ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 2327.
The test sample is FX-1003. It's purity was not sufficiently characterized, though current information indicates it is a solution of <45% ammonium perfluorooctanoate, 50% water, <3% inert perfluorinated compound and 1 - 2% C_5 and C_7 perfluoro analogue compounds.

The following summary applies to the test sample as a mixture of the test substance in water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD:

Method: OECD 203
Type: Static acute
GLP: Yes
Year completed: 1990
Species: *Pimephales promelas*
Supplier: Aquatic Research Organisms, Hampton, New Hampshire
Analytical monitoring: Temperature, pH, conductivity, and dissolved oxygen
Exposure period: 96-hours
Statistical methods: Non-linear interpolation, moving average, and/or probit analysis
Test fish age: Juvenile
Length and weight: Average length = 3.8 cm
Average weight = 0.45 g (wet)
Pretreatment: None.
Test conditions:
Dilution water: Hampton, New Hampshire well water
Dilution water chemistry:
pH: 7.4
Conductivity: 1500 µmhos/cm
Hardness: 88 mg/L (as $CaCO_3$)

Lighting: Cool-white fluorescent bulbs with an intensity of 35 $uEs^{-1}m^{-2}$. A daily photoperiod of 16 hours light and 8 hours dark was maintained throughout the testing period.

Test conditions remarks: The fish were fed a commercial fish food once or twice daily during the acclimation period, food being withheld throughout the test period. Fish were acclimated to test temperature for 14 days prior to use in assays.

Aeration was employed after 48 hours to maintain dissolved oxygen concentrations above acceptable levels.
Stock and test solution preparation: Test solutions were created by direct individual weight additions.
Concentrations dosing rate: Once
Stability of the test chemical solutions: Not noted
Exposure vessels: 19.6 L glass aquaria containing 15 liters test solution at an approximate depth of 17 cm.
Number of replicates: two
Number of fish per replicate: 10
Loading rate: 0.30 g/L
Number of concentrations: five plus a blank control
Water chemistry during the study:

Conductivity range: (0-96 hours)

1200 – 1500 µmhos/cm (control exposure)
1300 – 1600 µmhos/cm (1,000 mg/L exposure

pH range (0-96 hours):

7.4 – 8.4 (control exposure
7.8 – 8.2 (1,000 mg/L exposure)

Temperature range (0-96 hours):

21.0 – 22.0 °C (control exposure
21.0 – 22.0 °C (1,000 mg/L exposure)

Dissolved oxygen range (0-96 hours):

6.1 – 9.2 mg/L (control exposure
6.2 – 9.1 mg/L (1,000 mg/L exposure)

RESULTS

Nominal concentrations: Bk control, 150, 250, 400, 600, and 1,000 mg/L
Element value: 96-hour LC50 = >1,000 mg/L

Element value based on nominal concentrations

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The values reported apply to that mixture and not the test substance.

CONCLUSIONS

The test sample 96-hour LC_{50} for fathead minnow was determined to be >1,000 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 2. Testing meets the criteria for quality testing. However, sample purity was not properly characterized and it lacks analytical confirmation of test substance concentrations.

REFERENCES

Test was conducted by EnviroSystems, Inc. of Hampton, NH at the request of the 3M Company, 1990. The EnviroSystems study number was 9014-3

OTHER

Last changed: 5/25/00

Study Title

Static Acute Toxicity of FX-1003
to the Fathead Minnow, *Pimephales promelas*

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

March 15, 1990

Performing Laboratory

EnviroSystems Division
Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842

I. GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to OECD Good Laboratory Practice Regulations. Neither the Study Director nor the sponsor are aware of any circumstances that would affect the integrity of this study. Food was not witheld from fish during the 24 hours prior to the test initiation. This deviation is not believed to have affected the study. No other deviations existed that significantly affected the validity of the study.

