

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate, Lithium salt; may also be referred to as PFOS Li salt, FC-94, or FC-94-X. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, lithium salt, CAS # 29457-72-5)

Remarks: The test sample is a mixture of the test substance in water (approximately 24.5% test substance and 75.5% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: OECD 209

Test type: Static acute

GLP: No

Year Completed: 1994

Analytical monitoring: pH and DO concentrations.

Statistical methods: Not performed

Test organism source: Activated sludge mixed liquor collected from the Metropolitan Wastewater Treatment Plant, St. Paul, Minnesota.

Test conditions

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 per the OECD method. The pH of the reference solution was measured to be 7.4. The test solutions were created by direct addition of the test sample; final concentration of 1000 mg/L. Initial pH of the two 1000 mg/L test solutions was 7.1 and 7.2.

Test vessels: One liter glass fleakers.

Number of concentrations: 1 (done in duplicate) plus 1 reference control and 2 Blank controls

Temperature: 14.7-16.8°C

Total Suspended Solids and pH for sludge on day of testing:

Total Suspended Solids = 4.9 g/L (mixed liquor). Final test solution mixed liquor suspended solids = 1.92 g/L. pH of the activated sludge mixed liquor gave an initial reading of 6.4 and a final reading of 6.7.

Element Basis: Respiration inhibition as determined by oxygen consumption.

Method Remarks: Dissolved oxygen in the test solutions was quite low during the 30-minute exposure portion of the test. The technician realized the test reactors were not being sufficiently aerated after the 30-minute readings and adjusted the air flow. Because the 3-hour data is the most commonly used data, it was decided not to re-test due to this occurrence.

Test temperatures were not controlled during the test. The respiration rates were normalized to 20°C for the calculations.

RESULTS

Nominal concentrations: Two Blank controls, one reference substance (10 mg/L 3,5-DCP), two 1000 mg/L test material solutions.

Analytical Methodology: Analysis of DO concentrations in all test solutions were performed using a dissolved oxygen meter.

Control response: Satisfactory

Remarks: Values reported are for the test sample. No calculations were made to adjust for the concentration of the test substance in the test sample.

CONCLUSIONS

The test sample containing 24.5% Perfluorooctanesulfonate, Lithium salt exhibited a maximum inhibitory effect of 35% upon respiration at a nominal test substance concentration of 1000 mg/L at 3 hours exposure. The 3-hour EC50 (respiration inhibition) is >1000 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study lacks information on the sample purity, and the test was conducted at temperatures below those recommended by the method. Oxygen concentrations also were too low to allow an adequate measurement of oxygen uptake rate following the 30-minute exposure portion. These 30-minute results were considered invalid for the study. It was concluded that, since the 3-hour results show acceptable toxicity, the 30-minute results almost certainly would have shown the same.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number M1018, 2/22/94.

OTHER

Last changed: 5/3/00

001556

EVALUATION OF FC-94-X BY OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

Results

The inhibitory effect of FC-94-X, the lithium salt of perfluorooctanesulfonate, on activated sludge respiration was evaluated using OECD Test # 209. After calculated temperature corrections, measurements following a 3-hours exposure to FX-94-X at 1000 mg/L, showed activated sludge oxygen uptake rates were inhibited by 34%. The reference substance, 3,5-dichlorophenol, at 10 mg/L caused an inhibition of 56%.

Discussion

Though this study had deficits, the analyst considers that the results are sufficient to assess the potential for wastewater treatment system inhibition by FC-94-X.

One study deficit was that oxygen concentrations were too low to allow an adequate measurement of oxygen uptake rate following the 30-minute exposure portion of the OECD #209 study. These 30-minute results are thus invalid and should be ignored. The 30-minute results are not critical to the usefulness of the study, however, because inhibition is almost always greater following 3-hours of exposure. If the 3-hour results show acceptable toxicity, the 30-minute results almost certainly would have shown the same.

A second deficit is that temperature control was not used. Measured test temperatures were lower than that recommended by the method by as much as 5.5 °C. However, all tests were conducted within 1.5 degrees of each other. Also, prior to comparison of the oxygen uptake rates, calculations were made to adjust the rates to those likely to have occurred at 20 °C. The adjustment used was the rule of thumb that metabolic rates of mesophilic microbial populations typically double for each 10 °C increase in temperature.

July 22, 1998

TO: ERIC A. REINER

FROM: SUSAN A. BEACH

SUBJECT: Review of OECD 209 and BOD/COD Test Results for FC-94-X

DATE: July 8, 1998

I have reviewed the results from the test of FC-94-X on the inhibition of activated sludge respiration inhibition (OECD 209) and have a few comments.

