

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 the major component of FC-1015. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample is FC-1015. It's purity was not sufficiently characterized, though current information indicates is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205".

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. Data was used to compare toxicity of the branched/straight chain ammonium perfluorooctanoate homologue mixture in FC-143 vs. what is supposed to be the 100% straight carbon chain ammonium perfluorooctanoate in FC-1015.

METHOD:

Method: USEPA – TSCA 1993. 797.1400

Type: Static acute

GLP: No

Year completed: 1996

Species: *Pimephales promelas*

Supplier: Aquatic Biosystems, Fort Collins, Colorado

Analytical monitoring: Temperature, pH, conductivity, and DO

Exposure period: 96-hours

Statistical methods: LC₅₀ values calculated using the Stephan computer program, 1983

Test fish age: Juvenile

Length and weight: Average length = 35 mm
Average weight = 0.36 g (wet)

Loading: 0.24 g fish/L

Pretreatment: None.

Test conditions:

Dilution water: Deionized water adjusted to a hardness of 40 – 48 mg/L (as CaCO₃) and passed through a particulate filter, ultraviolet sterilizer, and activated carbon.

Dilution water chemistry:

Hardness: 44 mg/L as CaCO₃

Alkalinity: 29-30 mg/L as CaCO₃

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TOC: <1 mg/L

Lighting: Cool-white fluorescent lights at 30 foot-candles. A daily photoperiod of 16 hours light and 8 hours dark with a 15 minute transition period was maintained throughout the testing period.

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: 20 L glass aquaria containing 15 liters test solution at an approximate depth of 18 cm.

Number of replicates: two

Number of fish per replicate: 20

Number of concentrations: five plus a blank control

Water chemistry during the study:

Conductivity range (0-96 hours)

180 – 190 μ mhos/cm (control exposure)

300 – 320 μ mhos/cm (2100 mg/L exposure)*

pH range (0-96 hours):

7.3 – 7.9 (control exposure)

7.2 – 7.7 (2100 mg/L exposure)*

Temperature range (0-96 hours):

22.0 – 22.4 °C (control exposure)

21.8 – 22.5 °C (2100 mg/L exposure)*

Dissolved oxygen range (0-96 hours):

5.8 – 9.2 mg/L (control exposure)

5.7 – 9.2 mg/L (2100 mg/L exposure)*

* 2100 mg/L (second highest concentration) exposure values used because the highest concentration used resulted in total mortality at 72 hours.

RESULTS

Nominal concentrations: Bk control, 530, 830, 1330, 2100, and 3330 mg/L

Element values: 96-hour LC₅₀ = 2470 (2100 – 3330) mg/L

96-hour NOEC = 830 mg/L

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 values reported apply to that mixture and not the test substance.

CONCLUSIONS

The test sample 96-hour LC₅₀ for fathead minnow was determined to be 2470 mg/L with a 95% confidence interval of 2100 to 3330 mg/L.

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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 T.R. Wilbury Laboratories, Inc., of Marblehead, Massachusetts at the request of the 3M Company, Lab Request number P1624, 1996.

OTHER

Last changed: 5/25/00

004051

P1624

Study Title

**Acute Toxicity of FC-1015 to the
Fathead Minnow, *Pimephales promelas***

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1400

Authors

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Study Completed

May 15, 1996

Sponsor

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St. Paul, Minnesota 55144**

Testing Facility

**T.R. Wilbury Laboratories, Inc.
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Marblehead, Massachusetts 01945**

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III. SUMMARY

The acute toxicity of FC-1015 to the fathead minnow, *Pimephales promelas*, is described in this final report. The test was conducted for 3M Company for 96 hours from April 11 to 15, 1996, at T.R. Wilbury Laboratories, Inc. in Marblehead, Massachusetts. It was conducted by Jacqueline Nevius, Jeanne Magazu, Keven Stevens, Peter Kowalski, Robert Boeri, and Timothy Ward according to the procedures of the U.S. Environmental Protection Agency (1993). FC-1015 was supplied by the sponsor.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a temperature of 21.7 to 22.5°C. The dilution water was filtered deionized water collected at Marblehead, Massachusetts and adjusted to a hardness of 44 mg/L as CaCO₃.

Nominal concentrations of FC-1015 were 0 (control), 530, 830, 1,330, 2,100, and 3,330 mg/L. Fathead minnows used in the test were procured from a commercial supplier and acclimated to test conditions at T.R. Wilbury Laboratories. After 96 hours of exposure the control fish had an average wet weight (blotted dry) of 0.36 g and an average total length of 35 mm. All fish were in good condition at the beginning of the study.

Exposure of fathead minnows to FC-1015 resulted in a 96 hour median lethal concentration (LC50) of 2,470 mg/L, with a 95% confidence interval of 2,100 to 3,330 mg/L. The 96 hour no observed effect concentration (NOEC) is 830 mg/L.

