MICROTOX® TOXICITY 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: Azur Environmental Corp. Microtox® "BASIC" Procedure
Test type: Static acute
GLP: No
Year study completed: 1996
Year report completed: 1997
Species: *Vibrio fischeri* formerly *Photobacterium phosphoreum*
Analytical monitoring: pH and osmotic condition
Replicates: Two
Statistical methods: EC50 values calculated by statistical linear regression program provided for Microtox®
Test organism source: Azur Microtox® Reagent.
Test Conditions:

Dilution water: Azur Environmental Corp. diluent
Stock and test solution preparation: Water accommodated fractions. Test solutions were prepared for each test concentration by mass addition of test substance in Millipore Milli-Q™ water. The solutions were stirred overnight in the dark at ambient room temperature. A white precipitate was observed in the stock solutions after mixing. Prior to test initiation, a 10 mL aliquot of the clear liquid portion of each test solution was removed and each was osmotically adjusted. All test solutions were made by proportional dilution.
Exposure vessels: 4 mL glass cuvettes.

Number of replicates: Two
Number of concentrations: Four plus a control.
Element Basis: Percent light loss.

RESULTS

Nominal concentrations: Blank control, 125, 250, 500, 1000 mg/L
Element value: 5-minute EC_{50} = 600 (580-620) mg/L
15-minute EC_{50} = 510 (500-530) mg/L
30-minute EC_{50} = 350 (240-490) mg/L

All element values based on nominal concentrations.

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 30-minute EC_{50} for *Vibrio fischeri* was determined to be 350 mg/L with a 95% Confidence Interval of 240-490 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 in the test solutions and sample purity is not sufficiently characterized. Additionally, data is for a mixture and toxicity cannot be positively attributed to didecyldimethylammonium Perfluorooctylsulfonate salt alone.

REFERENCES

This study was conducted by the 3M Company Environmental Laboratory, St Paul, MN, Lab Request number P3025.

OTHER

Last changed: 5/24/00

3M ENVIRONMENTAL LABORATORY

INHIBITORY EFFECTS OF P3025 ON MICROTOX®

Azur Environmental Microtox® System

STUDY COMPLETED: December 19, 1996

FINAL REPORT COMPLETED: May 12, 1997

Prepared by:
Name: Susan A. Beach
Title: Advanced Environmental Biologist
Laboratory: 3M Environmental Laboratory
Address: Building 2-3E-09
935 Bush Avenue
St Paul, MN 55144

Env. Lab Request Number P3025

SUMMARY

The purpose of this study was to evaluate the inhibitory effect of P3025 on bioluminescent bacteria in accordance with the Azur Environmental Corp. Microtox® test. The median effective concentration, 30-minute EC_{50} was reported as 350 mg/L.

INTRODUCTION

Toxicity tests with Microtox® provide a rapid screening method and can be reliable predictors of the inhibitory effects of contaminants on microbial systems.

This toxicity study measured the light output of bioluminescent bacteria over a relatively short period (5, 15, and 30-minutes) 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 mmHg @ 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 solutions were prepared as Water-Accommodated Fractions (WAFs). Four stock solutions of 250, 500, 1000, and 2000 mg/L were prepared in Millipore Milli-Q water. Each was capped and allowed to mix overnight, in the dark, at ambient room temperature. Test substance was apparently not completely miscible with water. A few white particulates were observed in each stock solution after mixing. Just prior to test initiation, a 10 mL aliquot of the clear liquid portion of each stock solution was removed and each was osmotically adjusted with 200 mg reagent grade NaCl. Each initial concentration was diluted by 50% during the course of the test.
Reported values	The reported values are based on nominal concentrations in the test media at the beginning of the microbial assay.

Controls:

Blank control	Two controls containing Microtox® reagent (*Photobacterium phosphoreum*), but no test substance.

Exposure:

Test Date	12/19/97
Contact Time	5, 15, and 30-minutes
Vessels	4 mL Azur Environmental glass cuvettes
Number of Replicates	Two
Test Solution Vol.	1.0 mL
Starting Test Conc. Test Substance	125, 250, 500, and 1000 mg/L
Dilution Water	Azur Environmental Diluent (ultra pure water osmotically adjusted to 2% NaCl)
Temperature	Instrument temperature set at 15°C
Lighting	Ambient laboratory light
Measurements	Light readings were taken upon reconstitution of the Microtox® bioluminescent bacteria, and then 5, 15, and 30-minutes after test substance was added to the bacteria. All readings were captured electronically via computer interface with the Microtox® instrument.
Procedure	The test was conducted in general accordance with the Azur Environmental Microtox® Basic Test.

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 provided in the Azur Environmental software. The EC_{50} is defined as the concentration estimated to inhibit the rate of bioluminescence by 50% as compared to the controls, after 5, 15, and 30-minutes of exposure to the test substance.

RESULTS

The percent reduction in light output and the median effective concentration, EC_{50}, obtained after 5, 15 and 30-minutes exposure to the test substance P3025 are summarized in Table 1.

The median effective concentration, 30-minute EC_{50} and 95% confidence interval was reported as 350 (240 - 490) mg/L.

ACCEPTABILITY CRITERIA

This toxicity test meets quality criteria provided by Azur Environmental Corporation:

1) The 95% confidence factor for the EC_{50} was <1.3 for the 5 and 15-min exposure periods. It was > 1.3 at 30-min; however, this was probably due to complete inhibition at the highest concentration tested (1000 mg/L) .

2) The coefficient of determination was > 0.9 for the 5 and 15-min exposure periods. It was < 0.9 at 30-min; however, this was probably due to complete inhibition at the highest concentration tested (1000 mg/L) .

3) The correction factor was > 0.7 at 5-min and > 0.6 at 15 and 30-min.

This new version of Azur Environmental Corporation software does not provide the correlation coefficient.

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

REFERENCES

1. Microbics Corporation. 1992. Microtox® Manual. Volume 2. Detailed Protocols. Carlsbad, CA.

2. Microbics Corporation. 1993. Microbics Customer Training Manual. From February, 1993 workshop.

TABLE 1

SUMMARY OF TOXICITY OF P3025 TO MICROTOX

PERCENT LIGHT LOSS*

TEST CONC. mg/L	5-Min. Exposure	15-Min. Exposure	30-Min. Exposure
125	14	22	28
250	15	25	33
500	26	47	62
1000	95	98	100
EC_{50}	600	510	350
95% Confidence Interval	(580 - 620)	(500 - 530)	(240 - 490)
Statistical method used	Azur software	Azur software	Azur software

* Mean of two replicates