ACUTE TOXICITY TO AQUATIC INVERTABRATES (DAPHNIA MAGNA)

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 202
Type: Static acute
GLP: Yes
Year completed: 1990
Species: *Daphnia magna*
Supplier: EnviroSystems, Inc. in-house culture
Analytical monitoring: Temperature, pH, conductivity, and dissolved oxygen
Exposure period: 48-hours
Statistical methods: Non-linear interpolation, moving average, or probit analysis
Test organism age: <24 hours old and produced from daphnids that were maintained under test conditions for at least 14 days.
Pretreatment: None.

Test conditions:
Dilution water: Hampton, New Hampshire well water
Dilution water chemistry:

pH:	8.2
Conductivity:	900 µmhos/cm
Hardness:	180 mg/L (as $CaCO_3$)

Lighting: Cool-white fluorescent bulbs with an intensity of 45 $uEs^{-1}m^{-2}$. A daily photoperiod of 16 hours light and 8 hours dark was maintained throughout the testing period.

Test conditions remarks: Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels.

Daphnids were fed yeast, trout chow, and the freshwater alga *Selenastrum capricornutum* once daily during culturing, but were not fed during the test.

Stock and test solution preparation: Test solutions were created by direct weights addition.
Concentrations dosing rate: Once
Stability of the test chemical solutions: Not noted
Exposure vessels: 250 mL glass beakers containing 200 mL test solution (~7 cm depth).
Number of replicates: four
Number of daphnids per replicate: 5
Number of concentrations: five plus a blank control
Water chemistry during the study:

Conductivity range: (0-48 hours)
800 – 900 µmhos/cm (control exposure)
800 – 900 µmhos/cm (1,000 mg/L exposure)

pH range (0-48 hours):
8.1 – 8.2 (control exposure
8.3 – 8.4 (1,000 mg/L exposure)

Temperature range (0-48 hours):
19.5 – 20.1 °C (control exposure)
19.5 – 20.1 °C (1,000 mg/L exposure)

Dissolved oxygen range (0-48 hours):
9.0 – 9.5 mg/L (control exposure)
9.1 – 9.5 mg/L (1,000 mg/L exposure)

RESULTS

Nominal concentrations: Bk control, 150, 250, 400, 600, and 1,000 mg/L
Element value: 48-hour EC_{50} = 584 (400 – 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 48-hour EC_{50} for *Daphnia magna* was determined to be 584 mg/L with a 95% Confidence Interval of 400 to 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 9013-3

OTHER

Last changed: 5/25/00

Study Title

Static Acute Toxicity of FX-1003
to the Daphnid, *Daphnia magna*

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

April 9, 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. No deviations existed that significantly affected the validity of the study.

4/9/90
Timothy J. Ward
Author and Study Director

4-9-90
Robert L. Boeri
Coauthor

4-9-90
Jeanne P. Magazu
Aquatic Toxicologist

4-9-90
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 9013-3 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 original raw data and final report were sent to the sponsor. A certified copy of 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, and all data were verified by Quality Assurance Auditors. Quality assurance audits were performed on January 30, February 13, and March 9, 1990. Audits were reported to the Study Director on January 31, February 13, and March 9, 1990. Audits were reported to management on March 19 and April 18, 1990.

Danette Tekelenburg 4/23/90
Danette Tekelenburg
Quality Assurance Auditor

Doreen S. Newhouse 4-23-90
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 daphnid, *Daphnia magna*, is described in this final report. The test was conducted for 3M Company for 48 hours during February 12 to 14, 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 9013-3.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 19.9°C. The dilution water was filtered natural well water collected from wells at Hampton, New Hampshire. Aeration was not 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.

Daphnids used in the test were less than 24 hours old at the start of the test. They were produced from an in-house culture that was maintained under test conditions for at least 14 days. After 48 hours of exposure the control daphnids had an average wet weight (blotted dry) of 0.0007 g, resulting in a loading rate of 0.018 g/L. All daphnids were in good condition at the beginning of the study. Exposure of daphnids to the test substance resulted in a 48 hour EC50 of 584 mg/L FX-1003.

