in controls (4): 1.6

RaCm (5): 0.5834

- (1) R = Oxygen uptake rate (mg O₂/l-min)
 (2) Ra = R adjusted to 20°C = [R × 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

α = 1.072
 RaCm = mean adjusted R, controls
 Bk = blank control, S = sample
 Tobs = sample temp
 x = Tobs - 20°C

LR N2964
p37057

Chain of Custody Record
3M Environmental Laboratory

08672

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

Form 35176 - PWO

3M Facility MWCC Piquette	3M Project Title Household Liquor Pickup Sept 95	3M Project Number
Address	3M Facility Contact	3M Department Number
City, State, Zip Code	3M Template Number	Lab Request/Template Number

Sampled By (Print) Jim Krueger		Contractor PACE		Number Containers of each		Total Containers of Each Item	☐ Samples Split See Attachment Comments
Sampler Signature 		Date 9-14-95		Preservatives			
Item #	Sample Description	Date	Time	Matrix	None HNO ₃ H ₂ SO ₄ NaOH VOC		
1	Mixed liquor P.U.	9/14/95	0725	W	1	1	5 gallon Cubitainer
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	/ PACE	755	9/14/95	Self		7:55	9/14/95

Original - Accompanying Sample

Last Page - Original

See Reverse Side For Instructions