

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

Identity: Mixture containing Perfluorooctanesulfonate; may also be referred to as PFOS, FC-95, or as a component of FC-3072. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was not noted. The test sample is FC-3072. Current information indicates it is a mixture of 1.67% test substance, 33.33% diethylene glycol butyl ether, 29.23% water, 20.83% urea, 5% sodium octyl sulfate, 3.33% sulfonated alkyl ether sulfate salts, 4.60% acrylic foamer, 1.67% triethanolamine, and 0.33% tolyl triazole.

The following summary applies to the test sample as a mixture of the test substance in water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the fluorochemical component.

METHOD

Method: OECD Guideline 209

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

Test vessels: Not given.

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

Temperature: 20.7 – 21.7 °C

Total suspended solids and pH on day of testing: 4.0 g/L (TSS in final: 1.6 g/L), final pH 7.0

Element Basis: Respiration inhibition as determined by oxygen consumption.

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

RESULTS

Nominal concentrations: 2 Blank controls, 100, 180, 320, 560, and 1000 mg/L. 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 of the test substance 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 solution.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, Lab Request number M3597, 1995.

OTHER

Last changed: 6/25/00

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ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE MIXED LIQUOR

LR M 30747
Date 3/20/95
Analyst CWS
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Type Test OECD Activated Sludge Respiratory Inhibition Test #209

ACTIVATED SLUDGE MIXED LIQUOR

Date Obtained 3/20/95

Date Used 3/20/95

Mixed Liquor Suspended Solids (MLSS) 4.0 g/L Adjusted to g/L

MLSS in final test solution 1.6 g/L $\text{Calc: } (\text{MLSS} (3/L) \times 0.2 L) \times 2 = \text{MLSS Final Soln.}$

Initial pH NA (1st test soln. prepared)

Final pH 7.0 (last soln. prepared)

Comments Initial pH was not taken on Control I

TEST SUBSTANCE

Sample Description FC - 3072 AFFF Base (cont. Diethylene glycol -
cont. Methylcellulose / Alkyl Sulfates)

Initial pH of highest conc. tested (1000 mg/L) = 6.0

Adjusted to pH

Comments

REFERENCE SUBSTANCE

Sample Description 3,5-DCP Reference Substance

Initial pH of stock solution (10 mg/L) = 7.0

Adjusted to pH

Comments

EXPOSURE CONDITIONS 3/21/95

Test Duration 30 min / 3 hrs

Test Temperature Range: 20.7 - 21.7 °C

Comments Samples Froamed up considerably when aerated
with sludge during the 30 min readings
Samples did not foam as much at the readings
pH of Initial Activated Sludge was not taken
accurately

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LR M3597

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST # 209 TEST SOLUTIONS PREPARATION

Test Substance: EC-3072 AFFF Base

Sample Description: Cent. Diethylene glycol monobutyl ether / Alkyl Sulfate Salts
Dark Amber Liquid.

Analyst: GWJ

Date: 3/20/95

(mg/L) Test Conc. % by vol.	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
Blk Control I	—	284	16	200	500
100	50				
180	90				
320	160				
560	280				
1000	500				
Blk Control II					

Initial pH of High Conc (1000 mg/L)

pH Adjusted to Adj. with
7.4

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

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ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: EC-3072 AFFF Base

Source of Activated Sludge Mixed Liquor: Metropolitan Water Treatment Plant, St. Louis

Date Received: 3/20/95

Date Used: 3/20/95

Analyst: BWJ

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	5.9	5.5	5.1	4.8	4.5	4.1	3.7	3.4	3.0	2.7	2.4	21.2
100	4.4	4.1	3.7	3.3	3.0	2.6	2.2	1.9	1.4	1.0	0.6	20.8
200	5.0	4.7	4.3	4.0	3.5	3.1	2.7	2.4	2.0	1.6	1.2	20.7
320	1.0	0.6										21.5
500	0.6											21.6
600	0.8											21.7
BK CONTROL II	6.1	5.8	5.4	5.1	4.7	4.4	4.0	3.6	3.2	2.9	2.6	20.7

