ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

Identity: Perfluorooctylsulfonate, didecyldimethylammonium salt; may also be referred to as Fluoroalkyl ammonium derivative. [1-Decaminium, N-decyl-N,N-dimethyl-, salt with 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid (1:1), CAS # 251099-16-8]

Remarks: The 3M production lot number was Lot 1. The test sample is L-14394 referred to by the test laboratory as P3025. The sample was labeled F-11615, Lot 1. The test sample is a mixture of the test substance in water (approximately 30-40% test substance, 60-70% water, and 0-5% of residual perfluorochemicals). All values reported relate to this mixture. The test sample appears to be a 2-phase dispersion (clear liquid with opaque solid) which rapidly separates after agitation. 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: 1997
Analytical monitoring: Nominal concentrations. pH, temperature and dissolved oxygen.
Exposure period: 30-minutes and 3-hours
Statistical methods: not applicable
Test organism source: Activated sludge mixed liquor collected from Metro Wastewater Treatment Plant, St Paul, MN
Inoculum mixed liquor suspended solids: 4.38 g/L
Inoculum holding: None – used the day it was obtained

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 by dissolving 500 mg in 10 mL of 1N NaOH, diluted to 30 mL with Milli-Q® water, then brought to the point of incipient precipitation with 1N

H_2SO_4, and diluted to 1 L with Milli-Q® water. The pH of the reference solution was measured to be 7.3.

The test solutions were created by direct addition of the test substance to the test vessels. After test substance addition, the vessels were stirred with a Pyrex pipette for approximately one minute. A white precipitate was seen on the bottom of the vessels after stirring. The particles were suspended during aeration. Vigorous shaking was done before removing each sample aliquot for testing.
Test vessels: 1200 mL Pyrex® Fleakers™ containing 500 mL test solution
Number of concentrations: 3 plus one reference control and two blank controls
Temperature: 19-21°C
Total Suspended Solids and pH for sludge on day of testing: 4380 mg/L and 7.87 respectively.

Element basis: respiration inhibition as determined by oxygen consumption

Method Remarks: Test temperatures were not controlled. The respiration rates were normalized to 20°C for the inhibition calculations.

RESULTS

Nominal concentrations: Blank control, reference substance control (10 mg/L), 100, 500, and duplicated 1,000 mg/L test material solutions
Element Values: 30-minute EC_{50} = >1,000 mg/L
3-hour EC_{50} = >1,000 mg/L

Element value based on nominal concentrations

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

Control response: Satisfactory

Remarks: No oxygen inhibition seen in the 1,000 mg/L exposure concentration relative to the control in the 3-hour exposure.

CONCLUSIONS

The test substance exhibited only a slight inhibitory effect (<20%) upon respiration at a nominal test substance concentration of 1,000 mg/L at the 30-minute exposure and no inhibitory effect at the 3-hour exposure. The EC_{50} (respiration inhibition) is therefore greater than the highest exposure concentration, 1,000 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 P3025

OTHER

Last changed: 5/25/00

3M ENVIRONMENTAL LABORATORY

INHIBITORY EFFECTS OF P3025 ON ACTIVATED SLUDGE RESPIRATION

OECD Activated Sludge Respiration Inhibition Test, # 209

STUDY COMPLETED: January 21, 1997

FINAL REPORT COMPLETED: May 12, 1997

Prepared by:

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Env. Lab Request Number P3025

SUMMARY

The purpose of this study was to evaluate the inhibitory effect of P3025 on aerobic sludge systems in accordance with the "Activated Sludge Respiration Inhibition Test", OECD No. 209. The median effective concentration, 3-hour EC_{50} was reported as > 1000 mg/L.

INTRODUCTION

Toxicity tests with aerobic sludge provide a rapid screening method and are reliable predictors of the inhibitory effects of potential contaminants on the operation of activated sludge treatment systems.

