

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonylamido(ethyl)acetate; may also be referred to as PFOSAA, FC-128 or as the major component of FC-127, FC-129, FC-109, or FC-109-X. (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, potassium salt, CAS # 2991-51-7)

Remarks: The test sample is FC-109-X referred to by the test laboratory as E2566-2. Current information indicates it is a mixture of 19-23% test substance, 61% water, 12% 2-methoxymethylethoxypropanol, 2% ethanol, and 2-6% C₄-C₇ fluoroalkyl carboxylate compounds. Test material was from 3M production lot 2.

The following summary is abbreviated because the test sample is a mixture of the test substance in a water/organic solvent solution. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Methods: OECD 203

Test type: Static acute

GLP: Yes

Year Completed: 1988

Species: *Pimephales promelas*

Analytical monitoring: Temperature, pH, DO

Statistical analysis: LC₅₀ values were calculated using the program developed by Stephan et al.

RESULTS

Nominal concentrations: Bk control, 10, 18, 32, 56, 100, 180, 320 mg/L

Element values: 96-hour LC₅₀ = 57 (36-93) mg/L
96-hour NOEL = <10 mg/L

All element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvents or what portion of the toxicity can be contributed to the Perfluorooctanesulfonylamido(ethyl)acetate.

CONCLUSIONS

The FC-109-X 96-hour LC₅₀ for *Pimephales promelas* was determined to be 57 mg/L with a 95% confidence interval of 36-93 mg/L. The 96-hour no observed effect level was <10 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 at Analytical Bio-Chemistry Laboratories, Inc. of Columbia, Missouri at the request of the 3M Company.

OTHER

Last changed: 5/17/00



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St. Paul, Minnesota 55106

STUDY TITLE

Acute Toxicity of E2566-2
to Fathead Minnow (Pimephales promelas)

DATA REQUIREMENT

OECD Guideline #203

AUTHOR

Jane H. Bowman
Biologist II

STUDY COMPLETED ON

January 12, 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

#36617

Submitted By: Analytical Bio-Chemistry Laboratories, Inc.
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Date

Laura Muller
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TABLE OF CONTENTS

	<u>Page No.</u>
LIST OF ILLUSTRATIONS	36617-4
PROJECT PERSONNEL	36617-5
FISH ACUTE TOXICITY COMPENDIUM.	36617-6
SUMMARY	36617-7
INTRODUCTION.	36617-8
METHODS AND MATERIALS	36617-8
I. Test Fish	36617-8
II. Test Material	36617-8
III. Test Water.	36617-9
IV. Biological Test Procedure	36617-9
V. Biological Data Analysis.	36617-9
RESULTS	36617-10
LITERATURE CITED.	36617-16
QUALITY ASSURANCE STATEMENT	36617-17
STUDY COMPLIANCE STATEMENT.	36617-18

LIST OF ILLUSTRATIONS

Table No.

Page No.

- 1 Chemical Characteristics of Well Water Used
by ABC's Aquatic Toxicology Division. 36617-12
- 2 Acute Toxicity of E2566-2 to Fathead Minnow
(Pimephales promelas) 36617-13
- 3 Mortality Rates and Water Quality Measurements
During the Acute Toxicity Test of E2566-2 to
Fathead Minnow (Pimephales promelas) 36617-14

Figure No.

- 1 Slope of Dose-Response Line at 96 Hours for
E2566-2 to Fathead Minnow (Pimephales
promelas) 36617-15

PROJECT PERSONNEL

The study director of the project for Analytical Bio-Chemistry Laboratories, Inc. was Ms. Jane H. Bowman, 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>
Jane H. Bowman	Biologist II	<u>JHB</u>
Laura Mullen	Biologist I	<u>LMML</u>
Chris Brenneke	Biological Technician I	<u>CAB</u>
James P. Swigert, Ph.D.	Supervisor, Fish Acute/ Chronic Investigations	<u>JPS</u>

FISH ACUTE TOXICITY COMPENDIUM

Subject: "Acute Toxicity of E2566-2 to Fathead Minnow (Pimephales promelas).\" ABC Study Number 36617.