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	6.2	6.0	5.6	5.3	4.9	4.6	4.2	3.8	3.5	3.1	2.7	20.8
100	5.6	5.2	4.9	4.6	4.1	3.8	3.4	3.0	2.7	2.3	1.9	21.2
180	5.4	5.1	4.8	4.5	4.2	3.9	3.6	3.3	3.0	2.7	2.4	21.2
320	5.8	5.5	5.2	4.9	4.5	4.2	3.9	3.6	3.2	2.8	2.5	21.1
500	6.3	6.1	5.8	5.5	5.2	5.0	4.7	4.4	4.1	3.8	3.5	20.8
600	5.3	4.9	4.6	4.3	4.0	3.7	3.3	3.0	2.7	2.4	2.1	21.1
BK CONTROL II	6.1	5.7	5.4	5.1	4.6	4.3	4.0	3.6	3.2	2.8	2.5	20.7

pH of activated sludge mixed liquor: Initial 7.6 NA Final 6.8-7.0
 pH of highest concentration tested: Initial 6.0 Final 7.1
 Test temperature: 20.7 to 21.7 °C

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

Lr: M3597
Date: 3/20/95

Test Substance: FC-3072 AFFF BASE

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	5.9	5.5	5.1	4.8	4.5	4.1	3.7	3.4	3.0	2.7	2.4	21.2	0.6958	0.6401	
100.0	6.4	6.1	5.7	5.3	5.0	4.6	4.2	3.8	3.4	3.0	2.6	20.8	0.7745	0.7326	-10
180.0	5.0	4.7	4.3	4.0	3.5	3.1	2.7	2.4	2.0	1.6	1.2	20.7	0.7770	0.7401	0
320.0	1.0	0.6										21.5	* NA	NA	
560.0	0.6											21.6	NA	NA	
1000	0.8											21.7	NA	NA	
BK2	6.1	5.8	5.4	5.1	4.7	4.4	4.0	3.6	3.2	2.9	2.6	20.7	0.7212	0.6870	
% diff in controls (4): 7.1												EC50 (95% CI): > 1000 mg/L			
												RaCm (5): 0.6635			

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	6.2	6.0	5.6	5.3	4.9	4.6	4.2	3.8	3.5	3.1	2.7	20.8	0.7261	0.6868	
100.0	5.6	5.2	4.9	4.6	4.1	3.8	3.4	3.0	2.7	2.3	1.9	21.2	0.7406	0.6813	0
180.0	5.4	5.1	4.8	4.5	4.2	3.9	3.6	3.3	3.0	2.7	2.4	21.2	0.6000	0.5520	20
320.0	5.8	5.5	5.2	4.9	4.5	4.2	3.9	3.6	3.2	2.8	2.5	21.1	0.6703	0.6210	10
560.0	6.3	6.1	5.8	5.5	5.2	5.0	4.7	4.4	4.1	3.8	3.5	20.8	0.5709	0.5400	22
1000	5.3	4.9	4.6	4.3	4.0	3.7	3.3	3.0	2.7	2.4	2.1	21.1	0.6303	0.5839	15
BK2	6.1	5.7	5.4	5.1	4.6	4.3	4.0	3.6	3.2	2.8	2.5	20.7	0.7248	0.6904	
% diff in controls (4): 0.5												EC 50 (95% CI): > 1000 mg/L			
												RaCm (5): 0.6886			

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

* NA = Not enough information to calculate.

