

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA tetrabutylammonium salt, Ammonium perfluorooctanoate PFO, FC-116, FC-126, FC-169, FC-143 or as a major component of FC-1015 or FC-1015-X. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample is FC-1015-X. It's purity was not sufficiently characterized, though current information indicates is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205".

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 test substance. Data was used to compare toxicity of the branched/straight chain ammonium perfluorooctanoate homologue mixture in FC-143 vs. what is supposed to be the 100% straight carbon chain ammonium perfluorooctanoate in FC-1015-X.

METHOD

Method: OECD 209

Test type: Static acute

GLP: No

Year Completed: 1996

Analytical monitoring: Dissolved oxygen concentrations and pH.

Statistical methods: Not applicable

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 7.2. Test substance was added directly to test vessels.

Test vessels: Not stated

Number of concentrations: 4 plus 1 reference substance and 2 blank controls

Temperature: 19.1 – 22.1C

Total suspended solids and pH on day of testing: 3.22 g/L (TSS in final 1.3 g/L) and initial pH 7.8 and final pH 7.9

Element Basis: Respiration inhibition as determined by oxygen consumption.

Remarks: Values corrected to 20C for calculations

RESULTS

Nominal concentrations: 2 Blank controls, 420, 840, 1660 and 3320 mg/L

Element values: 30-minute EC₅₀ = >3320 mg/L
3-hour EC₅₀ = >3320 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 test substance.

CONCLUSIONS

The FC-1015-X 3-hour EC₅₀ for activated sludge respiration inhibition was determined to be >3320 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St Paul, MN, Lab Request N2169.

OTHER

Last changed: 5/25/00

ENVIRONMENTAL LABORATORY
ACTIVATED SLUDGE MIXED LIQUOR

LR P1626
Date 4-25-96
Analyst TKS
Page 1 of 8

Type Test OECD ACTIVATED SLUDGE RESPIRATION Inhibition Test # 209

ACTIVATED SLUDGE MIXED LIQUOR

Date Obtained 4-25-95

Date Used 4-25-95

Mixed Liquor Suspended Solids (MLSS) 3 220 g/L Adjusted to _____ g/L

MLSS in final test solution 1.3 g/L

Initial pH 7.8 (1st test soln. prepared)

Final pH 7.9 (last soln. prepared)

Comments _____

TEST SUBSTANCE

Sample Description FC - 1015 - X

Initial pH of highest conc. tested (3320 mg/L) = 7.2

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 19.1 - 22.1 °C

Comments _____

ENVIRONMENTAL LABORATORY

LA P1626
Pg 2 of 8

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
TEST SOLUTIONS PREPARATION

Test Substance: FC-1015-X

Sample Description: clear liquid

Analyst: TKS

Date: 4-25-96

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
Blank control	-	284	16	200	500
420	210	284	16	200	500
840	420	284	16	200	500
1660	830	284	16	200	500
3320	1660	284	16	200	500

Initial pH of highest conc. tested = 7.2

(1) Millipore Milli-QR high purity water

ENVIRONMENTAL LABORATORY

LR P1626

Pg 3 of 8

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-1015-XSource of Activated Sludge Mixed Liquor: Metro Waste Water Treatment Plant, St Paul, MNDate Received: 4-25-96Date Used: 4-25-96Analyst: TKS

30-MINUTE 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
BK CONTROL I	7.8	7.6	7.2	6.8	6.5	6.0	5.6	5.3	4.9	4.6	4.1	20.6
420	7.4	7.1	6.8	6.3	5.9	5.4	5.1	4.8	4.5	4.0	3.6	20.9
840	6.4	6.0	5.7	5.3	4.9	4.4	4.2	3.9	3.5	3.1	2.8	20.8
1660	8.0	7.8	7.6	7.3	7.0	6.6	6.4	6.0	5.7	5.4	5.0	20.7
3320	7.3	6.8	6.4	6.2	5.8	5.3	5.0	4.7	4.4	4.1	3.8	22.1
BK CONTROL II	7.1	6.8	6.4	6.0	5.6	5.2	4.8	4.6	4.1	3.7	3.4	20.7

