

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-3003. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was not given. The test sample is FC-3003. Current information indicates it is a mixture of 1% PFOS, 15% diethylene glycol butyl ether, 79.39% water, 2% Sultone foamer, 1.5% sodium octyl sulfate, 1% sodium decyl sulfate, 0.06% sodium lauryl sulfate, and 0.05% benzotriazole.

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

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

Test vessels: Not given.

Number of concentrations: five plus blank control

Temperature: 19.2 – 21.6°C

Total suspended solids and pH on day of testing: 4.04 g/L (TSS in final 1.61 g/L), initial pH 7.9 and final pH 8.6

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 test substance 3-hour EC₅₀ for activated sludge respiration inhibition was determined to be >1000 mg/L. This concentration inhibited respiration by ~24%.

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

DATA QUALITY

Reliability: Klimisch ranking = 2. The study meets all 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 R1331, 1997.

OTHER

Last changed: 6/26/00

002704

ENVIRONMENTAL LABORATORY

LR R1331
Pg 2058

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209 TEST SOLUTIONS PREPARATION

Test Substance: FC-3003 AFFF

Sample Description: golden brown liquid

Analyst: TKS

Date: 3-21-97

Test Conc. mg/L	mg added per 500 mL	well 3-24-97 TKS mL MQ(+) 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

Initial pH of highest conc. tested = 7.6

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

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-3003 AFFFSource of Activated Sludge Mixed Liquor: Metro Waste Water TreatmentPlant
St. Pa
mDate Received: 3-21-97Date Used: 3-21-97Analyst: 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	7.6	7.3	6.7	6.2	5.8	5.4	4.9	4.2	3.9	3.2	2.8	21.6
10	6.7	6.5	6.0	5.6	5.1	4.5	4.0	3.5	3.1	2.5	2.0	21.5
50	7.1	6.5	6.1	5.5	4.8	4.2	3.7	3.3	2.8	2.2	1.8	21.4
100	6.8	6.2	5.5	4.9	4.3	3.8	3.4	2.9	2.4	1.8	1.3	21.1
500	6.8	6.0	5.5	4.9	4.2	3.7	3.2	2.7	2.2	1.7	1.2	20.8
1000	5.8	5.2	4.3	3.4	2.9	2.4	2.0	1.1	0.5	—	—	21.0
1000	5.8	5.1	4.3	3.6	2.9	2.3	1.6	0.9	0.5	—	—	20.6
BK CONTROL II	5.1	4.6	4.2	3.7	3.3	2.7	2.4	1.9	1.4	1.0	0.6	21.0

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	9.0	8.7	8.5	8.2	7.8	7.5	7.0	6.5	6.1	5.7	5.3	19.2
10	8.4	8.3	8.0	7.5	7.1	6.7	6.1	5.7	5.4	4.8	4.4	20.0
50	8.7	8.5	8.1	7.8	7.4	6.9	6.5	6.2	5.7	5.4	4.9	19.5
100	8.2	7.9	7.6	7.1	6.7	6.3	6.0	5.6	5.1	4.7	4.2	19.9
500	8.4	8.1	7.7	7.4	7.0	6.8	6.4	6.0	5.7	5.3	5.0	19.8
1000	8.6	8.4	8.0	7.8	7.5	7.0	6.7	6.4	6.2	5.8	5.4	19.7
1000	8.6	8.4	8.1	7.9	7.7	7.4	7.0	6.8	6.5	6.1	5.8	19.8
BK CONTROL II	7.9	7.6	7.2	6.8	6.3	5.9	5.5	5.1	4.6	4.3	3.9	20.3

pH of activated sludge mixed liquor:

Initial 7.9Final 8.6

pH of highest concentration tested:

Initial 7.9Final 8.5Test temperature: 19.2-21.6

CALCULATION OF RESULTS

Test Substance: EC-3003 AFFFAnalyst: 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	7.6	7.3	6.7	6.2	5.8	5.4	4.9	4.2	3.9	3.2	2.8	21.6	0.9915	0.8871	
10	6.7	6.5	6.0	5.6	5.1	4.5	4.0	3.5	3.1	2.5	2.0	21.5	1.0036	0.9042	0
50	7.1	6.5	6.1	5.5	4.8	4.2	3.7	3.3	2.8	2.2	1.8	21.4	1.0679	0.9688	0
100	6.8	6.2	5.5	4.9	4.3	3.8	3.4	2.9	2.4	1.8	1.3	21.1	1.0558	0.9780	0
500	6.8	6.0	5.5	4.9	4.2	3.7	3.2	2.7	2.2	1.7	1.2	20.8	1.0703	1.0124	0
1000	5.8	5.2	4.3	3.4	2.9	2.4	2.0	1.1	0.5			21.0	1.2762	1.1905	0
1000	5.8	5.1	4.3	3.6	2.9	2.3	1.6	0.9	0.5			20.6	1.3286	1.2743	0
BK2	5.1	4.6	4.2	3.7	3.3	2.7	2.4	1.9	1.4	1.0	0.6	21.0	0.9018	0.8412	