Robert L Boeri 3-15-90
Robert L. Boeri
Coauthor and Study Director

Timothy J. Ward 3/5/90
Timothy J. Ward
Author

Jeanne Magazu 3/15/90
Jeanne P. Magazu
Aquatic Toxicologist

Peter Kowalski 3-15-90
Peter L. Kowalski
Aquatic Toxicologist

II. CERTIFICATION OF GOOD LABORATORY PRACTICES

Submitted by:

EnviroSystems Division
Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842

Certification: Data presented in this report were derived by methods and with materials identified in the section of the report entitled "Methods and Materials." The test was performed in accordance with EnviroSystems Protocol 9014-3 and the Product Registration Aquatic Toxicology Laboratory Standard Operating Procedures Manual. The toxicity test was performed by Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward. The raw data and final report will be archived at Resource Analysts Inc. for at least 10 years.

All data transcribed from the raw data to the report were checked for accuracy by two persons, and all data were verified by Quality Assurance Auditors. Quality assurance audits were performed (and reported to management) on February 1,1990, February 15, 1990, and March 14,1990(March 14, 1990).

Danette Tekelenburg 3/15/90
Danette Tekelenburg
Quality Assurance Auditor

Doreen Newhouse 3-19-90
Doreen Newhouse
Quality Assurance Officer

III. TABLE OF CONTENTS

IV. INDEX OF TABLES AND FIGURES

V. SUMMARY

The acute toxicity of FX-1003 to the fathead minnow, *Pimephales promelas*, is described in this final report. The test was conducted for 3M Company for 96 hours during February 12 to 16, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward according to the protocol developed for EnviroSystems Study Number 9014-3.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 21.8°C. The dilution water was filtered natural well water collected from wells at Hampton, New Hampshire. Aeration was employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of FX-1003 were: 0 mg/L (control), 150 mg/L, 250 mg/L, 400 mg/L, 600 mg/L, and 1,000 mg/L. Nominal concentrations were used for all calculations.

Fish used in the test were purchased from a commercial supplier (Aquatic Research Organisms, Hampton, New Hampshire) and acclimated under test conditions for 63 days. After 96 hours of exposure the control fish had an average wet weight (blotted dry) of 0.45 g (resulting in a loading rate of approximately 0.30 g/L), and an average total length of 3.8 cm. All fish were in good condition at the beginning of the study.

Exposure of fathead minnows to the test substance resulted in a 96 hour LC50 of >1,000 mg/L FX-1003, the highest tested concentration. The 96 hour no observed effect concentration is estimated to be >1,000 mg/L.

VI. INTRODUCTION

This study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of FX-1003 to the fathead minnow, a freshwater fish. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the test.

VII. METHODS AND MATERIALS

TEST SUBSTANCE:

FX-1003 (EnviroSystems Sample Number 2308E) was delivered to EnviroSystems on January 29, 1990. It was contained in a 500 mL plastic bottle that was labelled with the following information: G2882-1. FX-1003 (a yellow liquid) was supplied by 3M Company, 935 Bush Avenue, St. Paul, Minnesota. Prior to use the test material was stored in the dark at room temperature. A reserve sample (approximately 1 gram) will be archived at EnviroSystems for a minimum of 10 years.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was well water collected from wells at EnviroSystems in Hampton, New Hampshire. Water was adjusted to a hardness of 88 mg/L as $CaCO_3$ and stored in 500-gallon polyethylene tanks, where it was aerated. Results of chemical analysis of a representative sample of water are presented in Table 1.

TEST ORGANISM:

Juvenile fathead minnows employed as test organisms were from a single source and were identified using an appropriate taxonomic key. They were purchased from a commercial supplier (Aquatic Research Organisms, Hampton, New Hampshire) and acclimated at the EnviroSystems facility for 63 days. Control fish were weighed at the conclusion of the toxicity test. Prior to testing, fish were maintained in 100% dilution water under flow through conditions in an all glass aquarium. During acclimation fish were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. During the acclimation period 14 days prior to the test initiation the temperature ranged from 21.0° to 23.5°C, and the dissolved oxygen concentration was maintained above 8.0 mg/L. Fish were fed a commercial fish food (EnviroSystems lot number TM01) once or twice daily.

