

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-121-X, or 3M Sample E2112 and E2566-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafuoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and 2-propanol, 1-(1,1-dimethylethoxy). This mixture contains approximately 22-26% test substance, 33-37% propanol, 1-(1,1-dimethylethoxy), 0.5-1.5% perfluorononanesulfonate, ammonium salt and 38-42% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test sample was from 3M production lot 1.

METHOD

Method: EPA 660/3-75-009 and APHA (1980) Standard Methods for the Examination of Water and Wastewater

Test type: Static acute

GLP: Yes

Year Completed: 1988

Species: *Daphnia magna*

Analytical monitoring: pH, dissolved oxygen, temperature. Test substance concentration was not monitored.

Statistical methods: EC₅₀ values calculated using Binomial method

Test daphnid source: ABC Laboratories In-house cultures, Columbia, MO

Test daphnid age at study initiation: < 24-hours

Test conditions

Dilution water: Well water

Dilution water chemistry (from a representative sample):

hardness: 246 mg/L

alkalinity 178 mg/L

pH: 8.0

Stock and test solutions preparation: Not given. A cloudy appearance and surface foam present at preparation in the 560 and 1000 mg/L concentrations was not noted at the 24- and 48-hour observation periods.

Exposure vessels: 250 mL glass beakers containing 200 mL of test solution.

Number of replicates: 2
Number of daphnids per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study:
 Dissolved oxygen range (0-48 hours):
 8.7 – 9.2 mg/L (control exposure)
 8.6 – 9.2 mg/L (1000 mg/L exposure)
 pH range (0-48 hours):
 8.2 – 8.2 (control exposure)
 8.2 – 8.4 (1000 mg/L exposure)
 Test temperature range (0 – 48 hours)
 19 – 20 °C
Element basis: mortality and sublethal effects

RESULTS

Nominal concentrations: Bk control, 100, 180, 320, 560, 1000 mg/L
Element value: 24-hour LC₅₀ = 200 (100-320) mg/L
 48-hour LC₅₀ = 130 (100–180) mg/L
 48-hour NOEC (sublethal effects) = < 100 mg/L
 Sublethal effects included quiescence and/or
 daphnids tending to the bottom of test vessels.

Statistical Evaluation: The LC₅₀ value and 95% confidence interval was calculated using the binomial method.

All element values based on nominal concentrations.

Mortality of controls: None

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

Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours
Neg. Control	0	0
100	0	0
180	35	100
320	100	100
560	100	100
1000	100	100

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CONCLUSIONS

The test sample 48-hour EC₅₀ for *Daphnia magna* was determined to be 130 mg/L with a 95% confidence interval of 100 – 180 mg/L.

Testing was conducted on the “as-sold” mixture of the subject material in water (~22-26% subject material) The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component.

REFERENCES

This study was conducted by ABC Laboratories, Columbia, MO on behalf of the 3M Company.

OTHER

Last changed: 5/18/00

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SPONSOR

3M Company
Attn: Mr. Mohamed Elnabarawy
EE & PC Environmental Labs
Building 21-2W-05, 900 Bush Avenue
St. Paul, Minnesota 55106

STUDY TITLE

Acute Toxicity of E2566-1 to
Daphnia magna

DATA REQUIREMENT

OECD Guideline 202

AUTHOR

Scott Frazier
Biologist II

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STUDY COMPLETED ON

January 9, 1988

PERFORMING LABORATORY

Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
7200 East ABC Lane
P.O. Box 1097
Columbia, Missouri 65205

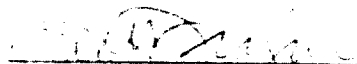
ABC LABORATORY PROJECT ID

#36614

Submitted By: Analytical Bio-Chemistry Laboratories, Inc.
7200 East ABC Lane
P. O. Box 1097
Columbia, Missouri 65205
(314) 474-8579

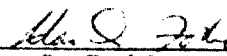
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Prepared By:

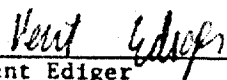


Scott Frazier
Biologist II/Study Director
1/15/88
Date

Approved By:



Alan D. Forbis
Supervisor, Invertebrate/
Bioconcentration Investigations
1/15/88
Date



Kent Ediger
Quality Assurance Officer
1/18/88
Date



Carl M. Thompson
Aquatic Toxicology Manager
1/18/88
Date

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PROJECT PERSONNEL

The Study Director of the project for Analytical Bio-Chemistry Laboratories, Inc. was Scott Frazier, Biologist II. Supervising the conduct of the study for 3M Company was Mr. Mohamed Elnabarawy. The following ABC personnel assisted with various phases of the study.