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 daphnid, a freshwater invertebrate. 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 collected from wells at EnviroSystems in Hampton, New Hampshire. Water was adjusted to a hardness of 180 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 daphnids employed as test organisms were from a single source and were identified using an appropriate taxonomic key. Daphnids used in the test were less than 24 hours old at the start of the test. They were produced from an in-house culture that was maintained under test conditions for at least 14 days. Control daphnids were weighed at the conclusion of the toxicity test. Prior to testing, daphnids were maintained in 100% dilution water under static conditions in 4 liter glass jars. During acclimation daphnids 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 24 hours prior to the test initiation the temperature was 19.8°C. Daphnids were fed yeast, trout chow, and the freshwater alga *Selenastrum capricornutum* once daily before the test.

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	--	8.2
Conductivity	umhos/cm	--	900
Hardness	mg/L as $CaCO_3$	--	180
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 - 8, 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 48 hours of exposure there was 60% survival at 1,000 mg/L and 100% survival at all other tested concentrations.

The definitive toxicity test was performed during February 12 - 14, 1990, according to EnviroSystems Test Protocol 9013-3 (Static, Acute Toxicity of FX-1003 To The Daphnid *Daphnia magna*), which was signed by the Study Director on February 6, 1989. It is based on procedures of the OECD (1984).

The test was conducted at a target temperature of 20 ± 1°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 daphnids were randomly and equally distributed among four replicates of each treatment. The test was performed in 250 ml glass beakers that contained 200 ml of test solution (water depth was approximately 7 cm). Test vessels were randomly arranged in an incubator during the 48 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 45 $uEs^{-1}m^{-2}$. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Daphnids were not fed during the test.

The number of surviving organisms and the occurrence of immobilization (inability to swim within 15 seconds after gentle agitation) or other 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 and 48 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 were interpreted by standard statistical techniques (Stephan, 1983). The probit, moving average, or linear interpolation method was used by the author to calculate the 48 hour EC50 (based on immobilization) using nominal concentrations of test substance.

VIII. RESULTS

All test vessels containing FX-1003 maintained a clear appearence 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 daphnids had an average wet weight (blotted dry) of 0.0007 g at the end of the test. Loading rate during the toxicity test was approximately 0.018 g/L. Exposure of daphnids to the reference toxicant sodium dodecyl sulfate resulted in a 48 hour LC50 of 33 mg/L, and a 48 hour EC50 of 16 mg/L.

The 24 and 48 hour EC50s for daphnids exposed to FX-1003 are presented in Table 3. The 48 hour EC50 is 584 mg/L FX-1003.

Table 2. Survival data from toxicity test

Nominal Concentration (mg/L)	rep.	Number Alive			Number Affected		
		0 hr	24 hr	48 hr	0hr	24hr	48hr
0 (control)	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
150	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
250	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
400	1	5	5	5	0	0	0
	2	5	5	5	0	0	0
	3	5	5	5	0	0	0
	4	5	5	5	0	0	0
600	1	5	5	5	0	0	3
	2	5	5	4	0	0	2
	3	5	5	4	0	0	2
	4	5	5	5	0	0	2
1,000	1	5	5	3	0	0	3
	2	5	5	4	0	1	4
	3	5	5	5	0	0	5
	4	5	5	3	0	1	3

Note: Affected organisms were immobilized (unable to swim within 15 seconds after gentle agitation)

Table 3. Median effective concentrations (EC50s) from toxicity test

Exposure period	EC50	95 percent confidence limits	Calculation method
24 hours	>1,000 mg/L	--	--
48 hours	584 mg/L	400 - 1,000 mg/L	nonlinear interpolation

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 202, Daphnid sp., Acute Immobilisation Test and Reproduction Test. Adopted April 4, 1984.

Stephan, C.E. 1983. Computer program for calculation of LC50 values. Personal communication.

