

ACUTE TOXICITY TO FISH

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 203

Type: Static acute

GLP: No

Year completed: 1996

Species: *Pimephales promelas*

Supplier: Not noted.

Analytical monitoring: DO, pH, temperature, and conductivity were monitored daily.

Exposure period: 96-hours

Statistical methods: LL₅₀ values calculated using the Trimmed Spearman-Kärber method. The NOEL was calculated using Fisher's Exact tests.

Test fish age: Not given.

Length and weight: Average length = 11.3 mm, Average weight = 7.8 mg

Loading: 0.26 g/L

Pretreatment: None

Test conditions:

Dilution water: Dechlorinated City of Duluth, MN tap water. Water was aerated for 24-hours prior to use in the test.

Dilution water chemistry:

hardness: 48 mg/L as CaCO₃

pH: 8.08

Lighting: Cool-white fluorescent bulbs. Photoperiod of 16-hours light, 8-hours dark used. No transition period noted.

Stock and test solution preparation: Water accommodated fractions. Test solutions were prepared individually for each test replicate concentration by mass addition of vigorously shaken test substance in 4 L of dilution water. The solutions were vigorously stirred for 21-hours (vortex 1/2 to 1/3 solution depth). The aqueous phase was siphoned from the vessel at mid-depth.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Not noted.

Exposure vessels: 4-L glass jars containing 3-L of test solution. The jars were sealed with Teflon-lined lids fitted with stoppers to accommodate oxygen flushing of headspace.

Number of replicates: two

Number of fish per replicate: ten

Number of concentrations: three plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

9.1 – 14.6 mg/L (control exposure)

8.7 – 18.2 mg/L (700 mg/L exposure)

pH range (0 – 96 hours)

7.80 – 8.08 (control exposure)

7.78 – 7.99 (700 mg/L exposure)

Test temperature range (0 – 96 hours)

20.8 – 20.9°C

Conductivity range (0 – 96 hours):

128 – 142 µmhos/cm (control exposure)

118 – 154 µmhos/cm (700 mg/L exposure)

Remarks: Oxygen was added to the headspace in the jars before sealing initially and at each observation period. The dissolved oxygen concentrations were super-saturated in the test vessels, particularly in the 700 mg/L exposure concentration.

RESULTS

Nominal loading concentrations: Bk control, 400, 700, 1,000 mg/L.

Element value: 24-hour LL₅₀ = 618 (568 - 673) mg/L

48-hour LL₅₀ = 607 (554 - 664) mg/L

72-hour LL_{50} = 595 (551 - 643) mg/L
96-hour LL_{50} = 562 (523 - 604) mg/L
96-hour NOEL = <490 mg/L

All element values based on nominal concentrations.

Biological observations after 96-hours: No mortality or abnormal behavior observed in the negative control during the test. Mortality was observed in the remaining exposure concentrations. Surfacing was observed in half of the fish at the 700 mg/L exposure concentration at 24-hours, and 2 fish were quiescent at 96-hours. No abnormal behavior was observed in the 400 mg/L exposure concentration.

Cumulative percent mortality:

Nominal Loading Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Neg. Control	0	0	0	0
490	10	15	15	25
700	75	75	80	90
1,000	100	100	100	100

Lowest concentration causing 100% mortality: 1,000 mg/L
Mortality of controls: None

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 96-hour LL_{50} for fathead minnow was determined to be 562 mg/L with a 95% confidence interval of 523 –604 mg/L. The 96-hour no observed effects level (NOEL) was <490 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is

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not sufficiently characterized. Additionally, data is for a mixture and toxicity cannot be positively attributed to didecyldimethylammonium Perfluorooctylsulfonate salt alone. Also, supersaturation of the test solutions with oxygen could also have contributed to the toxicity.

REFERENCES

This study was conducted at AScl Corporation, Environmental Testing Division, Duluth, MN, at the request of the 3M Company.

OTHER

Last changed: 5/24/00

STUDY TITLE

ACUTE TOXICITY OF ~~P3025~~ DEVELOPMENTAL MATERIAL
TO FATHEAD MINNOW (*PIMEPHALES PROMELAS*)

DATA STANDARD

OECD GUIDELINE 203

STUDY COMPLETED

January 2, 1997

TESTING FACILITY

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

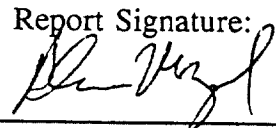
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STUDY IDENTIFICATION NUMBERS

ASCI Study ID# 5010-068

3M Company Lab Request# P3025

1.0 GENERAL INFORMATION

Study Title	Acute Toxicity of P3025 Developmental Material to Fathead Minnow (<i>Pimephales promelas</i>).	
Data Standard	OECD Guideline 203.	
Sponsor	3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133.	
Sponsor's Representative	Susan A. Beach, 3M Environmental Technology and Safety Services, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55133. Tel No. (612) 778-7452.	
Sponsor's Lab Request#	P3025.	
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 Dates	December 17-21, 1996.	
Data Validation and Report Review	Alan Mozol.	Report Signature: 
Retention of Raw Data and Final Report	Two 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(S)

To determine the 96-h LL₅₀ (lethal loading) and 96-h NOEL (no observed effect loading) of the test substance, P3025 Developmental Material, for fathead minnow (*Pimephales promelas*).

This study was conducted according to the ASCI study plan retained by the Sponsor and OECD Guideline 203 (OECD 1993). The test solutions were water-accommodated fractions (WAF) prepared following Girling, Whale, & Adema 1994.