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
St. Paul, Minnesota 55106

Test Material: E2566-2

Test Concentrations: Control, 10, 18, 32, 56, 100, 180 and 320 mg/l

Dilution Water: 42 mg/l (hardness), 32 mg/l (alkalinity),
7.4 (pH)

Test Dates: Initiation - January 8, 1988

Termination - January 12, 1988

Length of Study: 96-hours

Results: 96-hour LC_{50} = 57 mg/l (95% C.I. = 36-93 mg/l)

96-hour NOEL = <10 mg/l

96-hour Slope of the Dose-Response Line was 16

Test Species: Fathead Minnow (Pimephales promelas)

Source of Organisms: ABC Laboratories in-house culture

Mean Weight: 0.061 g (± 0.037 g)

Mean Length: 17 mm (± 2.2 mm)

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3M Company
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Test Material: E2566-2

Test Concentrations: Control, 10, 18, 32, 56, 100, 180 and 320 mg/l

Dilution Water: 42 mg/l (hardness), 32 mg/l (alkalinity),
7.4 (pH)

Test Dates: Initiation - January 8, 1988

Termination - January 12, 1988

Length of Study: 96-hours

Results: 96-hour LC_{50} = 57 mg/l (95% C.I. = 36-93 mg/l)

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Test Species: Fathead Minnow (Pimephales promelas)

Source of Organisms: ABC Laboratories in-house culture

Mean Weight: 0.061 g (± 0.037 g)

Mean Length: 17 mm (± 2.2 mm)

SUMMARY

The acute toxicity of E2566-2 to fathead minnow (Pimephales promelas) 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 throughout the test and were within acceptable limits. Culture and acclimation records indicated the fish were in good condition for testing.

The study was conducted at the following nominal concentrations of E2566-2: 10, 18, 32, 56, 100, 180 and 320 mg/l. At 0-hour, the 18, 32, 56, 100, 180 and 320 mg/l solutions had a white surface film. The amount of surface film increased with the concentration from a very slight amount in the 18 mg/l solution to a thicker, nearly complete covering over the 320 mg/l solution. After 24 hours, a slight surface film also appeared over the 10 mg/l solution while the other solutions had a surface film increasing with the concentration. At the 72- and 96-hour observations the 100, 180 and 320 mg/l solutions were cloudy and all solutions had a surface film that increased in proportion to the concentration. Ten fish, with a mean weight of 0.061 (± 0.037) g and a mean standard length of 17 (± 2.2) mm, were exposed to each test concentration and control.

The results of the four day static fish toxicity study using E2566-2 are summarized below. The 24-, 48- and 72-hour LC_{50} values were also determined. The 96-hour slope of the dose response line was 16.

<u>Compound</u>	<u>96-Hour LC_{50}</u> <u>(95% C.I.)</u>
E2566-2	57 mg/l (36-93 mg/l)

Also, the results indicated a 96-hour, no-observed effect concentration could be estimated at <10 mg/l, the lowest concentration tested. Abnormal effects of mortality, surfacing, loss of equilibrium, dark discoloration, fish on the bottom of test chamber, quiescence and/or excitability were observed during the 96-hour exposure period in the 10, 18, 56, 100, 180 and 320 mg/l test concentrations. One fish in the control chamber was quiescent and on the bottom of the test chamber at the 96-hour observation.

INTRODUCTION

This static bioassay was performed by the Aquatic Toxicology Division of Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri, for 3M Company, from January 8, 1988 to January 12, 1988. The purpose of this test was to determine the 24-, 48- and 96-hour LC₅₀ levels for E2566-2 to fathead minnow (Pimephales promelas). A preliminary range-finding study was conducted from December 31, 1987 to January 4, 1988 to determine the concentration range for the definitive bioassay. The study was conducted following the procedures outlined in ABC Protocol Number 7601 (Raw Data), as approved by Mr. Mohamed Elmahrawy, 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 Fish

The fathead minnow (ABC Lot #2287 FHM) used in the test were obtained from an ABC Labs in-house culture. All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least fourteen days prior to testing. Fish culture techniques used were basically those described by Brauhn et al. (3). A daily record of fish observations during the holding period, along with any prophylactic or therapeutic disease treatments, is included in the Raw Data. During this period, the fish received a standard commercial fish food occasionally supplemented with brine shrimp nauplii (Artemia sp.) daily until 48-96 hours prior to testing at which time feeding was discontinued. The fathead minnow used for this experiment had a mean weight of 0.061 (± 0.037) g and a mean standard length of 17 (± 2.2) mm. This gave a test chamber loading biomass of 0.041 g/l for the definitive study. Weight and length measurements were made on the control group of fish at the termination of the test and are included in the Raw Data.