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	pH 0 hr	24 hr	48 hr
0 (control)	1	900	800	900	8.2	8.1	8.2
	2	900	800	900	8.2	8.2	8.2
	3	900	800	900	8.2	8.2	8.2
	4	900	800	900	8.2	8.2	8.2
150	1	900	800	900	8.2	8.2	8.2
	2	900	800	900	8.2	8.2	8.2
	3	900	800	900	8.2	8.2	8.2
	4	900	800	900	8.2	8.2	8.2
250	1	900	800	900	8.3	8.3	8.2
	2	900	800	900	8.3	8.3	8.2
	3	900	800	900	8.3	8.3	8.2
	4	900	800	900	8.3	8.3	8.2
400	1	900	800	900	8.3	8.3	8.2
	2	900	800	900	8.3	8.3	8.2
	3	900	800	900	8.3	8.3	8.2
	4	900	800	900	8.3	8.3	8.2
600	1	900	800	900	8.3	8.3	8.3
	2	900	800	900	8.3	8.3	8.3
	3	900	800	900	8.3	8.3	8.3
	4	900	800	900	8.3	8.3	8.3
1,000	1	900	800	900	8.4	8.3	8.3
	2	900	800	900	8.4	8.3	8.3
	3	900	800	900	8.4	8.3	8.3
	4	900	800	900	8.4	8.3	8.3

Table A.1. Continued.

Nominal concentration (mg/L)	Rep.	Temperature (°C) 0 hr	24 hr	48 hr	Dissolved Oxygen (mg/L) 0 hr	24 hr	48 hr
0 (control)	1	20.1	20.0	19.5	9.5	9.5	9.0
	2	20.1	20.0	19.5	9.5	9.5	9.0
	3	20.1	20.0	19.5	9.5	9.5	9.0
	4	20.1	20.0	19.5	9.5	9.5	9.0
150	1	20.1	20.0	19.5	9.5	9.3	9.1
	2	20.1	20.0	19.5	9.5	9.3	9.1
	3	20.1	20.0	19.5	9.5	9.3	9.1
	4	20.1	20.0	19.5	9.5	9.3	9.1
250	1	20.1	20.0	19.5	9.5	9.3	9.1
	2	20.1	20.0	19.5	9.5	9.3	9.1
	3	20.1	20.0	19.5	9.5	9.3	9.1
	4	20.1	20.0	19.5	9.5	9.3	9.1
400	1	20.1	20.0	19.5	9.3	9.3	9.1
	2	20.1	20.0	19.5	9.3	9.3	9.1
	3	20.1	20.0	19.5	9.3	9.3	9.1
	4	20.1	20.0	19.5	9.3	9.3	9.1
600	1	20.1	20.0	19.5	9.3	9.3	9.1
	2	20.1	20.0	19.5	9.3	9.3	9.1
	3	20.1	20.0	19.5	9.3	9.3	9.1
	4	20.1	20.0	19.5	9.3	9.3	9.1
1,000	1	20.1	20.0	19.5	9.5	9.3	9.1
	2	20.1	20.0	19.5	9.5	9.3	9.1
	3	20.1	20.0	19.5	9.5	9.3	9.1
	4	20.1	20.0	19.5	9.5	9.3	9.1

RESOURCE ANALYSTS, INCORPORATED
ENVIROSYSTEMS DIVISION

PRODUCT REGISTRATION LABORATORY
AQUATIC TOXICOLOGY STUDY PROTOCOL

Static Acute Toxicity Test With FX-1003 And The Daphnid, *Daphnia magna*

Sponsor

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

Sponsor Approval of Protocol Susan A. Beach Date 1-24-90

Testing Facility

EnviroSystems, Inc.
1 Lafayette Road
Hampton, NH 03842

Estimated Test Initiation Date: January, 1990

Estimated Test Completion Date: March, 1990

Study Director: Timothy J. Ward

Study Director Approval of Protocol Timothy J. Ward Date 2/6/90

1.0 TITLE

Static Acute Toxicity Test With FX-1003 And The Daphnid, *Daphnia magna*

2.0 PURPOSE

To determine the 24 hour and 48 hour median lethal concentrations (LC50s) and median effective concentrations (EC50s) of the test substance to the daphnid, when exposed for 48 hours 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 requested 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 substance 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 (free from known diseases) from a single source will be used to initiate the test. They will be obtained from an in-house culture with a known history and will be less than 24 hours old at the initiation of the test. The 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 Daphnids will be maintained under static or replacement 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 a mixture of yeast, trout chow, and cerophyl and/or freshwater algae. Daphnid cultures will be maintained in dilution water at test conditions (temperature, hardness, and photoperiod) for at least 14 days prior to testing.