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LR 113597

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
TEST SOLUTIONS PREPARATION

Test Substance: 3,5-Dichlorophenol Reference Solution
 Sample Description: Red Label Aldrich Chemical Co. Lot # CC-100KZ
 Analyst: GLJ
 Date: 5/20/95

Stock Solution Preparation: dissolved 0.5g 3,5-DCP in 10ml of 1M NaOH, diluted to approximately 200 ml with Milli-Q H₂O, adding water to bring to 1L. pH 8.5 to 9.0 to prevent precipitation. Final pH of Stock soln = 7.0
Stock Soln = 500 mg/L 1 ml = 0.5 mg

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	1	1	1
Blank Control	—	284	1	1	1

5/21/95
~~Initial pH of 10 mg/L 3,5-DCP~~ ~~pH~~ ~~Adj to~~ ~~Adj with~~
~~7.0~~

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

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LR M3597

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: 3,5-DichlorophenolSource of Activated Sludge Mixed Liquor: Metro Waste Water Treatment Plant at Bldg.Date Received: 7/20/95Date Used: 7/20/95Analyst: GOS

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	5.9	5.5	5.1	4.8	4.5	4.1	3.7	3.4	3.0	2.7	2.4	21.2
16	6.6	6.4	6.2	5.9	5.7	5.5	5.2	5.0	4.8	4.6	4.4	21.4
BK CONTROL II	6.1	5.8	5.4	5.1	4.7	4.4	4.0	3.6	3.2	2.9	2.6	20.7

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	6.2	6.0	5.6	5.3	4.9	4.6	4.2	3.8	3.5	3.1	2.7	20.8
16	7.2	7.1	7.0	6.8	6.6	6.6	6.4	6.3	6.1	5.9	5.8	21.2
BK CONTROL II	6.1	5.7	5.4	5.1	4.6	4.3	4.0	3.6	3.2	2.8	2.5	20.7

pH of activated sludge mixed liquor:

Initial 7.0 NaOHFinal 6.8 7.0

pH of highest concentration tested:

Initial 7.0Final 7.7Test temperature: 20.7 - 21.4 °C

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

Lr: M3597
Date: 3/20/95

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	5.9	5.5	5.1	4.8	4.5	4.1	3.7	3.4	3.0	2.7	2.4	21.2	0.6958	0.6401			
10	6.6	6.4	6.2	5.9	5.7	5.5	5.2	5.0	4.8	4.6	4.3	21.4	0.4606	0.4179	37		
BK2	6.1	5.8	5.4	5.1	4.7	4.4	4.0	3.6	3.2	2.9	2.6	20.7	0.7212	0.6870			
% diff in controls (4):			7.1										RaCm (5):			0.6635	

% diff in controls (4): 7.1

RaCm (5): 0.6635

3-Hour Exposure			Dissolved Oxygen Concentrations, mg/L										Temp, °C	R (1)	Ra (2)	% Inh. (3)
mg/L	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5					
BK1	6.2	6.0	5.6	5.3	4.9	4.6	4.2	3.8	3.5	3.1	2.7	20.8	0.7261	0.6868		
10	7.2	7.1	7.0	6.8	6.6	6.6	6.4	6.3	6.1	5.9	5.8	21.2	0.2909	0.2676	61	
BK2	6.1	5.7	5.4	5.1	4.6	4.3	4.0	3.6	3.2	2.8	2.5	20.7	0.7248	0.6904		

% diff in controls (4): 0.5

RaCm (5): 0.6886

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

(2) Ra= R adjusted to 20 °C = [R/a 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

a= 1.072

RaCm= mean adjusted R, controls

Bk= blank control, S= sample

Tobs= sample temp

x= Tobs-20 °C

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Chain of Custody Record

3M Environmental Laboratory

02663

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

Form 3500-1-90

3M Facility	3M Main Plant	Material Type	Mixed Liquor
Address		Material Contact	
City/State/Zip Code		Material Quantity	

Signature/Print		Date		Number Containers of each		Total Containers of Each Item	☐ Samples Split See Attachment Comments			
Signature		Date		Preservatives						
Item #	Sample Description	Date	Time	Matrix	None	HNO ₃	H ₂ SO ₄	NaOH	VOC	Substances
1	Mixed Liquor	3/20/95	9:37							2
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. Fawcett	9:37	3/20/95	Self	Debra L. Fawcett	9:37	3/20/95

See Reverse Side For Instructions