<u>Name</u>	<u>Title</u>	<u>Initials</u>
Jeffrey Latham	Biological Technician I	<u> JL </u>
Michael Ruddy	Biological Technician II	<u> MR </u>
Scott Frazier	Biologist II	<u> SF </u>

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INVERTEBRATE ACUTE TOXICITY COMPENDIUM

Subject: "Acute Toxicity of E2566-1 to Daphnia magna,"
ABC Study Number 36614.

Sponsor: 3M Company

Testing Facility: Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
P.O. Box 1097
Columbia, Missouri 65205 (314-474-8579)

Location of Original Raw Data and Final Report:

3M Company
EE & PC Environmental Labs
Building 21-2W-05, 900 Bush Avenue
St. Paul, Minnesota

Test Material: E2566-1

Test Concentrations: Control, 100, 180, 320, 560 and 1000 mg/l.

Dilution Water: 246 mg/l (hardness), 178 mg/l (alkalinity),
8.0 (pH).

Test Dates: Initiation - January 7, 1988

Termination - January 9, 1988

Length of Study: 48 hours

Results: 24-hour LC_{50} = 200 mg/l (95% C.I. = 100-320 mg/l)

24-hour NOEL = 100 mg/l

48-hour LC_{50} = 130 mg/l (95% C.I. = 100-180 mg/l)

48-hour NOEL = <100 mg/l (estimated)

Test Species: Daphnia magna

Source of Organisms: ABC Laboratories in-house culture.

Age of Organisms at Study Initiation: First-instar (<24 hours old)

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SUMMARY

The acute toxicity of E2566-1 to Daphnia magna was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen and pH were measured at the termination of the test and were within acceptable limits.

The results of the 48-hour static Daphnia magna toxicity study are summarized below. All reported values were based upon nominal concentrations.

<u>Compound</u>	<u>24-Hour LC₅₀</u> <u>(95% C.I.)</u>	<u>48-Hour LC₅₀</u> <u>(95% C.I.)</u>
E2566-1	200 mg/l (100-320 mg/l)	130 mg/l (100-180 mg/l)

The no-effect level observed for E2566-1 at 24 hours was 100 mg/l based on the absence of all abnormal effects. The 48-hour no-effect level was estimated to be <100 mg/l since abnormal effects were observed in all treated test concentrations.

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INTRODUCTION

This definitive static bioassay was performed by the Aquatic Toxicology Division of Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri, for 3M Company, from January 7, 1988 to January 9, 1988. The purpose of this test was to determine the 24- and 48-hour LC₅₀ levels for E2566-1 to Daphnia magna. The study was performed using ABC Protocol #7806 as approved by Mr. Mohamed Elnabarawy, 3M Company, on January 7, 1988.

METHODS AND MATERIALS

The procedures for static bioassay, as described in Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1) and Standard Methods for Examination of Water and Wastewater (2), were used in this experiment.

I. Test Organisms

The Daphnia magna used in the test were cultured at the ABC facilities in well water (Table 1). The adult daphnids were fed algae (Selenastrum capricornutum) at least every three days prior to testing and supplemented with a suspension of Tetramin®, cereal leaves, vitamin solution and yeast. The daphnids were identified to species using the taxonomic key presented by Pennak (3).

II. Test Procedure

The static Daphnia bioassay was conducted in 250-ml glass beakers containing 200 ml of daphnid culture/test water. Test vessels were covered with loose-fitting petri dish covers to minimize evaporation and protect against contamination during the study. These vessels were kept at 20 (± 2.0) °C in a temperature controlled area. The lighting was maintained at 50-70 footcandles on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods.