II. Test Material

The E2566-2 sample was received on December 29, 1987, in good condition. The sample upon receipt was observed to be a foam yellow liquid and was stored at room temperature. Sample purity was not specified. The definitive test concentrations were obtained by transferring appropriate weights of test compound directly to the test chambers. All test concentrations were based on the total compound, i.e. not corrected for sample purity. All standard weights and dilution values are listed in the Raw Data.

III. Test Water

The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of soft reconstituted water composed of the following compounds in the amounts stated per liter of deionized water:

48 mg NaHCO_3
30 mg $\text{CaSO}_4 \cdot 2 \text{H}_2\text{O}$
30 mg MgSO_4
2 mg KCl

This reconstituted water was prepared to yield a total hardness of 40-48 mg/l as CaCO_3 , a total alkalinity of 25-35 mg/l as CaCO_3 and an initial pH of 7.2 to 7.6. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 mg/l and pH 7.4. The well water source from which this dilution water was prepared had the characteristics shown in Table 1.

The test vessels were kept in a water bath at 22°C (± 1.0). The test fish were acclimated to the dilution water and test temperature and held without food for 48-96 hours prior to testing. An acclimation record is included in the Raw Data.

IV. Biological Test Procedure

A range-finding test of 96-hours duration was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 30, 100 and 320 mg/l. Based on the results of preliminary testing, seven concentrations of the test compound, ranging in a logarithmic series from 10 to 320 mg/l, with ten fish per concentration were selected for the definitive bioassay. Also included was a dilution water control. The fish were added to the test chambers by random assignment within 30 minutes after addition of test material. All test organisms were observed once every 24 hours for mortality and abnormal (sub-lethal) effects. Any dead individuals were removed from the test chambers after each 24-hour observation.

V. Biological Data Analysis

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. The concentration-mortality curve was constructed for the 96-hour mortality data. The slope of the dose-response line was reported as calculated by least squares regression analysis (log of concentration vs. probit transformation of percent mortality).

RESULTS

The results of the 96-hour static toxicity test with fathead minnow (*Pimephales promelas*) exposed to E2566-2 are presented in Table 2. The 24-, 48- and 96-hour LC_{50} values for E2566-2 were >320, 160 and 57 mg/l, respectively. The 96-hour slope of the dose-response curve was 16 (Figure 1). All results were based on the nominal concentrations of 10, 18, 32, 56, 100, 180 and 320 mg/l. At 0-hour, the 18, 32, 56, 100, 180 and 320 mg/l solutions had a white surface film. The amount of surface film increased with the concentration from a very slight amount in the 18 mg/l solution to a thicker, nearly complete covering over the 320 mg/l solution. After 24 hours a slight surface film also appeared over the 10 mg/l solution while the other solutions had a surface film increasing with the concentration. At the 72- and 96-hour observations the 100, 180 and 320 mg/l solutions were cloudy and all solutions had a surface film that increased in proportion to the concentration.

The 96-hour no-observed effect concentration was estimated to be <10 mg/l, the lowest concentration tested. The abnormal effects of mortality, surfacing, loss of equilibrium, dark discoloration, fish on the bottom of test chamber, quiescence and/or excitability were observed in the 10, 18, 56, 100, 180 and 320 mg/l test concentrations during the 96-hour exposure period. One fish in the control chamber was quiescent and on the bottom of the test chamber at the 96-hour observation. The 10 mg/l chamber also had 20% mortality at 96 hours while the 18 and 32 mg/l chambers had 0% mortality. An examination of the fish culture and acclimation records for this test indicated that the fish were in good condition for testing (Raw Data).

Nominal test concentrations, mortality rates and water quality data are presented in Table 3. The dissolved oxygen concentrations ranged from 6.8 to 9.8 mg/l during the test. These values represented 81 and 117% saturation at 22°C, respectively, and were considered adequate for testing (1), therefore, the test chambers were not aerated during testing. The pH values ranged from 7.2 to 7.9.