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
BK CONTROL I	7.9	7.7	7.4	7.0	6.7	6.4	6.0	5.6	5.3	4.9	4.6	19.8
420	7.0	6.7	6.5	6.1	5.7	5.3	4.9	4.6	4.2	3.9	3.5	20.4
840	6.0	5.7	5.4	5.0	4.6	4.3	3.9	3.5	3.3	2.9	2.5	20.5
1660	6.4	6.1	5.9	5.6	5.3	5.0	4.7	4.4	4.1	3.8	3.5	20.5
3320	8.7	8.5	8.3	8.1	7.9	7.7	7.5	7.3	7.0	6.8	6.5	20.9
BK CONTROL II	8.8	8.6	8.2	7.9	7.6	7.2	6.8	6.6	6.3	5.9	5.6	19.7

pH of activated sludge mixed liquor:

Initial: 7.8Final: 7.9

pH of highest concentration tested:

Initial: 7.3Final: 7.5

Test temperature:

19.8

ENVIRONMENTAL LABORATORY

OECD ACTIVATED SLUDGE RESPIRATION INHI ON TEST 209

Lr: P1828
Date: 4/25/96

CALCULATION OF RESULTS

Test Substance: EC-1015-X
Analyst: 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	7.8	7.6	7.2	6.8	6.5	6.0	5.6	5.3	4.9	4.6	4.1	20.6	0.7661	0.7348	
420	7.4	7.1	6.8	6.3	5.9	5.4	5.1	4.8	4.5	4.0	3.6	20.9	0.7721	0.7253	0
840	6.4	6.0	5.7	5.3	4.9	4.4	4.2	3.9	3.5	3.1	2.8	20.8	0.7176	0.6788	6.1
1680	8.0	7.8	7.6	7.3	7.0	6.6	6.4	6.0	5.7	5.4	5.0	20.7	0.6167	0.5874	18.7
3320	7.3	6.8	6.4	6.2	5.8	5.3	5.0	4.7	4.4	4.1	3.8	22.1	0.6752	0.5834	19.3
BK2	7.1	6.8	6.4	6.0	5.6	5.2	4.8	4.6	4.1	3.7	3.4	20.9	0.7564	0.7105	

% diff in controls (4): 3.4 EC50(95% CI) = > 3320 mg/L

RaCm (5): 0.7226

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	7.9	7.7	7.4	7.0	6.7	6.4	6.0	5.6	5.3	4.9	4.6	19.8	0.6982	0.7080	
420	7.0	6.7	6.5	6.1	5.7	5.3	4.9	4.6	4.2	3.9	3.5	20.4	0.7297	0.7067	0
840	6.0	5.7	5.4	5.0	4.6	4.3	3.9	3.5	3.3	2.9	2.5	20.5	0.7091	0.6849	2.9
1680	6.4	6.2	5.9	5.6	5.3	5.0	4.7	4.3	4.1	3.8	3.5	20.7	0.6036	0.5750	18.5
3320	8.7	8.5	8.3	8.1	7.9	7.7	7.5	7.2	7.0	6.8	6.6	20.8	0.4291	0.4059	42.5
BK2	8.8	8.6	8.2	7.9	7.6	7.2	6.8	6.6	6.3	5.9	5.6	19.1	0.6606	0.7033	

% diff in controls (4): 0.7 EC50(95% CI) = > 3320 mg/L

RaCm (5): 0.7056

(1) The Oxygen uptake rate (mg O₂/min)(2) Rm adjusted to 20°C (Rm E₂₀)

(3) % Inhibition: [(RaCm - Ra)/(RaCm)]100

(4) % difference in controls: [(BK1 - BK2)/RaCm]100 (Two controls must be within 10% of each other.)