Table 1: Chemical characterization of a representative sample of natural well water used as dilution water for toxicity test

Parameter	Unit of Measurement	Reporting Limit	Measured Value
pH	pH units	--	7.4
Conductivity	umhos/cm	--	1500
Hardness	mg/L as $CaCO_3$	--	88
Organochlorine pesticides	ug/L	0.5	ND[a]
Organophosphorus pesticides	ug/L	0.5	ND[a]
Polychlorinated biphenyls	ug/L	0.5	ND[a]

[a]Not detected above the reporting limit.

TOXICITY TESTING:

A screening test with the test substance was conducted during February 6 - 10, 1990. Nominal concentrations of test substance were 0.1 mg/L, 1 mg/L, 10 mg/L, 100 mg/L, and 1,000 mg/L. After 96 hours of exposure there was 100% survival at all tested concentrations.

The definitive toxicity test was performed during February 12 - 16, 1990, according to EnviroSystems Test Protocol 9014-3 (Acute Toxicity of FX-1003 To The Fathead Minnow *Pimephales promelas*), which was signed by the Study Director on February 6, 1990. It is based on procedures of the OECD (1984).

The test was conducted at a target temperature of 23 ± 2°C with five concentrations of test substance and a dilution water control. No stock solution was prepared as test material was added directly to dilution water contained in the test vessels without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L (control), 150 mg/L, 250 mg/L, 400 mg/L, 600 mg/L, and 1,000 mg/L.

Twenty fish were randomly and equally distributed among two replicates of each treatment. The test was performed in 19.6 liter glass aquaria (approximately 20 cm in width, 40 cm in length, and 25 cm in height) that contained 15 liters of test solution (water depth was approximately 17 cm). Test vessels were randomly arranged in a water bath during the 96 hour test (a random numbers table was used to select the location of each vessel). A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 35 $uEs^{-1}m^{-2}$. Aeration was employed after 48 hours to maintain dissolved oxygen concentrations above acceptable levels. Fish were not fed during the test.

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-3), pH (Beckman model pHI 12 meter; instrument number PRL-4), conductivity (Labcomp SCT meter, instrument number PRL-9), and temperature (ASTM mercury thermometer; thermometer number 2040) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test could not be interpreted by standard statistical techniques due to 100% survival at the highest tested concentration.

VIII. RESULTS

All test vessels containing FX-1003 remained clear throughout the test. Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Appendix A, respectively. One hundred percent survival occurred in the control exposure. Control fish had an average total length of 3.8 cm, and an average wet weight (blotted dry) of 0.45 g at the end of the test. Loading rate during the toxicity test was approximately 0.30 g/L.

The 24, 48, 72, and 96 hour LC50s for fish exposed to FX-1003 are presented in Table 3. The 96 hour LC50 is >1,000 mg/L FX-1003, the highest tested concentration. The estimated 96 hour no observed effect concentration is >1,000 mg/L FX-1003.

Table 2. Survival data from toxicity test

Nominal Concentration (mg/L)	rep.	Number Alive					Number Affected				
		0hr	24hr	48hr	72hr	96hr	0hr	24hr	48hr	72hr	96hr
0 (control)	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
150	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
250	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
400	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
600	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
1,000	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0

Note: Sublethal effects include loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, and change in behavior

Table 3. Median lethal concentrations (LC50s) from toxicity test

Exposure period	LC50	95 percent confidence limits	LC50 calculation method
24 hours	>1,000 mg/L	--	--
48 hours	>1,000 mg/L	--	--
72 hours	>1,000 mg/L	--	--
96 hours	>1,000 mg/L	--	--

IX. REFERENCES

OECD. 1981. Decision of the Council Concerning the Mutual Acceptance of Data in the Assessment of Chemicals. Annex 2. OECD Principles of Good Laboratory Practice. C (81) 30 (Final).

OECD. 1984. Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 203, Fish Acute Toxicity Test. Adopted April 4, 1984.