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 was provided to 3M Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

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 hos/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

^aAfter aeration

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

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

^dTotal monthly range of organic carbon

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

TABLE 2

Acute Toxicity of E2566-2 to Fathead Minnow
(Pimephales promelas)

Compound	LC ₅₀ in milligrams/liter (ppm)		
	24 hours	48 hours	96 hours
E2566-2 ^a	>320 ^(b)	160 ⁽³⁾ (120-210) ^c	57 ⁽²⁾ (36-93) ^c

N = 10 fish per concentration.

^a Bioassay as conducted at 22°C (±1.0), mean fish weight and length, 0.061 (±0.037)g and 17 (±2.7)mm.

^b LC₅₀ could not be calculated due to insufficient mortality data.

^c 95% confidence limits.

The 96-hour no-observed effect concentration could be estimated at <10 mg/l.

The 96-hour slope of the dose-response line was 16 as calculated by least squares regression analysis.

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

LC₅₀ calculated using:

- (1) Binomial Method
- (2) Moving Average Method
- (3) Probit Method

TABLE 3

Mortality Rates and Water Quality Measurements During the Acute Toxicity Test
of E2566-2 to Fathead Minnow (*Pimephales promelas*)

Nominal Concentration (mg/l)	Percent Mortality Hours			Water Quality								
				0-hours			48-hours			96-hours		
	24	48	96	Temp. °C	D.O. ^a mg/l	pH ^b	Temp. °C	D.O. mg/l	pH	Temp. °C	D.O. mg/l	pH
Control	0	0	0	22	9.3	7.4	22	8.2	7.4	22	8.1	7.5
10	0	0	20	22	9.2	7.5	22	8.2	7.4	22	7.4	7.4
18	0	0	0							22	7.5	7.4
32	0	0	0	22	9.3	7.5	22	7.5	7.3	22	6.8	7.2
56	0	10	30							22	7.0	7.3
100	0	10	100	22	9.5	7.6	22	7.5	7.4			
180	0	50	100				22	7.4	7.3			
320	10	100	100	22	9.8	7.9						

^a Dissolved oxygen concentrations - Dissolved Oxygen Probe (YSI Model 54).

^b pH - pH Probe (Corning Model 476182) used with a Corning Model 125 pH and mV meter.

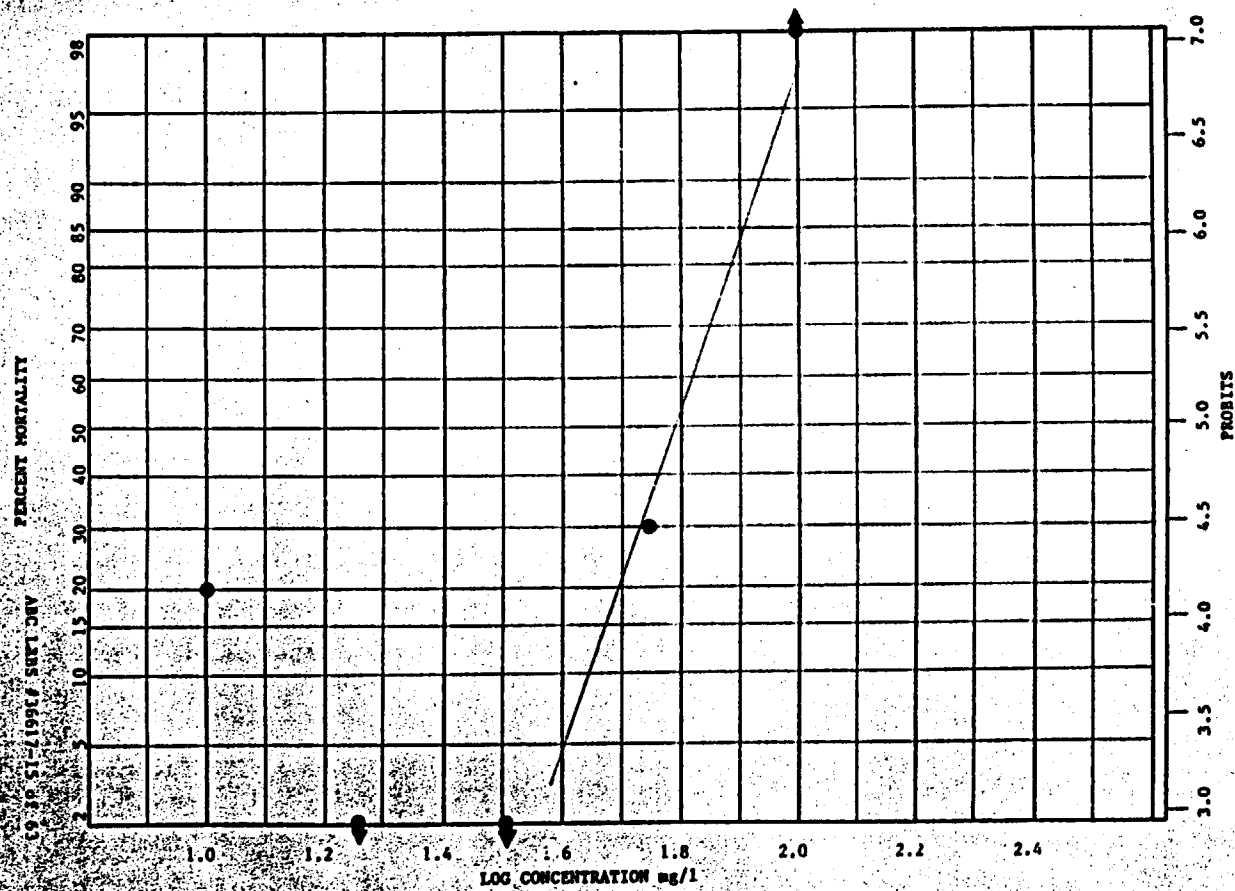
NOTE: Dissolved oxygen saturation corrected for altitude at the test temperature of 22°C is 8.4 mg/l.

