

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

TEST SUBSTANCE

Identity: N-Ethylperfluorooctanesulfonamide; may also be referred to as U1464, EtFOSA, EtPOSA, F-6309, or FX-12. (1-Octanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptafluoro-N-ethyl-, CAS # 4151-50-2)

Remarks: Material is a white solid. Sample purity and lot number were not recorded.

METHOD

Method: OECD 209

Test type: Static acute

GLP: No

Year Completed: 1998

Analytical monitoring: Dissolved oxygen concentrations.

Test organism source: Activated sludge collected from the Western Lake Superior Sanitary district, Duluth, MN

Test conditions:

Dilution water: Carbon-treated dechlorinated City of Duluth, MN, tap water

Synthetic Sewage:

1 liter DI water
16.0 g Bacto. peptone
11.0 g Beef extract
3.0 g urea
0.7 g NaCl
0.4 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
2.8 g K_2HPO_4

Reference and test solution preparation: A 500 mg/L stock solution of the reference substance 3,5-dichlorophenol was prepared using Millipore water. Concentrations tested were 4, 16, and 32 mg/L, prepared by diluting the stock solution with Millipore® water.

EtFOSA was added directly to test vessels rather than by volumetric addition of a stock solution.

Test vessels: Mixtures were prepared (500 mL final volume) and aerated in 1000 mL borosilicate glass beakers and then transferred

into 300 mL Biochemical Oxygen Demand (BOD) bottles to obtain oxygen uptake rates.

Number of concentrations: One, in duplicate plus three reference substance concentrations and two Blank controls

Temperature: 20.1-21.8°C

Total Suspended Solids of sludge on day of testing: 6.5 g/L (TSS in test solutions = 3.3 g/L). Sludge was used one day after collection.

Element Basis: Respiration inhibition as determined by dissolved oxygen concentration.

Test mixtures were prepared at 7-minute intervals and aerated. After 30-minutes and 3-hours of contact time, dissolved oxygen was measured every 0.5 minutes for up to 5.5 minutes.

RESULTS

Nominal concentrations: Two blank controls, three reference substance concentrations, 1000mg/L (in duplicate) test material solutions

Statistical Analyses: EC₅₀ values were calculated for the reference material by the Trimmed Spearman-Kärber Method. An EC₅₀ value could not be calculated for the test substance.

Analytical Methodology: Analysis of DO concentrations in all test solutions were performed at ASCL Corporation using a YSI Model 54A dissolved oxygen meter. Dissolved oxygen readings were recorded every 30 seconds for 5.5 minutes. Respiration rates were normalized for 20°C prior to calculating percent inhibition.

Respiration Rates and Percent Inhibitions after 3-Hours Exposure

Treatment	Respiration Rate mg O ₂ /L/min. (@ 20°C)	Percent Inhibition
Control 1	0.2714	NA
Control 2	0.3134	NA
Test substance 1000 mg/L	0.3981	-36
Test substance 1000 mg/L (duplicate)	0.4081	-40

Control response: satisfactory; difference between controls ranged from 6.8% at 30-minutes to 14.4% after 3-hours of exposure.

Reference Substance: 3-hr EC₅₀ = 18.4 mg/L indicating the sludge had normal sensitivity.

CONCLUSIONS

The test substance exhibited a no inhibitory effect on respiration at a nominal test substance concentration of 1000 mg/L after 3-hours of exposure. The EC₅₀ (respiration inhibition) is therefore greater than 1000 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted using the methodology acceptable at the time, lacks analytical data for determination of the test substance concentration in the test solutions and determination of the sample purity. Testing was also done well above the solubility limit of the test substance

REFERENCES

This study was conducted at AScl Corporation of Duluth, Minnesota at the request of the 3M Company.