1. Although the dissolved oxygen in the test solutions was quite low during the 30-minute exposure portion of the test, it was adequate after 3-hours exposure. The technician realized the test reactors were not being sufficiently aerated after the 30-minute readings, and adjusted the air flow. Because the 3-hour data is the most commonly used data, it was decided not to re-test due to this occurrence.
2. At the time this test was run, the laboratory did not have a procedure in place for controlling the temperature during exposure. The temperature of the test reactors fluctuated with the temperature in the hood. Because this was tested in February in Minnesota, it is not surprising that the test temperature was so low. The respiration rates were normalized to 20°C for the calculations.
3. The software used to produce the data reduction sheets did not have an easy way to depict exponents, so the equation for the temperature-adjusted respiration rates (Ra) is difficult to understand. It is as follows:

$$Ra = R \text{ adjusted to } 20^{\circ}\text{C} = \frac{R}{\theta^x}$$

Where $\theta = 1.072$
 $x = T (\text{observed}) - 20^{\circ}\text{C}$

*This method assumes
the rate doubles
for each 10°C
rise in temperature
SAR: 7/16/98*

4. Even though the 30-minute data is in question and the test was conducted at lower than normal temperatures, the data are useful for the interpretation of the toxicity of FC-94-X to activated sludge. An average of 34% inhibition was seen at 1000 mg/L. We generally do not consider inhibition less than or equal to 15% to be significant when using this procedure due to the inherent variability of the method.

As for the BOD/COD testing, we have been unable to locate the data at this time.

Please see me if you have any questions.

Regards,

Susan A. Beach

Susan A. Beach

001558

ENVIRONMENTAL LABORATORY
ACTIVATED SLUDGE MIXED LIQUOR

LR M1018
Date 2/22/94
Analyst RJW
Page 1 of 4

Type Test : OECD ACTIVATED SLUDGE RESPIRATION INHIBITION, # 209

ACTIVATED SLUDGE MIXED LIQUOR

Date Obtained 2/22/94 Date Used 2/22/94
Mixed Liquor Suspended Solids (MLSS) 4.9 g/L Adjusted to — g/L
MLSS in final test solution 1.92 g/L
Initial pH 6.4 (1st test soln. prepared) Final pH 6.7 (last soln. prepared)
Comments —

TEST SUBSTANCE

Sample Description FC-94-X
Initial pH of highest conc. tested (1000 mg/L) = 7.2 Adjusted to pH —
Comments —

REFERENCE SUBSTANCE

Sample Description 3,5-Dichlorophenol
Initial pH of stock solution (500 mg/L) = 7.4 Adjusted to pH —
Comments —

EXPOSURE CONDITIONS

Test Duration 3 Hours
Test Temperature Range: 14.7-16.8 C
Comments —
—
—
—

001559

ENVIRONMENTAL LABORATORY

LR M1018
Pg 20f4

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209 TEST SOLUTIONS PREPARATION

Test Substance: FC-94-X

Sample Description: Brownish-yellow Liquid.

Analyst: RJW

Date: 2/22/94

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
BKQWZL	-	284	16	200	500

1000	500	284	16	200	500
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1000	500	284	16	200	500
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INITIAL 2/21/94 RJW

CONC. TEST	INITIAL
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Soln. v/v	PH
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1000	7.2
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1000	7.1
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(1) Millipore Milli-Q^R high purity water

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: FC-94-XSource of Activated Sludge Mixed Liquor: Metro Waste Water Treatment Plant, St. Paul, Minn.Date Received: 2/22/94Date Used: 2/22/94Analyst: PSW

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	
EK CONTROL I	7.0	6.9	6.4	6.0	5.7	5.4	5.0	4.7	4.3	4.0	3.6	16.6
1000	2.3	1.7	1.5	1.2	1.0	0.8	0.5	—	—	—	—	16.8
1000	1.2	0.9	0.6	0.5	—	—	—	—	—	—	—	16.8
EK CONTROL II	8.0	7.7	7.4	7.1	6.8	6.5	6.2	5.9	5.6	5.4	5.1	15.2

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	
EK CONTROL I	7.9	7.6	7.4	7.1	6.8	6.5	6.3	6.0	5.7	5.4	5.1	14.7
1000	6.6	6.3	6.0	5.9	5.7	5.5	5.3	5.1	4.9	4.7	4.5	16.0
1000	5.3	5.1	4.9	4.7	4.5	4.3	4.1	3.9	3.7	3.5	3.3	15.9
EK CONTROL II	7.4	7.1	6.9	6.5	6.2	6.0	5.7	5.4	5.2	4.9	4.6	14.9

pH of activated sludge mixed liquor:

Initial 6.4Final 6.7

pH of highest concentration tested:

Initial 6.5Final 6.5Test temperature: 14.7-16.8°C

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

Lr: M1018
Date: 2/22/94
Pg 4 of 4.

