

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

ENVIRONMENTAL LABORATORY
ACTIVATED SLUDGE MIXED LIQUOR

LR M 3-747
Date 3/20/95
Analyst CUCJ
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Type Test CECD Activated Sludge Respiratory Inhibition Test #209

ACTIVATED SLUDGE MIXED LIQUOR

Date Obtained 2/20/95 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 Calc. $(MLSS(3/L) \times 0.2L) \times 2 = MLSS \text{ Final Sol.}$
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 AFFE Base (cont. Diethylene glycol -
cont. monobutylether/Alkal. Sulfate S.
Initial pH of highest conc. tested 1.0 = -6.0 Adjusted to pH
Comments

REFERENCE SUBSTANCE

Sample Description 3,5-DCP Reference Substance
Initial pH of stock solution (1.0 mg/L) = 7.0 Adjusted to pH
Comments

EXPOSURE CONDITIONS

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 30min 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
Analyst: GWJ Dark Amber Liquid.
Date: 3/20/95

Test Conc. (mg/L) % 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				
150	90				
320	160				
500	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

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

LR 415347

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Test Substance: FC-3072 AFFF Base

Source of Activated Sludge Mixed Liquor: Metcalf & Eddy Treatment Plant St. Paul

Date Received: 3/30/95

Date Used: 3/30/95

Analyst: EWJ

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	6.4	6.1	5.7	5.3	5.0	4.6	4.2	3.8	3.4	3.0	2.6	20.8
180	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
560	0.6											21.6
1000	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
560	6.3	6.1	5.8	5.5	5.2	5.0	4.7	4.4	4.1	3.8	3.5	20.8
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
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 ± 0.1 °C

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	0.7408	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 E_s]
 (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
 s = Tobs - 20°C

* NA = Not enough information to calculate.

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LR #13597

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
TEST SOLUTIONS PREPARATION

Test Substance: 3,5-Dichlorophenol Reference Substance

Sample Description: Red Label Aldrich Chemical Co. Lot # C0010KZ

Analyst: G.W.J.

Date: 5/20/95

Stock Solution

Preparation: disolved 0.5g 3,5-DCP in 10ml of 1N H₂SO₄ dilute
to approximately 300 ml with distilled H₂O, adding more
1N H₂SO₄ to the point of incipient precipitation = 500 ml.
Final pH of stock soln = 7.0

Stock Soln = 500mg/L 1 ml = 0.5mg

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	↓	↓
Blank Control	—	284	↓	↓	↓

3/21/95 pH of 10 mg/L 3,5-DCP = 7.0 Adj. to Adj. to

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

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LR M3547

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: 3,5-Dichlorophenol

Source of Activated Sludge Mixed Liquor: Metro Waste Water Treatment Plant St. Paul

Date Received: 3/20/95

Date Used: 3/20/95

Analyst: GLOS

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	5.4	5.5	5.1	4.8	4.5	4.1	3.7	3.4	3.0	2.7	2.4	21.2
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
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
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
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

NAOH

3/20/95

Final

6.8

7.0

3/20/95

pH of highest concentration tested:

Initial

7.0

Final

1.1

Test temperature: 20.7 - 21.4

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

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/θ^{T-20}]

(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

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Chain of Custody Record
3M Environmental Laboratory

02663

3M Requester
3M Project Lead

Form 3517E EVO

3M Facility 3M - Main Plant	3M Project Name Mixed Liquor	3M Project Number
Address	3M Facility Contact	3M Department Number
City, State, Zip Code	3M Facility Phone Number	Lab Request/Template Number

Signed By (Print) Debra L. Fawcett		Date 3/20/95		Number Containers of each						Total Containers of Each Item	☐ Samples Split See Attachment Comments
Signature <i>Debra L. Fawcett</i>				Preservatives							
Item #	Sample Description	Date	Time	Matrix	None	HNO ₃	H ₂ SO ₄	NaOH	VOC	5 other substances	
1	Mixed Liquor	3/20/95	11:37							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	<i>Debra L. Fawcett</i>	11:37	3/20/95	Self	<i>J. L. [Signature]</i>	9:37	3/20/95

See Reverse Side For Instructions