Appendix A: WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Conductivity, pH, temperature, and dissolved oxygen concentration measured during toxicity test

Nominal concentration (mg/L)	Rep.	Conductivity (umho/cm) 0 hr	24 hr	48 hr	72 hr	96 hr	pH 0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	1500	1300	1200	1400	1300	7.4	7.7	8.0	8.3	8.4
	2	1500	1300	1200	1400	1300	7.4	7.5	8.0	8.3	8.4
150	1	1500	1300	1200	1400	1300	7.4	7.6	7.9	8.0	8.3
	2	1500	1300	1200	1400	1300	7.4	7.6	7.9	8.1	8.3
250	1	1500	1400	1200	1400	1300	7.4	7.6	7.9	8.1	8.3
	2	1500	1400	1200	1400	1300	7.5	7.6	7.9	8.1	8.3
400	1	1500	1400	1200	1400	1300	7.5	7.6	7.9	8.2	8.3
	2	1500	1400	1200	1400	1300	7.5	7.6	7.9	8.1	8.3
600	1	1500	1400	1300	1400	1300	7.6	7.7	8.0	8.1	8.2
	2	1500	1400	1300	1400	1300	7.6	7.8	8.0	8.2	8.3
1,000	1	1600	1400	1300	1500	1400	7.8	7.8	8.0	8.1	8.2
	2	1600	1400	1300	1500	1400	7.8	7.8	8.0	8.1	8.2

Table A.1. Continued.

Nominal concentration (mg/L)	Rep.	Temperature (°C) 0 hr	24 hr	48 hr	72 hr	96 hr	Dissolved Oxygen (mg/L) 0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	21.0	22.0	22.0	22.0	21.8	9.1	8.5	6.1	8.6	9.2
	2	21.0	22.0	22.0	22.0	21.8	9.1	8.3	6.3	8.8	9.2
150	1	21.0	22.0	22.0	22.0	21.8	9.1	8.0	6.0	8.5	9.2
	2	21.0	22.0	22.0	22.0	21.8	9.1	8.1	6.5	8.7	9.2
250	1	21.0	22.0	22.0	22.0	21.8	9.1	8.1	6.1	8.6	9.0
	2	21.0	22.0	22.0	22.0	21.8	9.1	8.0	6.7	8.6	8.8
400	1	21.0	22.0	22.0	22.0	21.8	9.1	8.2	6.4	8.6	8.9
	2	21.0	22.0	22.0	22.0	21.8	9.1	8.1	6.4	8.9	9.0
600	1	21.0	22.0	22.0	22.0	21.8	9.1	8.0	6.8	8.8	9.0
	2	21.0	22.0	22.0	22.0	21.8	9.1	8.1	6.5	9.0	9.0
1,000	1	21.0	22.0	22.0	22.0	21.8	9.1	8.2	6.2	8.9	9.1
	2	21.0	22.0	22.0	22.0	21.8	9.1	8.2	6.2	8.9	9.0

EnviroSystems Study Number 9014-3
Effective Date: January, 1990

RESOURCE ANALYSTS, INCORPORATED
ENVIROSYSTEMS DIVISION

THIS IS A CERTIFIED
TRUE COPY.
SIGNATURE [signature] DATE 09/27/1

49 pages

PRODUCT REGISTRATION LABORATORY
AQUATIC TOXICOLOGY STUDY PROTOCOL

Acute Toxicity of FX-1003 To the Fathead Minnow, *Pimephales promelas*

Sponsor

3M Company
PO Box 33428
St. Paul, Minnesota 55133

Sponsor Approval of Protocol [signature] Date 1-24-90

Testing Facility

EnviroSystems, Inc.
41 Lafayette Road
Hampton, NH 03842

Estimated Test Initiation Date: January, 1990
Estimated Test Completion Date: March, 1990
Study Director: Robert L. Boeri

Study Director Approval of Protocol [signature] Date 2-6-90

1.0 TITLE

Acute Toxicity of FX-1003 to the Fathead Minnow, *Pimephales promelas*

2.0 PURPOSE

To determine the 24, 48, 72, and 96 hour median lethal concentrations (LC50s), of test substance to fish exposed under static conditions.