(5) Rm adjusted to 20°C (Rm E₂₀)

ENVIRONMENTAL LABORATORY

LA P1626
Pg 5 of 8

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST # 200
TEST SOLUTIONS PREPARATION

Test Substance: 3,5-Dichlorophenol
Sample Description: Aldrich - Reo LABEL lot 00410KZ
Analyst: TKS
Date: 4-25-96

Stock Solution
Preparation: 500mg/L; 1.0ml = 0.5mg
500mg 3,5-DCP was added to 10ml 1.0N NaOH
Dilution was made with approx 30ml Milli-Q water
Approx 8 ml 1.0N H₂SO₄ was added under stirring until incipient
precip.
Diluted to 1.0L with Milli-Q water
Stock 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
<u>CONTROL</u>	<u>-</u>	<u>284</u>	<u>16</u>	<u>200</u>	<u>500</u>
<u>10</u>	<u>10</u>	<u>284</u>	<u>16</u>	<u>200</u>	<u>500</u>

Initial pH of highest conc tested = 7.3

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

ENVIRONMENTAL LABORATORY

LA P1626
pg 6 of 8

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: 3,5-Dichlorophenol

Source of Activated Sludge Mixed Liquor: Metrol Waste Water Treatment Plant, St Paul, MN

Date Received: 4-25-96

Date Used: 4-25-96

Analyst: TCS

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	7.2	6.8	6.5	6.0	5.6	5.3	4.9	4.6	4.1	20.6
10	7.9	7.8	7.6	7.4	7.1	6.8	6.6	6.4	6.2	5.9	5.6	21.0
BK CONTROL II	7.1	6.8	6.4	6.0	5.6	5.2	4.8	4.6	4.1	3.7	3.4	20.9

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	7.9	7.4	7.4	7.0	6.7	6.4	6.0	5.6	5.3	4.9	4.6	19.8
10	9.2	8.9	9.2	9.0	9.0	8.8	8.7	8.5	8.4	8.4	8.1	20.3
BK CONTROL II	8.8	8.6	8.2	7.9	7.6	7.2	6.8	6.6	6.3	5.9	5.6	19.1

pH of activated sludge mixed liquor: Initial 7.9 Final 7.9
 pH of highest concentration tested: Initial 7.6 Final 7.6
 Test temperature: 19.8

ENVIRONMENTAL LABORATORY

CALCULATION OF RESULTS

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST 209.

Lr: P1626
Date: 4/25/96

Test Substance: 3,5-DICHLOROPHENOL

Analyst: 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	7.8	7.6	7.2	6.8	6.5	6.0	5.6	5.3	4.9	4.6	4.1	20.6	0.7661	0.7348		
10	7.9	7.8	7.6	7.4	7.1	6.8	6.6	6.4	6.2	5.9	5.8	21.0	0.4848	0.4523	37.4	
BK2	7.1	6.8	6.4	6.0	5.6	5.2	4.8	4.6	4.1	3.7	3.4	20.9	0.7564	0.7105		
% diff in controls (4): 3.4																

% diff. in controls (4): 3.4

RaCm (5): 0.7226

3-Hour Exposure

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	7.9	7.7	7.4	7.0	6.7	6.4	6.0	5.6	5.3	4.9	4.6	19.8	0.6982	0.7080							
10	9.2	9.2	9.2	9.0	9.0	8.8	8.7	8.5	8.4	8.4	8.1	20.3	0.2436	0.2386							
BK2	8.8	8.6	8.2	7.9	7.6	7.2	6.8	6.6	6.3	5.9	5.6	19.1	0.6806	0.7033							
% diff. in controls (4): 0.7																					

% diff. in controls (4): 0.7

RaCm (5): 0.7056

- (1) Oxygen uptake rate (mg O₂/L-min)
- (2) R adjusted to 20°C (R₂₀)
- (3) RaCm = (R₂₀ - R₀) / (R₂₀ - R₀)
- (4) % diff. in controls = [(R₂₀ - R₀) / (R₂₀ - R₀)] * 100 (Two controls must be within 15% of each other)
- (5) Mean adjusted R₂₀ = (R₂₀ + R₂₀) / 2