

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-203CF-X or FC-203CF. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was 501. The test sample is FC-203CF-X. Current information indicates it is a mixture of 1% PFOS, 20% diethylene glycol monobutyl ether, 70.2% water, 2.75% Acrylic foamer, 3% sodium octyl sulfate, 1% triethanolamine, 2% 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 toxicity of the fluorochemical component of the test sample.

METHOD

Method: OECD Guideline 209

GLP: No

Year Completed: 1992

Analytical monitoring: Dissolved oxygen concentrations, pH and temperature.

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 10 mL 1N NaOH, diluted to 30 mL with Millipore Milli-Q™ water, then brought to the point of incipient precipitation with 1N H₂SO₄ and diluted to 1 L with Milli-Q™ water. The pH of the reference solution was measured to be 7.6. Test substance was added directly to test vessels, initial pH 6.5.

Test vessels: Not given

Number of concentrations: one (duplicated) plus 1 reference substance and 2 blank controls

Temperature: 18.6 – 21.6 °C

Total suspended solids and pH on day of testing: 2.6 g/L (TSS in final 1.04 g/L) and initial pH 6.9 and final pH 7.9.

Element Basis: Respiration inhibition as determined by oxygen consumption.

Remarks: Values corrected to 20 °C for calculations.

RESULTS

Nominal concentrations: 2 Blank controls, reference substance and 1000 mg/L (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. The 1000 mg/L treatment caused ~19% respiration inhibition at 3 hours.

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

DATA QUALITY

Reliability: Klimisch ranking 2. The study meets all criteria for quality testing. However, the 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 K1905-1, 1992.

OTHER

Last changed: 6/28/00

ENVIRONMENTAL LABORATORY
ACTIVATED SLUDGE MIXED LIQUOR

LR K1905-1+2
Date 6/26/92
Analyst SAB
Page 1 of 4

Type Test OECD Activated Sludge Respiration Inhibition #209

ACTIVATED SLUDGE MIXED LIQUOR

Date Obtained 6/26/92

Date Used 6/26/92

Mixed Liquor Suspended Solids (MLSS) 2.6 g/L Adjusted to — g/L

MLSS in final test solution 1.04 g/L

Initial pH 6.9 (1st test soln. prepared)

Final pH 7.9 (last soln. prepared)

Comments _____

TEST SUBSTANCE

Sample Description FC-203CF-X

Initial pH of highest conc. tested (1000 mg/L) = 6.5

Adjusted to pH —

Comments _____

REFERENCE SUBSTANCE

Sample Description 3,5-Dichlorophenol

Initial pH of stock solution (500 mg/L) = 7.6

Adjusted to pH —

Comments _____

EXPOSURE CONDITIONS

Test Duration 3-Hours

Test Temperature Range: 18.6-21.6 °C

Comments _____

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
TEST SOLUTIONS PREPARATION

LR K1905-1

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Test Substance: FC-203CF-X Lot 501

Sample Description: Amber Liquid

Analyst: SAB

Date: 6/26/92

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
Control	—	284	16	200	500
1000	500	284	16	200	500
Duplicate	500	284	16	200	500

Initial pH = 6.5

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

ENVIRONMENTAL LABORATORY

LR K1905-1
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OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-203CF-XSource of Activated Sludge Mixed Liquor: Metrol Waste Treatment Plant St Paul, MNDate Received: 6/26/92Date Used: 6/30/92Analyst: August A. Beach
R.D. Wright Jr

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	7.2	7.0	6.8	6.6	6.4	6.2	6.0	5.7	20.1
SUB 1000												
1000 Duplicate ^{SUB}	3.4	3.0	2.7	2.3	2.0	1.6	1.2	.9	.5			21.6
Duplicate	3.7	3.3	2.9	2.5	2.2	1.8	1.5	1.1	0.7			21.3
BK CONTROL II	7.0	6.7	6.5	6.3	6.1	5.9	5.7	5.4	5.2	5.1	4.8	20.7

3-HOUR EXPOSURE

DISSOLVED OXYGEN CONCENTRATIONS, mg/L

Test Substance Concentration ()	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	8.4	8.2	8.1	7.9	7.6	7.5	7.4	7.1	7.0	6.9	6.7	18.6
1000	6.7	6.4	6.2	6.1	5.9	5.8	5.6	5.4	5.3	5.1	5.0	21.0
Duplicate	7.1	6.9	6.8	6.6	6.4	6.2	6.1	5.9	5.8	5.6	5.3	20.8
BK CONTROL II	7.3	7.1	6.9	6.7	6.6	6.4	6.2	6.0	5.8	5.6	5.4	19.6

pH of activated sludge mixed liquor:

Initial 6.9Final 7.9

pH of highest concentration tested:

Initial 6.4Final 6.5Test temperature: 18.6 - 21.6 °C

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

Lr: K1905-1
Date: 6/26/92

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Test Substance: EC-203CE-X

Analyst: SAB/RJW

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	7.8	7.6	7.4	7.2	7.0	6.8	6.6	6.4	6.2	6.0	5.7	20.1	0.4109	0.4081	
1000.0	3.4	3.0	2.7	2.3	2.0	1.6	1.2	0.9	0.5			21.6	0.7190	0.6433	0
1000.0	3.7	3.3	2.9	2.5	2.2	1.8	1.5	1.1	0.7			21.3	0.7286	0.6656	0
BK2	7.0	6.7	6.5	6.3	6.1	5.9	5.7	5.4	5.2	5.1	4.8	20.7	0.4206	0.4006	
% diff in controls (4): 1.8												EC50 (95% CI): > 1000 mg/L			
												RaCm (5): 0.4043			