3.0 TEST MATERIAL

3.1 The test substance and related stability and purity data will be supplied by the sponsor. Test material will be stored at 2-4°C in the original shipping container, unless alternate storage conditions are specified by the sponsor. Handling of the test material will be in accordance with information contained in the sponsor-supplied Material Safety Data Sheet and RAI Standard Operating Procedure 01-02-1106. A subsample of test substance (approximately 1 gram) will be removed from the original container and transferred to a glass vial for archiving. All unused test substance will be returned to the sponsor.

3.2 Calculations are based on nominal concentrations of the test substance. Test material stock solutions are prepared in deionized or dilution water without the use of a solvent (carrier) if possible. If a solvent is required, dimethylformamide, triethylene glycol, or acetone will be used. The concentration of solvent will be limited to 100 mg/L.

4.0 TEST SPECIES

4.1 The selection of test species is determined by the sponsor. Healthy juveniles from a single source will be used to initiate the test. They will be obtained from a commercial supplier. Fish should be 1-3 cm long at the start of the test. The length and wet weight of control organisms will be determined at the end of the test.

4.2 Identification of the test animals shall be verified using appropriate taxonomic keys.

5.0 PRETEST OBSERVATIONS AND PROCEDURES

5.1 Pretest observation data concerning the source, handling procedures, receipt date, disease treatment (if any), health, feeding, and mortality of test animals will be recorded and reported.

5.2 Fish will be maintained under static, replacement, or flow-through conditions prior to test initiation in the same water and at the same photoperiod and temperature that will be used for testing. The animals will be fed at least once daily with commercial fish food and/or brine shrimp nauplii during acclimation except for the 24 hour period immediately preceeding the test initiation. Each batch of food will be analyzed to verify that it is free of measurable concentrations of pesticides. Fish will be maintained in dilution water at test conditions (temperature, hardness, and photoperiod) for at least 12-15 days prior to testing.

6.0 EXPOSURE CONDITIONS

6.1 The study will be conducted under static conditions and test media will only be provided at the beginning of the test. The test animals will be added to test media after the toxicant is added to the test media.

6.2 Dilution water will be natural ground water. It will have a hardness of 50-250 mg/L as $CaCO_3$, a pH of 6.0-8.5, be free of measurable concentrations of pesticides, and meet the requirements specified in U.S.EPA (1986,1987). Water will be passed through a <20 micron filter and chemically characterized (at least twice yearly analysis at RAI) prior to use.

6.3 Water temperature will be 23 $\pm$ 2°C.

6.4 Dissolved oxygen concentration will be maintained above 80% saturation. If aeration is required it will be supplied to all test chambers. Sponsor approval will be obtained prior to aeration.

6.5 Photoperiod will be automatically controlled and adjusted to 16 hours light and 8 hours dark.

6.6 The test vessels will be 19.6 L glass aquaria containing at least 15 L of solution. Loading rate will be less than 1.0 g/L.

7.0 STUDY CONDUCT

7.1 Range Finding Investigation

7.1.1 The results of the range finding investigation and/or previous testing data will be used to select the concentration range needed for determination of the definitive LC50s.

7.1.2 If a range finding test is conducted, the animals will be exposed to a series of concentrations of test substance plus a control (dilution water without test substance) and a solvent control (if necessary).

7.1.3 At least 10 animals will be exposed to each treatment. The animals will be exposed in groups of 10 animals per vessel, each containing at least 15 L of solution. The animals will be equally and randomly assigned to the test vessels (a random numbers table will be used to choose the test vessel for each specimen), and they will not be fed.

7.2 Definitive Investigation

7.2.1 The animals will be exposed for 96 hours to a series of at least five concentrations of test substance, a control, and a solvent control (if necessary) under static conditions. The highest tested concentration will be 1 g/L. The dilution factor between concentrations will be ≤1.8.

7.2.2 Each treatment group will consist of 20 animals with 10 animals per duplicate test vessel. The animals will be randomly assigned to the test vessels.

7.2.3 Dissolved oxygen concentration, pH, temperature, and conductivity will be measured and recorded at the beginning of the test and daily thereafter in all test vessels, as long as living animals are present in those vessels.