OTHER

Last changed: 5/18/00

STUDY TITLE

INHIBITION OF U1464 FOR ACTIVATED SLUDGE RESPIRATION

DATA STANDARD

OECD GUIDELINE 209

STUDY COMPLETED

MAY 15, 1998

TESTING FACILITY

ASCI Corporation/ASCI-Duluth
Environmental Testing Division
4444 Airpark Boulevard
Duluth, MN 55811

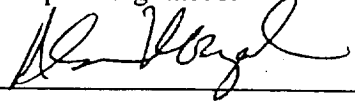
Tel. No. (218) 722-4040

PROJECT IDENTIFICATION NUMBERS

ASCI Study ID# 5010-068

3M Company Lab Request# U1464

1.0 GENERAL INFORMATION

Study Title	Inhibition of U1464 For Activated Sludge Respiration.	
Data Standard	OECD Guideline 209.	
Sponsor	3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7452.	
Sponsor's Representative	Rochelle R. Robideau, 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133; Tel No. (612) 778-7065.	
Sponsor's Lab Request#	U1464.	
Testing Facility	ASCI Corporation/ASCI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	
Principal Investigator	Joe Dierkes	
Project Director	Joe Amato	
Testing Facility Director	Donald Mount	
Definitive Test Date	May 1, 1998	
Data Validation and Report Review	Alan Mozol	Report Signature: 
Retention of Raw Data and Final Report	Five years from the date study is completed.	
Location of Raw Data and Final Report	ASCI Corporation/ASCI-Duluth Environmental Testing Division, 4444 Airpark Boulevard, Duluth, MN 55811; Tel. No. (218) 722-4040.	

2.0 OBJECTIVE

To determine, if possible, the 30-minute and 3-h (contact times) EC_{50} 's of the test substance U1464 for activated sludge respiration.

This study was conducted according to the ASCI study plan retained by the Sponsor and OECD Guideline 209 (OECD 1993).

3.0 TEST METHODS

Test Substance	<i>Properties:</i> U1464, white crystals, specific gravity = 1.7 (water = 1), stable, and negligible solubility in water. <i>Test concentrations:</i> 0 and 1000 mg/L prepared by direct addition.																
Reference Substance	<i>Name:</i> 3,5-dichlorophenol, purity 97%, Aldrich Chemicals, Lot# 09615KG. <i>Test concentrations:</i> 0, 4, 16, and 32 mg/L prepared by direct addition of 500 mg/L stock solution in Millipore® water. The reference test was performed concurrently with the exposure for U1464. Results and control chart appended to report.																
Test Organisms	<i>Source:</i> Activated sludge from the Western Lake Superior Sanitary District Duluth, Minnesota collected on April 30, 1998. <i>Activated sludge characteristics:</i> The sludge was determined to have a total suspended solids concentration of 6.5 g/L at test time. The total suspended solids concentration in the test solutions was 3.3 g/L. The mixture was fed synthetic sewage feed and aerated at 21°C until test initiation. The sludge was used one day after collection.																
Synthetic Sewage Feed	<i>Composition:</i> <table> <tr> <th><u>Chemical</u></th><th><u>g dissolved/L of deionized water</u></th></tr> <tr> <td>Bacto. peptone</td><td>16.0</td></tr> <tr> <td>Beef extract</td><td>11.0</td></tr> <tr> <td>Urea</td><td>3.0</td></tr> <tr> <td>NaCl</td><td>0.7</td></tr> <tr> <td>CaCl₂·2H₂O</td><td>0.4</td></tr> <tr> <td>MgSO₄·7H₂O</td><td>0.2</td></tr> <tr> <td>K₂HPO₄</td><td>2.8</td></tr> </table>	<u>Chemical</u>	<u>g dissolved/L of deionized water</u>	Bacto. peptone	16.0	Beef extract	11.0	Urea	3.0	NaCl	0.7	CaCl ₂ ·2H ₂ O	0.4	MgSO ₄ ·7H ₂ O	0.2	K ₂ HPO ₄	2.8
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Dilution Water	<i>Source:</i> Carbon-treated dechlorinated City of Duluth, MN, tap water. The most recent chemical analysis of post carbon (PC) water is in the Raw Data Package.
Test Setup	<i>Exposure vessels:</i> 4, 1-L borosilicate glass beakers; two inoculum controls and 1 test concentration exposure in duplicate. <i>Order of preparation:</i> At 7-minute time intervals: (1) first inoculum control, (2) test exposure concentration replicates, and (3) second inoculum control. The final volume in each beaker was 500 ml. <i>Preparation:</i> (1) Added required amount of carbon-treated water, (2) added 16 ml of synthetic sewage feed to each beaker, and (3) in test exposures added the required amounts of the test substance, (4) and added 200 ml of the sludge, for a final test solution volume of 500 ml. <i>Incubation and aeration:</i> 20.1° to 21.8°C and at an aeration rate of 0.5 to 1.0 liters per minute.
Determination of Respiration	<i>Sequence:</i> Same as exposures were prepared. <i>Determination:</i> At 30-min and 3-h contact times: (1) filled one 300-ml BOD bottle with the exposure medium and continued to aerate the remaining medium, (2) using a YSI 54A oxygen meter with a self-stirring probe, determined the DO concentration in the exposure medium every 0.5 minutes for up to 5.5 minutes (the first determination was made after 0.5 minutes), and (3) the sub-sample was placed back into the 1-L exposure chamber. DO meter calibration checks were made between the sets of measurements for each control or treatment.
Calculation of Respiration Rate	For each exposure, correlated DO depletion and time interval, and calculated the slope of the regression line as the respiration rate.
Endpoint Calculation	<i>EC</i> ₅₀ : Trimmed Spearman-Kärber Analysis (Hamilton, 1977).