001562

Test Substance: FC-94-x

Analyst: 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	7.0	6.9	6.4	6.0	5.7	5.4	5.0	4.7	4.3	4.0	3.6	16.6	0.7079	0.8966	0
1000.0	2.3	1.7	1.5	1.2	1.0	0.8	0.5					16.8	0.4743	0.5925	31
1000.0	1.2	0.9	0.6	0.5								16.8	0.4000	0.4997	41
BK2	8.0	7.7	7.4	7.1	6.8	6.5	6.2	5.9	5.6	5.4	5.1	15.2	0.5806	0.8106	0

% diff in controls (4): 10.1

EC50 (95% CI): ≥ 1000 mg/L

RaCm (5): 0.8536

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	7.6	7.4	7.1	6.8	6.5	6.3	6.0	5.7	5.4	5.1	14.7	0.5588	0.8078	0
1000.0	6.6	6.3	6.0	5.9	5.7	5.5	5.3	5.1	4.9	4.7	4.5	16.0	0.3915	0.5170	35
1000.0	5.3	5.1	4.9	4.7	4.5	4.3	4.1	3.9	3.7	3.5	3.3	15.9	0.4000	0.5319	33
BK 2	7.4	7.1	6.9	6.5	6.2	6.0	5.7	5.4	5.2	4.9	4.6	14.9	0.5539	0.7897	0

% diff in controls (4): 2.3

EC 50 (95% CI): ≥ 1000 mg/L

RaCm (5): 0.7987

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

(2) Ra= R adjusted to 20°C= $[R/\theta^{(T-20)}]$

(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= $[RaBk1+RaBk2]/2$

$\theta = 1.072$

RaCm= mean adjusted R, controls

Bk= blank control, S= sample

Tobs= sample temp

x= Tobs-20°C

LR M1018 & M1149.
Pg 1 OF 3.

Test Substance: 3,5-Dichlorophenol Reference Substance

Analyst: RSW

Date: 2/22/14

Preparation: 500 mg/L Each 1 mL = 0.5 mg
Dissolved 500mg 3,5-DCP w 10 ml 1.0N NaOH
ADDED ~ 30 ml MQ water
ADDED 1.0 N H₂SO₄, DROPWISE UNDER STIRRING UNTIL INSIPIENT PPT.
DILUTED to 1.0 LITER with MQ water

Stock Solution pH = 7.4

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

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

OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

RAW DATA

Test Substance: 3,5-Dichlorophenol Reference Substance
 Source of Activated Sludge Mixed Liquor: Metrol Waste Water Treatment Plant ST. PAUL MN
 Date Received: 2/22/94 Date Used: 2/22/94
 Analyst: RSW

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	
EK CONTROL I	7.0	6.9	6.4	6.0	5.7	5.4	5.0	4.7	4.3	4.0	3.6	16.6
10	8.6	8.4	8.3	8.1	7.9	7.7	7.5	7.3	7.2	7.0	6.8	14.7
EK CONTROL II	8.0	7.7	7.4	7.1	6.8	6.5	6.2	5.9	5.6	5.4	5.1	15.2

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	
EK CONTROL I	7.9	7.6	7.4	7.1	6.8	6.5	6.3	6.0	5.7	5.4	5.1	14.7
10	8.2	8.1	8.0	7.9	7.8	7.7	7.5	7.4	7.3	7.2	7.0	14.5
EK CONTROL II	7.4	7.1	6.9	6.5	6.2	6.0	5.7	5.4	5.2	4.9	4.6	14.9

pH of activated sludge mixed liquor:
 pH of highest concentration tested:
 Test temperature: 14.5-16.6 °C

Initial 6.4
 Initial 6.6

Final 6.7
 Final 6.7

001564

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

Lr: M1018 & M1149

Date: 2/22/94

Pg 3 of 3

001565

Test Substance: 3,5-Dichlorophenol Reference Substance

Analyst: RW

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	7.0	6.9	6.4	6.0	5.7	5.4	5.0	4.7	4.3	4.0	3.6	16.6	0.7079	0.8966	0
10.0	8.6	8.4	8.3	8.1	7.9	7.7	7.5	7.3	7.2	7.0	6.8	14.7	0.3636	0.5257	38
BK2	8.0	7.7	7.4	7.1	6.8	6.5	6.2	5.9	5.6	5.4	5.1	15.2	0.5806	0.8106	0

% diff in controls (4): 10.1

EC50 (95% CI): ≥ 10 mg/L

RaCm (5): 0.8536

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	7.6	7.4	7.1	6.8	6.5	6.3	6.0	5.7	5.4	5.1	14.7	0.5588	0.8078	0
10.0	8.2	8.1	8.0	7.9	7.8	7.7	7.5	7.4	7.3	7.2	7.0	14.5	0.2412	0.3536	56
BK2	7.4	7.1	6.9	6.5	6.2	6.0	5.7	5.4	5.2	4.9	4.6	14.9	0.5539	0.7897	0

% diff in controls (4): 2.3

EC 50 (95% CI): ≤ 10 mg/L

RaCm (5): 0.7987

(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: $[|RaBk1-RaBk2|/RaCm]100$ (Two controls must be within 15% of each other.)

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

s= 1.072

RaCm= mean adjusted R, controls

Bk= blank control, S= sample

Tobs= sample temp

x= Tobs-20°C