3.0 TEST METHODS

Test Substance	<i>Properties:</i> P3025, two-phase liquid before agitated, light colored, nearly odorless slurry after agitation, specific gravity ca. 1.2 g/cc, and insoluble in water. <i>Test loadings:</i> 0 (dilution water control), 490 mg/L, 700 mg/L and 1000 mg/L. Test loading solutions were prepared individually for each test replicate. The 490 mg/L solutions were prepared by placing 1960 mg of vigorously shaken test substance in 4 L of dilution water. The 700 mg/L and 1000 mg/L solutions were prepared in the same manner using 2800 mg and 4000 mg of test substance, respectively. The solutions were vigorously stirred (vortex 1/3 to 1/2 solution depth) for 21 h. The aqueous phase was siphoned from the vessel at mid depth.
Test Organisms	<i>Source:</i> Healthy, juvenile fathead minnows (<i>Pimephales promelas</i>) obtained from a commercial supplier. <i>Holding/acclimation:</i> Held and acclimated for 12 days in the test dilution water. For the 48 hours preceding the test 0% mortality occurred. For the entire 12 days, mortality was 0%. At test termination, the fish were 1.1 cm in mean length and 7.8 mg in mean weight. <i>Use in test:</i> Used 10 organisms/replicate and 2 replicates/test concentration.

Dilution Water	<i>Source:</i> Dechlorinated City of Duluth, MN, tap water. The most recent chemical analysis is in Raw Data Package. <i>Characteristics:</i> At test time, the water had a hardness of 48 mg/L (as CaCO₃) and a pH of 8.08. Aerated for 24 h prior to using in the test.
Exposure Chambers	<i>Type:</i> 4-L glass jars sealed with Teflon-lined lids fitted with stoppers to accommodate oxygen flushing of headspace. <i>Use in test:</i> 3 L of dilution water or test solution/chamber. During the test, kept covered, except when experimental observations were made or headspace was flushed with oxygen. Solutions were not aerated during the exposures.
Incubation	<i>Duration:</i> 96 h. <i>Daily photoperiod:</i> 16 h light and 8 h dark, maintained using cool-white fluorescent bulbs. <i>Temperature:</i> 21°C.
Observations	<i>Biological:</i> Daily- Test organisms with abnormal behavior and signs of stress, and dead test organisms. <i>Water chemistry:</i> (1) At test initiation- total hardness and alkalinity, (2) daily- temperature, DO, conductivity, and pH. All determinations were made according to APHA methods (1995).
Endpoint Calculations	<i>24-, 48-, 72-, and 96-h median lethal loading (LL₅₀'s), and 96-h no observable effect loading (NOEL)</i> <i>LL₅₀:</i> Trimmed Spearman-Kärber method. <i>NOEL:</i> TOXSTAT statistical software, Version 3.2, University of Wyoming.

3.0 RESULTS

Test Substance Toxicity (Pertinent data are in Table 1)	24-h LL_{50}: 618 mg/L (568-673 mg/L). 48-h LL_{50}: 607 mg/L (554-664 mg/L). 72-h LL_{50}: 595 mg/L (551-643 mg/L). 96-h LL_{50}: 562 mg/L (523-604 mg/L). 96-h $NOEL$: < 490 mg/L.
Ranges of Water Chemistry Parameters	Total hardness (as $CaCO_3$ mg/L): 44-48. Alkalinity (as $CaCO_3$ mg/L): 44. Temperature ($^{\circ}C$): 20.8-20.9$^{\circ}C$. DO (mg/L): 8.6-20.0. pH: 7.71-8.08. Specific conductivity ($\mu mhos/cm$): 118-487.

4.0 TEST QA/QC

QA/QC Criteria	Data
Mortality in control exposure must not be more than 10%.	0% dead.
The lowest DO concentration at test termination must be at least 60% of the air saturation value at test temperature.	The lowest DO concentration measured was 97% of the air saturation value at 21.0$^{\circ}C$. After initial 0-hour routine chemistries had been performed the chambers were sealed and each chamber's headspace was flushed with oxygen daily to maintain adequate DO. Ensuing DO concentrations were 100% of saturation or greater.

Test duration must be 96 h.	The test duration was 96 h.
Reference substance toxicity test (this test is optional).	Control chart for sodium chloride reference toxicity testing is in Raw Data Package.

5.0 REFERENCES

American Public Health Association (APHA). 1995. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, D.C.

Girling, A.E., G.F. Whale, and D.M.M. Adema. 1994. A Guideline Supplement for Determining the Aquatic Toxicity of Poorly Water-Soluble Complex Mixtures using Water-Accommodated Fractions. Chemosphere, Vol. 29, No. 12, pp. 2645-2649.

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.

University of Wyoming. 1996. Toxstat Version 3.2. Laramie, Wyoming.

TABLE 1. ACUTE TOXICITY OF *Promelas* DEVELOPMENTAL MATERIAL TO FATHEAD MINNOWS (*P. promelas*)

Test Substance Loading (mg/L)	Cumulative Number of Dead Organisms and Percent Mortality ^a			
	24 h	48 h	72 h	96 h
0 (Control)	0 (0)	0 (0)	0 (0)	0 (0)
490	2 (10)	3 (15)	3 (15)	5 (25)
700	15 (75)	15 (75)	16 (80)	18 (90)
1000	20 (100)	20 (100)	20 (100)	20 (100)

^a Percentages are in parenthesis.

**EFFECT AND NO-EFFECT LOADINGS WITH
CORRESPONDING 95% CONFIDENCE INTERVALS**

LL ₅₀ and NOEL	Data (mg/L)	95% Confidence Interval
24-h LL ₅₀	618	568-673
48-h LL ₅₀	607	554-664
72-h LL ₅₀	595	551-643
96-h LL ₅₀	562	523-604
96-h NOEL	<490	Not applicable