3.0 RESULTS

Test Substance Toxicity (Pertinent data are in Table 1)	30-minute EC_{50} : >1000 mg/L. 3-h EC_{50} : >1000 mg/L.
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4.0 TEST QA/QC

QA/QC Criteria	Data
Respiration rates of two inoculum controls must not vary by more than 15%.	Variations after 30-minute and 3-h contact times were 7% and 14%, respectively.
3-h EC_{50} of reference substance must be between 5-30 mg/L.	3-h EC_{50} was 18.4 mg/L, indicating the sludge had normal sensitivity.
Test duration must be 3 h.	The test duration was 3 h.

5.0 REFERENCES

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Kärber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ. Sci. Technol. 11(7): 714-719; Correction 12(4):417. 1978.

Organization for Economic Cooperation and Development (OECD). 1993. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, Washington, D.C.

TABLE 1.0
OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209
CALCULATION OF RESULTS

ASol Corporation/ASol-Duluth
Environmental Testing Division
Submitted to 3M Co. - 5/98
ASol Study ID# 5010-068

Test Substance: U1464

Analyst: DIERKES

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	8.5	8.2	7.9	7.7	7.5	7.2	6.8	6.6	6.3	5.9	5.7	21.2	0.5648	0.5196	
1000A	8.1	7.8	7.5	7.2	6.9	6.6	6.3	6.0	5.7	5.3	5.0	21.2	0.6194	0.5698	-6
1000B	8.4	8.1	7.9	7.6	7.3	7.1	6.8	6.5	6.2	5.9	5.6	21.2	0.5600	0.5152	4
BK2	8.5	8.2	7.9	7.6	7.3	7.0	6.6	6.3	6.0	5.7	5.4	21.8	0.6303	0.5562	

% diff in controls(4): 6.8

EC50 (95% CI): ≥ 1000 mg/L

RaCm (5): 0.5379

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	8.8	8.7	8.6	8.5	8.4	8.3	8.2	8.1	7.9	7.6	7.4	20.2	0.2752	0.2714	
1000A	8.7	8.6	8.5	8.3	8.1	7.9	7.7	7.4	7.3	7.1	6.8	20.2	0.4036	0.3981	-36
1000B	9.0	8.8	8.7	8.5	8.2	8.1	7.9	7.7	7.4	7.2	7.0	20.1	0.4109	0.4081	-40
BK2	8.7	8.6	8.4	8.3	8.1	7.9	7.6	7.5	7.4	7.3	7	21.5	0.3479	0.3134	

% diff in controls 4): 14.4

EC 50 (95% CI): ≥ 1000 mg/L

RaCm (5): 0.2924

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

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

(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