This toxicity study measured the respiration rate of activated sludge obtained from a municipal wastewater treatment system and followed decreases in the rate of oxygen consumption over a relatively short period (30-minutes to 3-hours) in the presence of different concentrations of P3025.

MATERIALS AND METHODS

Test Substance:	P3025, date 10/7/96, Lot 1
Appearance	Two-phase dispersion (clear liquid with opaque solid)
Aqueous Solubility	Apparently not completely soluble in water at tested concentrations.
Available Physicochemical Properties:	Boiling Point: 100°C Vapor Pressure: ca. 18 mm Hg @ 20°C Vapor Density: < 1 Evaporation Rate: <1 Solubility in water: Insoluble Specific Gravity: ca. 1.2 Percent Volatile: 60 - 70% pH: 6 - 8
Date Received	10/16/96
Storage	In the dark at ambient room temperature (22 ± 2°C)
Test Solutions	Test substance weighed out and added directly to test vessels. After test substance addition, vessels were stirred with a Pyrex pipette for approximately one minute. Test substance formed white particles which sank to the bottom of the Fleaker™. Particles were suspended during aeration.
Reported values	The reported values are based on nominal concentrations in the test media at the beginning of the microbial assay.
Controls:	
Functional Control (Reference substance)	3,5-dichlorophenol (3,5-DCP). Aldrich red label; 97%. Lot 09615KG DQ. Received 11/18/96.

Controls (Cont.):

Blank control (untreated)	Two controls containing inoculum and nutrient medium, but no test or reference chemicals, one at the start and the other at the end of the test series.

Inoculum/Nutrient Mixture:

Microbial Inoculum	Activated sludge mixed liquor was obtained from the Metropolitan Wastewater Treatment Plant, St Paul, Minnesota on test day. The suspended solids level was 4.38 ± .072 g/L; initial pH 7.6. The sludge was continuously aerated after it was received in the laboratory.
Nutrient Medium	Synthetic sewage feed prepared on test day as outlined in OECD 209.

Exposure:

Test Date	1/13/97
Contact Time	30-minutes and 3-hours.
Vessels	1200 mL Pyrex® Fleakers™
Test Solution Vol.	500 mL
Starting Test Conc.	
Test Substance	100 500 1000 mg/L (in duplicate at 30-min; 3-hour duplicate lost due to lab accident)
Reference Substance	10 mg/L
Mixed Liquor Suspended Solids Level in Test Media	1.75 g/L
Dilution Water	Millipore Milli-Q® water
Temperature	15.6 - 18.0°C over the course of the study
Lighting	Ambient laboratory light
Mixing and Aeration	Test solutions were continually aerated with clean, oil-free air.

Measurements: Dissolved oxygen concentrations were measured after 30-minutes and 3-hours contact time. A sub-sample of test solution was quickly poured from the exposure vessel into a BOD bottle and dissolved oxygen concentrations were recorded using a dissolved oxygen meter every 30-seconds over a five-minute period. The sub-sample was stirred while recording oxygen. The sub-sample was returned to the main test solution after the 30-minute readings.

Procedure

The test was conducted in general accordance with the "Activated Sludge Respiration Inhibition Test", OECD No. 209 (1). Values were reported to different levels of significance depending on the accuracy of the measuring devices involved in any one process.

CALCULATIONS

The reported values are based on nominal concentrations in the test media at the beginning of the test. The concentrations tested and the corresponding response data derived from the toxicity test are used to calculate, if possible, the medial effective concentration, EC_{50} and the 95% confidence interval by standard logistic regression methods (i.e., Probit Analysis, Trimmed Spearman-Karber). The EC_{50} is defined as the concentration estimated to inhibit the rate of respiration by 50% as compared to the controls, after 30-minutes and 3-hours of exposure to the test substance.