% diff in controls (4): 5.3

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

RaCm (5): 0.8642

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	9.0	8.7	8.5	8.2	7.8	7.5	7.0	6.5	6.1	5.7	5.3	19.2	0.7891	0.8342	
10	8.4	8.3	8.0	7.5	7.1	6.7	6.1	5.7	5.4	4.8	4.4	20.0	0.8824	0.8824	0
50	8.7	8.5	8.1	7.8	7.4	6.9	6.5	6.2	5.7	5.4	4.9	19.5	0.7976	0.8258	0
100	8.2	7.9	7.6	7.1	6.7	6.3	6.0	5.6	5.1	4.7	4.2	19.9	0.8145	0.8202	0.5
500	8.4	8.1	7.7	7.4	7.0	6.8	6.4	6.0	5.7	5.3	5.0	19.8	0.6861	0.6957	15.6
1000	8.6	8.4	8.0	7.8	7.5	7.0	6.7	6.4	6.2	5.8	5.4	19.7	0.6545	0.6683	18.9
1000	8.6	8.4	8.1	7.9	7.7	7.4	7.0	6.8	6.5	6.1	5.8	19.8	0.5758	0.5838	29.2
BK2	7.9	7.6	7.2	6.8	6.3	5.9	5.5	5.1	4.6	4.3	3.9	20.3	0.8315	0.8144	

% diff in controls (4): 2.4

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

RaCm (5): 0.8243

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

(2) Ra= R adjusted to 20°C= [R/s.Ex]

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

(4) % difference in controls: [(IRaBk1-RaBk2)/RaCm]100 (Two controls must be within 15% of each other.)

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

s= 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 R1331
Pg 45 of 8

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST # 209
TEST SOLUTIONS PREPARATION

Test Substance: 3,5-Dichlorophenol

Sample Description: ALDRICH (RED LABEL) LOT # 09615KG DQ

Analyst: TKS

Date: 3-21-97

Stock Solution

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

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

Diluted to approx 30ml with Milli-Q water

Add 8.0ml 1.0N H₂SO₄ to incipient ppt

Dilute to 1.0L with Milli-Q water

Initial pH stock sol = 6.3

Test Conc. mg/L	mL added per 500 mL	well 3-21-97 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

Initial pH highest conc tested = 7.8

(1) ~~Millipore Milli-Q~~ high purity water TKS 3-21-97

002708

ENVIRONMENTAL LABORATORY

LR R 1331

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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. Pa
NDate Received: 3-21-97Date Used: 3-21-97Analyst: TXS

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	7.6	7.3	6.7	6.2	5.8	5.4	4.9	4.2	3.9	3.2	2.8	21.6
10	8.2	7.9	7.6	7.2	6.9	6.7	6.4	6.0	5.7	5.4	5.0	21.0
BK CONTROL II	5.1	4.6	4.2	3.7	3.3	2.7	2.4	1.9	1.4	1.0	0.6	21.0

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	9.0	8.7	8.5	8.2	7.8	7.5	7.0	6.5	6.1	5.7	5.3	19.2
10	9.0	8.9	8.8	8.5	8.4	8.2	8.0	7.8	7.5	7.4	7.2	20.1
BK CONTROL II	7.9	7.6	7.2	6.8	6.3	5.9	5.5	5.1	4.6	4.3	3.9	20.3

pH of activated sludge mixed liquor:

Initial 7.9Final 8.6

pH of highest concentration tested:

Initial 8.0Final 8.4Test temperature: 19.2 - 21.6

002705

CALCULATION OF RESULTS

Test Substance: 3,5-DichlorophenolAnalyst: Trudy Stiefenhofer

30-Minute 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	7.6	7.3	6.7	6.2	5.8	5.4	4.9	4.2	3.9	3.2	2.8	21.6	0.9915	0.8871					
10	8.2	7.9	7.6	7.2	6.9	6.7	6.4	6.0	5.7	5.4	5.0	21.0	0.6303	0.5880	32.0				
BK2	5.1	4.6	4.2	3.7	3.3	2.7	2.4	1.9	1.4	1.0	0.6	21.0	0.9018	0.8412					

RaCm (5): 0.8642

% diff in controls (4): 5.3

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	9.0	8.7	8.5	8.2	7.8	7.5	7.0	6.5	6.1	5.7	5.3	19.2	0.7891	0.8342					
10	9.0	8.9	8.8	8.5	8.4	8.2	8.0	7.8	7.5	7.4	7.2	20.1	0.3891	0.3864	53.1				
BK2	7.9	7.6	7.2	6.8	6.3	5.9	5.5	5.1	4.6	4.3	3.9	20.3	0.8315	0.8144					

RaCm (5): 0.8243

% diff in controls (4): 2.4

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

(2) Ra= R adjusted to 20°C= [R/e.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

e= 1.072

RaCm= mean adjusted R, controls

Bk= blank control, S= sample

Tobs= sample temp

x= Tobs-20°C

Chain of Custody Record

3M Environmental Laboratory

Form 35176 - PWO

07659

3M Requester

3M Project Lead

3M Facility 3M-STMP	3M Project Title 3M-Mixed Lig.	3M Project Number Vary
Address	3M Facility Contact	3M Department Number Vary
City, State, Zip Code	3M Telephone Number	Lab Request Template Number

Sampled By (Print) Mike Harold		Contractor Pace		Number Containers of each		Total Containers of Each Item	☐ Samples Split See Attachment Comments					
Sampler Signature <i>[Signature]</i>		Date 3/21/97		Preservatives								
Item #	Sample Description	Date	Time	Matrix	None			HNO ₃	H ₂ SO ₄	NaOH	VOC	
1	Mixed Lig. P.V.	3/21/97	0730	H ₂ O	1						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	0810	3/21/97	Self	STWoj	8:13	3/21/97

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1453-21-47