An initial range-finding experiment was conducted using ten Daphnia each in exposure concentrations of 1.0, 10, and 100 mg/l. From these results, five treatment concentrations in duplicate with ten Daphnia (first instar less than 24 hours old) per beaker were selected for the definitive bioassay. These concentrations were a logarithmic series ranging from 100 to 1000 mg/l and included a control. All concentrations were observed once every 24 hours for mortality and abnormal effects such as surfacing, clumping of the daphnids together and daphnids tending to the bottom of test chambers. Water chemistry parameters of temperature, dissolved oxygen and pH were measured in the control, low, middle and high concentrations at 0 and 48 hours.

III. Test Compound

The E2566-1 sample was received on December 29, 1987 as a light brown liquid and was stored at room temperature in darkness. Sample purity was unspecified. Test concentrations were prepared based on total product. All standard weights and dilutions can be found in the Appendix. Daphnid culture/test water was used in the preparation of all working stock solutions.

IV. Statistics

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC_{50} program developed by Stephan et al. (4). This program calculated the LC_{50} statistic and its 95 percent confidence limits using the binomial, the moving average and the probit tests. However, if no mortality occurred or if a dose response could not be demonstrated over a reasonable range (<37 to >63%) an LC_{50} and/or its 95 percent confidence limits could not be calculated. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC_{50} (4, 5) although all three models are valid.

RESULTS

Table 2 presents the calculated LC_{50} values and 95-percent confidence intervals for E2566-1. The 24- and 48-hour LC_{50} values were 200 and 130 mg/l, respectively. All results were based on the nominal concentrations of 100, 180, 320, 560 and 1000 mg/l. The no-effect concentration based on the absence of mortality and abnormal effects was 100 mg/l at 24 hours. At 48 hours the no-effect level was estimated to be <100 mg/l since all treated concentrations exhibited abnormal effects. The abnormal effects observed during the study were mortality, quiescence and/or daphnids tending to the bottom of test vessels. A cloudy appearance and surface foam present at preparation in the 560 and 1000 mg/l concentrations was not noted at the 24- and 48-hour observation periods.

Table 3 presents the percent mortality and water quality parameters measured during the test. The dissolved oxygen concentrations ranged between 8.6 and 9.2 mg/l. These values represented 99 and 103-percent saturation at 20 and 19 °C, respectively (corrected for local altitudinal pressure), and were considered adequate for testing (1). The pH values of the test solutions ranged from 8.2 to 8.4 and were consistent with the control.

The study was conducted following the intent of the Good Laboratory Practice Regulations (6) and the final report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data were provided to 3M Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

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TABLE 1
Chemical Characteristics of Well Water Used by
ABC's Aquatic Toxicology Division.

Parameter	Concentration
Temperature	17 - 22°C ^b
Dissolved Oxygen ^a	7.4 - 9.1 ppm ^b
pH	7.6 - 8.4 ^b
Hardness (CaCO ₃)	206 - 275 ppm ^b
Alkalinity (CaCO ₃)	224 - 336 ppm ^b
Conductivity	500 - 650 μ mhos/cm ^b
Total Organic Carbon	1.4 ppm ^c (0.9 - 2.8 ppm) ^d
Suspended Solids	0 - 1.3 ppm ^b
Aluminum	<10 ppb
Arsenic	<1 ppb
Boron	2.25 ppm
Cadmium	<3 ppb
Chromium	<7 ppb
Cobalt	<3 ppb
Copper	<3 ppb
Iron	14 ppb
Lead	<30 ppb
Mercury	0.43 ppb
Nickel	<8 ppb
Silver	<4 ppb
Zinc	2 ppb
Measured organophosphorus pesticides	<0.14 ppb
Measured organochlorine pesticides plus PCB's	<1.0 ppb

^a After aeration

^b Represents seasonal variation, difference from one month to the next, will not exceed 10%

^c Average of all well water values from January-September, 1987

^d Total monthly range of organic carbon

Sample dates: Trace elements = June 3, 1987, organophosphate and organochlorine analyses = May 28, 1987.