RESULTS

The percent reduction in respiration rate and the median effective concentration, EC_{50}, obtained after 30-minutes and 3-hours exposure to the test substance P3025 are summarized in Table 1. The percent reduction in respiration rate of the reference substance 3,5-Dichlorophenol is also reported in Table 1.

The median effective concentration, 3-hour EC_{50} of P3025 to activated sludge was determined as >1000 mg/L. The 95% Confidence Interval could not be calculated.

ACCEPTABILITY CRITERIA

This toxicity test meets quality criteria provided in test guidelines:

1) The two blank control respiration rates were within 15 percent of each other after 3-hours exposure. The percent differences were 22.2 and 4.1 after 30-minutes and 3-hours of exposure, respectively.

2) The 3-hour percent inhibition induced by the reference substance, "3,5-DCP", was within the normal range seen with this mixed liquor in this laboratory (see Table 2).

3) The reported values were based on nominal concentrations in the test media at the beginning of the microbial assay.

This test was conducted by Trudy K. Stiefenhoefer as requested by Pam Wolf of 3M SCD Division.

REFERENCES

1. OECD (1984). Activated Sludge Respiration Inhibition Test, Test No. 209. In: Guidelines for Testing of Chemicals. Organization for Economic Cooperation and Development, Paris, France.

2. OECD. 1981. Principles of Good Laboratory Practice, Annex 2, C(81)30 (Final): 7-28. OECD Guidelines for Testing of Chemicals. Organization for Economic Cooperation and Development, Paris, France.

TABLE 1

SUMMARY OF TOXICITY OF P3025 TO ACTIVATED SLUDGE

TEST CONC. mg/L	PERCENT INHIBITION IN RESPIRATION RATE	
	30-min. Exposure	3-hours Exposure
100	1.8	0
500	0	0
1000	17	0
1000 (dup)	19	na - lab accident
EC_{50}	> 1000	> 1000
95% Confidence Interval	Not calculable	Not calculable
Statistical method used	Not applicable	Not applicable

SUMMARY OF TOXICITY OF 3,5-DICHLOROPHENOL REFERENCE SUBSTANCE TO ACTIVATED SLUDGE

TEST CONC. mg/L	PERCENT INHIBITION IN RESPIRATION RATE	
	30-min. Exposure	3-hours Exposure
10	36	54

TABLE 2

OECD 209

Results from Reference Toxicant Testing with 3,5-Dichlorophenol

Test Concentration = 10 mg/L

Percent Inhibition in Oxygen Uptake Rate

Date Tested	30-min Exposure	3-hrs Exposure	MLSS*, g/L
11/27/95	38	58	5.5
1/25/96	36	53	7.2
2/26/96	32	58	5.2
3/4/96	27	65	2.4
3/7/96	34	57	3.4
3/11/96	34	58	3.7
4/4/96	29	58	5.9
4/8/96	25	60	3.9
4/17/96	36	56	4.7
4/25/96	37	66	3.2
5/16/96	55	52	3.6
8/14/96	50	73	3.3
8/19/96	54	68	3.0
8/26/96	46	52	4.1
8/28/96	52	57	4.0
9/5/96	48	49	3.1
9/9/96	69	37	4.2
9/16/96	40	58	3.7
9/30/96	36	45	3.8
10/2/96	44	54	3.9
10/9/96	48	61	4.1
10/14/96	30	46	3.8
10/23/96	28	55	3.0
10/29/96	45	59	4.1
10/31/96	42	48	3.6
11/4/96	55	54	4.4
11/6/96	53	64	4.7
11/18/96	30	42	4.3
11/25/96	52	59	5.2
12/2/96	28	50	5.6
1/6/97	40	47	3.3
1/8/97	49	41	3.8
1/13/97	36	54	4.4
1/21/97	37	56	4.4
MEAN	**41**	**55**	**4.1**
Standard Deviation	10.3	7.8	0.96

*Mixed liquor suspended solids

Metro Wastewater Treatment Plant, St Paul, MN