PERCENT MORTALITY

AHC LABS # 36617-15 of 63

2 5 10 15 20 30 40 50 60 70 80 85 90 95 98

Figure 1
Slope of Dose-Response Line at 96 Hours for E2566-2
to Fathead Minnow (*Pimephales promelas*)



LITERATURE CITED

- (1) Committee on Methods for Toxicity Tests with Aquatic Organisms (C. E. Stephan, Chairman). 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. 61 p.
- (2) American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. Washington, DC. 1134 p.
- (3) Brauhn, J. L. and R. A. Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May, 1975. 45 p.
- (4) Stephan, C. E., K. A. Busch, R. Smith, J. Burke and R. W. Andrew. 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) (a) U. S. Food and Drug Administration. 1978. Nonclinical Laboratory Studies, Good Laboratory Practice Regulations (21 CFR, Part 58). Federal Register, Vol. 43, No. 247:59986-60025.
- (b) U. S. Environmental Protection Agency. 1983. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, Vol. 48, No. 230: 53946-53969.
- (c) U. S. Environmental Protection Agency. 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). Federal Register, Vol. 48; No. 230:53922-53944.
- (d) 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.

QUALITY ASSURANCE STATEMENT

Quality Assurance Statement for final report #36617 entitled, "Acute Toxicity of E2566-2 to Fathead Minnow (Pimephales promelas)," 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 E2566-2 was conducted and found to be in an acceptable form by a member of our Quality Assurance Unit. An inspection of the daily mortality rate of the test organisms prior to the initiation of the study indicated they were in good health and should not bias the observed mortality in the study. A procedure audit was conducted on January 8, 1988. Results were reported to the study director on January 8, 1988. Results were reported to management on January 20, 1988. A final inspection of all data and records on January 20, 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.

Phillip M. Buckler 12/9/88
Phillip M. Buckler Date
Quality Assurance Supervisor

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC report #36617 entitled, "Acute Toxicity of E2566-2 to Fathead Minnow (Pimephales promelas)," 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 Principles of Good Laboratory Practice Annex 2, C(81), OECD Guidelines for Testing of Chemicals.

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

Jane H. Bowman
Jane H. Bowman
ABC Study Director

2/83
Date

Raw Data Report
#36617

Acute Toxicity of E2566-2 to Fathead
Minnow (Pimephales promelas)

NOTE: Some of the records which appear in this raw data appendix have been provided as photocopies of original records on file at ABC. This has been done by necessity for certain data which are used commonly in several studies at ABC. Such records include acclimation records, organism culture logs and well water chemical screen results.