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TABLE 2

Acute Toxicity of E2566-1
to Daphnia magna^a

<u>Compound</u>	<u>LC₅₀ (mg/l)</u>	
	<u>24 hours</u>	<u>48 hours</u>
E2566-1	200 (1) (100-320) ^b	130 (1) (100-180) ^b

^aBicassay conducted at 20°C (±2.0).

^b95% confidence limits.

The 24-hour no-effect concentration was 100 mg/l, based on the absence of all abnormal effects. The 48-hour no-effect concentration was estimated to be <100 mg/l since abnormal effects were observed in all test concentrations.

All values were rounded to two significant figures following the technique provided in ABC S.O.P. #8.7.

LC₅₀ calculated using:

(1) Binomial Method

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TABLE 3

Percent Mortality and Water Quality Measurements During the
Acute Toxicity Test of E2566-1 to Daphnia magna.

Nominal Concentration (mg/l)	Percent Mortality		Water Quality					
			0 hours			48 hours		
			Temp. ^a °C	D.O. ^b mg/l	pH ^c	Temp. °C	D.O. mg/l	pH
Control	0	0	19	9.2	8.2	20	8.7	8.2
100	0	0	19	9.2	8.2	20	8.7	8.2
180	35	100						
320	100	100	19	9.2	8.2	20	8.8	8.4
560	100	100						
1000	100	100	19	9.2	8.2	20	8.6	8.4

^aTemperature - mercury thermometer.

^bDissolved oxygen concentrations - YSI 54 ARC dissolved oxygen meter.

^cpH - Corning 140 pH/mV meter with Beckman 39831 electrode.

NOTE: Dissolved oxygen saturations at the test temperatures of 19 and 20°C are 8.9 and 8.7 mg/l, respectively (corrected for altitudinal pressure at ABC Labs).

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LITERATURE CITED

- (1) Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. 1975. U.S. EPA, Ecol. Res. Ser. 660/3-75-009.
- (2) American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. Washington, DC. 1134 p.
- (3) Pennak, R. W. 1978. Freshwater Invertebrates of the United States. John Wiley & Sons, New York, 2nd ed. 803 p.
- (4) Stephan, C. E., K. A. Busch, R. Smith, J. Burke and R. W. Andrews. 1978. A Computer Program for Calculating an LC_{50} . U. S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.
- (5) Stephan, C. 1977. Methods for Calculating an LC_{50} , p. 65-84. In F. L. Mayer and J. L. Hamelink (eds.). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.
- (6) Organization for Economic Cooperation and Development. 1981. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30(Final):7-28.

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QUALITY ASSURANCE STATEMENT

Quality Assurance Statement for report #36614 entitled, "Acute Toxicity of E2566-1 to Daphnia magna," for Mr. Mohamed Elnabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories' intent that all studies conducted at our facilities are designed and function in conformance with good laboratory practice regulations and the protocols for individual laboratory studies, an inspection of the final report for E2566-1 was conducted and found to be in an acceptable form by a member of our Quality Assurance Unit. A procedure audit was conducted on December 14, 1987. Results were reported to the study director on the same day and to management on December 30, 1987. A final inspection of all data and records on January 13, 1988, indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories.

Should you have any questions relating to the information provided in this statement or the function of our Quality Assurance Unit, please contact the Quality Assurance Unit at your convenience.

Kent Ediger
Kent Ediger
Quality Assurance Officer

1/15/88
Date

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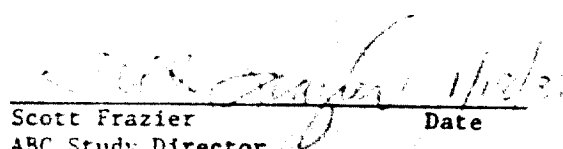
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STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC report #36614 entitled, "Acute Toxicity of E2566-1 to Daphnia magna," for Mr. Mohamed Elnabarawy, 3M Company, St. Paul, Minnesota.

In accordance with ABC Laboratories' intent that all aquatic toxicity tests conducted by our facility follow good laboratory practices, ABC's study director for the above test herein confirms that the study was conducted in compliance with the OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30(Final):7-28.

All original raw data were provided to 3M Company, and a copy retained at Analytical Bio-Chemistry Laboratories.



Scott Frazier

Date

ABC Study Director

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