7.2.4 Hardness in the dilution water will be measured at 0 and 96 hours.

7.2.5 Mortality and sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change of behavior) will be recorded at 24 hour intervals during the test. Dead animals will be removed when first observed.

7.3 Data Analysis and Statistical Methods

The 24, 48, 72 and 96-hour LC50 values and their 95% confidence intervals will be calculated by the non-linear interpolation, moving average and/or probit methods. If toxicity occurs the slope of the dose-response curve will be provided.

8.0 QUALITY ASSURANCE AND QUALITY CONTROL

8.1 The test will be repeated if any of the following conditions occur:

8.1.1 Control survival at the end of the test is less than 90%, or if behavioral abnormalities are observed in the controls.

8.1.2 Temperature in any test vessel is outside of the acceptable range or dissolved oxygen concentrations fall below 80% saturation.

8.2 The test will be conducted according to OECD Good Laboratory Practice Standards.

8.3 All critical phases of the test conducted at Resource Analysts Inc. will be audited by the Quality Assurance Unit.

8.4 The Study Director will be responsible for reviewing all data and for recording any changes to procedures outlined in this protocol in a protocol amendment. Protocol amendments will be approved by the sponsor.

8.5 Original data will be archived at Resource Analysts Inc. for at least 10 years or it will be transferred to the sponsor for archiving.

9.0 REPORT

9.1 Three original reports will be prepared after review of a draft report by the sponsor. The final report will be signed by the Study Director and staff that conducted the study and prepared the report, and by the Quality Assurance Unit Representative.

9.2 The final report will consist of at least the following information:

9.2.1 Title page, including the study title, data requirement, author, study completion date, testing facility, and sponsor.

9.2.2 Sponsor-supplied confidentiality claims.

9.2.3 Good Laboratory Practice Statement.

9.2.4 Certification of Good Laboratory Practices.

9.2.5 Table of Contents.

9.2.6 Summary of test procedures and results.

9.2.7 Introduction.

9.2.8 Methods and Materials, including a description of test methods, organisms, dilution water, and statistical techniques.

9.2.9 Results, including all data from range-finding and definitive tests, and LC50 values.

9.2.10 References.

9.2.11 Appendices, including all water quality data and the test protocol with amendments (if any).

10.0 REFERENCES

OECD. 1984. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 203, Fish Acute Toxicity Test. Adopted April 4, 1984.

U.S. EPA. 1986. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Federal Register, Monday, January 6, 1986. Section 797.1400.

U.S. EPA. 1987. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Amendments. Federal Register, Wednesday, May 20, 1987. Section 797.1400.

SUMMARY OF TEST CONDITIONS

Acceptable Species:	*Pimephales promelas*
Reference:	OECD 203
Test Type:	Static
Duration:	96 Hours
Flow Rate:	Not Applicable
Dilution Water:	Natural Ground Water
Age/Size of Test Organisms:	Juveniles; 2.0 ± 1.0 cm long
Acclimation Period:	12-15 days
Temperature:	23 ± 2°
Photoperiod:	16 Hours Light/8 Hours Dark
Light Intensity:	Ambient Laboratory Lighting
Number of Concentrations:	5 or more
Dilution Factor:	≤1.8
Number of Replicates:	2 or more
Number of Organisms/Replicate:	10
Test Vessel Size:	19.6 L
Test Vessel Volume:	15 L
Solvent:	Dimethylformamide, Triethylene Glycol, or Acetone (all less than 100 mg/L)
Loading Rate:	<1.0 g/L
Feeding:	None During Exposure
Dissolved Oxygen Levels:	≥80% saturation
Effects Measured:	Death; note sublethal effects
Test Concentration Analysis:	None
Water Quality Measurements:	DO, pH, Temperature and Conductivity at 0, 24, 48, 72, and 96 Hours Hardness in dilution water at 0 and 96 hours
Acceptability Criteria:	≤10% Control Mortality Acceptable Temperature Range Acceptable Dissolved Oxygen Concentrations Absence of Behavioral Abnormalities in Controls