6.0 EXPOSURE CONDITIONS

6.1 The test will be conducted under static conditions, and test media will be added only at the beginning of the test.

6.2 Dilution water will be natural ground water. It will 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 20 ± 1°C.

6.4 Dissolved oxygen concentration will be maintained above 70% saturation. Aeration will not be utilized.

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

6.6 The test vessels will be 250 ml glass beakers that contain 200 ml of test media. Loading rate will be adjusted to assure an adequate dissolved oxygen concentration.

7.0 STUDY CONDUCT

7.1 The results of a range finding investigation and/or previous testing data will be used to select the concentration range for the definitive test. If a range finding test is conducted animals will be exposed to a series of concentrations of test substance plus a control and a solvent control (if required).

7.2 During the definitive test the animals will be exposed for 48 hours to at least five concentrations of test substance plus a control (dilution water without test substance) and a solvent control (if necessary) under static conditions. Test concentrations will be chosen to bracket the anticipated LC50 and EC50.

7.3 At least 20 animals will be exposed to each treatment. The animals will be exposed in groups of 5 animals per vessel, each containing 200 ml of solution. The animals will be equally and randomly assigned to the replicate test vessels (a random numbers table will be used to choose the test vessel for each specimen), and they will not be fed.

7.4 Animals will be added to test media after test substance has been added and aeration will not be employed.

7.5 The highest tested concentration will be 1 g/L.

7.6 Volatile test substances will be tested in completely filled and sealed containers.

7.7 A toxicity test should also be conducted with a reference toxicant and the batch of daphnids used for the test.

7.8 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.

7.9 Mortality and sublethal effects (immobility) will be recorded at 24 hour intervals during the test. Dead animals will be removed when first observed.

7.10 Data Analysis and Statistical Methods

7.10.1 The LC50 and EC50 values and their 95% confidence intervals will be calculated by the non-linear interpolation, moving average and/or probit methods.

7.10.2 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 more than 10% of the control daphnids are stressed or immobilized, or any control animals are trapped at the surface.

8.1.2 Temperature in any test vessel is outside of the range of 19 - 21°C, or dissolved oxygen falls below 70% 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, LC50 values and EC50 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 202, Daphnia sp., Acute Immobilisation Test and Reproduction Test. Adopted 4 April 1984.

U.S. EPA. 1986. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Federal Register, Monday, January 6, 1986. Section 797.1300.

U.S. EPA. 1987. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Amendments. Federal Register, Wednesday, May 20, 1987. Section 797.1300.

SUMMARY OF TEST CONDITIONS

Acceptable Species:	*Daphnia magna*
Test Type:	Static
Test Duration:	48 Hours
Flow Rate:	Not Applicable
Dilution Water:	Natural Ground Water
Age/Size of Test Organisms:	Juveniles; Less Than 24 Hours Old
Acclimation Period:	Continuous
Temperature:	20 ± 1°C
Photoperiod:	16 Hours Light/8 Hours Dark
Light Intensity:	Ambient Laboratory Lighting
Number of Concentrations:	5 or More
Dilution Factor:	≤1.6
Number of Replicates:	4 or More
Number of Organisms/Replicate:	5
Test Vessel Size:	250 ml or larger
Test Vessel Volume:	200 ml or larger
Solvent:	Dimethylformamide, Acetone, or Triethylene Glycol. Less than or equal to 100 mg/L
Loading Rate:	<0.5 g/L
Feeding:	None During Exposure
Aeration:	None
Effects Measured:	Death; Immobilization
Test Concentration Analysis:	None
Water Quality Measurements:	DO, pH, Temperature and Conductivity at 0, 24, and 48 Hours
Acceptability Criteria:	≤10% Control Mortality, Stress, or Immobilization Acceptable Temperature Range