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	8.4	8.2	8.1	7.9	7.6	7.5	7.4	7.1	7.0	6.9	6.7	18.6	0.3394	0.3741	
1000.0	6.7	6.4	6.2	6.1	5.9	5.8	5.6	5.4	5.3	5.1	5.0	21.0	0.3152	0.2940	23
1000.0	7.1	6.9	6.8	6.6	6.4	6.2	6.1	5.9	5.8	5.6	5.3	20.8	0.3442	0.3256	14
BK2	7.3	7.1	6.9	6.7	6.6	6.4	6.2	6.0	5.8	5.6	5.4	19.6	0.3745	0.3851	
% diff in controls (4): 2.9												EC 50 (95% CI): > 1000 mg/L			
												RaCm (5): 0.3796			

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

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

ENVIRONMENTAL LABORATORY

LR K1905
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OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST # 209
TEST SOLUTIONS PREPARATION

Test Substance: 3,5-Dichlorophenol Reference Substance

Sample Description: Aldrich Red label Lot D7,060-0

Analyst: SAB

Date: 6/26/92

Stock Solution

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

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

Diluted to 30 ml with Milli-Q water

Added 1.0 N H₂SO₄ dropwise until incipient ppt

Diluted to one liter with Milli-Q water

Stock Soln pH = 7.6

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
BK Control	—	284	16	200	500

10	10	274	16	200	500
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(1) Millipore Milli-QR high purity water

ENVIRONMENTAL LABORATORY

LR K1905

Pg 2 of 3

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: 3,5-Dichlorophenol Reference SubstanceSource of Activated Sludge Mixed Liquor: Metra Waste Treatment Plant St Paul, MNDate Received: 6/26/92Date Used: 6/26/92Analyst: James A. Beach
R. J. Wright Jr.

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	7.3	7.6	7.4	7.2	7.0	6.8	6.6	6.4	6.2	6.0	5.7	20.1
<u>10</u>	7.8	7.5	7.5	7.3	7.2	7.0	6.9	6.8	6.6	6.5	6.3	20.8
BK CONTROL II	7.0	6.7	6.5	6.3	6.1	5.9	5.7	5.4	5.2	5.1	4.8	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	8.4	8.2	8.1	7.9	7.6	7.5	7.4	7.1	7.0	6.9	6.7	18.6
<u>10</u>	8.1	8.0	8.0	7.9	7.8	7.7	7.6	7.6	7.5	7.4	7.3	20.7
BK CONTROL II	5.3	4.1	6.9	6.7	6.6	6.4	6.2	6.0	5.8	5.6	5.4	19.6

pH of activated sludge mixed liquor: 7.3 7.1pH of highest concentration tested: 7.0Test temperature: 18.6 - 20.8 °CInitial 6.9
Initial 7.0Final 7.9
Final 7.7

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

Lr: K1905
Date: 6/26/92
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Test Substance: 3,5-DICHLOROPHENOL

Analyst: SAB/RJW

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	7.8	7.6	7.4	7.2	7.0	6.8	6.6	6.4	6.2	6.0	5.7	20.1	0.4109	0.4081	
10	7.8	7.5	7.5	7.3	7.2	7.0	6.9	6.8	6.6	6.5	6.3	20.8	0.2739	0.2591	36
BK2	7.0	6.7	6.5	6.3	6.1	5.9	5.7	5.4	5.2	5.1	4.8	20.7	0.4206	0.4006	

% diff in controls (4): 1.8

EC50 (95% CI):

RaCm (5): 0.4043

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	8.4	8.2	8.1	7.9	7.6	7.5	7.4	7.1	7.0	6.9	6.7	18.6	0.3394	0.3741	
10	8.1	8.0	8.0	7.9	7.8	7.7	7.6	7.6	7.5	7.4	7.3	20.7	0.1600	0.1524	60
BK2	7.3	7.1	6.9	6.7	6.6	6.4	6.2	6.0	5.8	5.6	5.4	19.6	0.3745	0.3851	

% diff in controls (4): 2.9

EC 50 (95% CI):

RaCm (5): 0.3796

(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

RaCm= mean adjusted R, controls

Bk= blank control, S= sample

Tobs= sample temp

x= Tobs-20°C

112654

CHAIN-OF-CUSTODY RECORD

Analytical Request

Client 5/12

Report To: _____

Page Client No. _____

Address _____

Bill To: _____

Pace Project Manager _____

P.O. # / Billing Reference

Pace Project No.

Phone _____

Project Name / No. _____

*Requested Due Date: _____

Sampled By (PRINT):

Sample Signature

Date Sampled

PRESERVATIVES

ANALYSES
REQUEST

REMARKS

Additional Comments

REINQUIRED BY AFFILIATION

ACCEPTED BY AFFILIATION

SEE REVERSE SIDE FOR INSTRUCTIONS