IV. GENERAL INFORMATION

Material Tested:	FC-1015
Sponsor:	3M Company Environmental Laboratory 935 Bush Avenue Building 2-3E-09 St. Paul, Minnesota 55144
Test Substance Shipped From:	3M Company Environmental Laboratory 935 Bush Avenue Building 2-3E-09 St. Paul, Minnesota 55106
Reported Purity:	100%
Experimental Start Date:	April 11, 1996
Experimental Completion Date:	April 15, 1996
Archive Location for the Biological Raw Data and a Copy of the Final Report:	T.R. Wilbury Laboratories, Inc. Marblehead, Massachusetts

V. INTRODUCTION

This study was sponsored by 3M Company, St Paul, Minnesota. The objective of the study was to determine the 24, 48, 72, and 96 hour median lethal concentrations (LC50s) of the test substance to the fathead minnow, *Pimephales promelas*, a freshwater fish, under static conditions. The study was conducted following U.S. EPA (TSCA) guidelines but at the sponsor's request it was not conducted according to Good Laboratory Practice (GLP) rules. The report contains sections that describe the methods and materials employed in the study, and results of the investigation. The report also contains an appendix that presents water quality data collected during the test.

VI. METHODS AND MATERIALS

TEST SUBSTANCE:

The sample of FC-1015 (T.R. Wilbury Laboratories Sample Number 604) was delivered to T.R. Wilbury Laboratories on April 4, 1996. The sample was delivered in a metal can within a cardboard box at ambient temperature. The test substance was contained in 1 250-ml plastic bottle. The label attached to the bottle contained the following information: "FC-1015, (LR P1624), See MSDS, Susan A. Beach, 2-3E-09, (612) 778-7452, 4/2/96".

FC-1015 (a clear liquid) was supplied by 3M Company, St Paul, Minnesota. Prior to use the test substance was stored in the dark at room temperature. The test substance was assumed to have a purity of 100% active ingredient and to be stable under storage and testing conditions. Unused test substance is returned to the sponsor.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was deionized water collected at T.R. Wilbury Laboratories in Marblehead, Massachusetts. Water was adjusted to a hardness of 40-48 mg/L as CaCO₃ and stored in 500-gallon polyethylene tanks where it was aerated and continuously passed through a particle filter, ultraviolet sterilizer, and activated carbon. Water used for the test had a hardness of 44 mg/L, and acidity of 24 mg/L and an alkalinity of 29-30 mg/L as CaCO₃, and it contained <10 mg/L particulate matter and <1 mg/L total organic carbon. Results of chemical analysis of a representative sample of water are presented in Table 1. A sample of dilution water was sent to Lancaster Laboratories, Lancaster, PA for analysis of total organic carbon. The sample was determined to contain < 1.0 mg/L total organic carbon.

Table 1. Chemical characterization of a representative sample of the deionized water used to formulate the freshwater dilution water for the toxicity test with fathead minnows, *Pimephales promelas*, and FC-1015.

Parameter ¹	Unit of Measurement	Detection Limit	Measured Value
Metals			
Aluminum	mg/L	0.1	ND ²
Arsenic	mg/L	0.01	ND
Boron	mg/L	0.5	ND
Cadmium	mg/L	0.0002	ND
Chromium	mg/L	0.01	ND
Cobalt	mg/L	0.03	ND
Copper	mg/L	0.005	ND
Iron	mg/L	0.03	0.03
Lead	mg/L	0.005	ND
Mercury	mg/L	0.0003	ND
Nickel	mg/L	0.03	ND
Silver	mg/L	0.02	ND
Zinc	mg/L	0.02	ND
Nitrate	mg/L as N	0.05	ND
Chloride	mg/L	1	
Fluoride	mg/L	0.1	ND
Total Organic Carbon	mg/L	1	ND
Total Phosphorus	mg/L	0.03	ND
Organochlorine Pesticides	µg/L	0.5	ND
Toxaphene	µg/L	2	ND
Organophosphorus Pesticides	µg/L	0.5	ND
Dimethoate	µg/L	2.0	ND
TEPP	µg/L	2.0	ND
Monocrotophos	µg/L	2.0	ND
PCBs	µg/L	0.5	ND

Notes: 1. Parameters were measured in deionized water (used to formulate dilution water) that was collected during August, 1995 and analyzed by Pace, Inc. as part of routine water quality testing.

2. ND = not detected at or above the detection limit.

TEST ORGANISM:

Juvenile fathead minnows, *Pimephales promelas*, employed as test organisms were from a single source and were procured from Aquatic Biosystems, Fort Collins, Colorado on March 26, 1996. The species of the fish was verified using an appropriate taxonomic key. Prior to testing the fish were maintained under flow through conditions in a 270 liter fiberglass tank. During acclimation fish were not treated for disease, and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. Mortality during the final 48 hours of acclimation was less than 3%.

During the 14 day period before the start of the test the acclimation temperature range was 22.0 to 22.9°C and the dissolved oxygen concentration was always at least 7.6 mg/L. During acclimation fish were fed a dry commercial food (Tetra Min® Staple Food, Lot # TM06) daily except during the 48 hours immediately preceding the test. Fish were not fed during the test. At the conclusion of the test the control fish had an average total length of 35 mm and an average wet weight of 0.36 g (loading during the toxicity test was 0.24 g/L).

TOXICITY TESTING:

The toxicity test was performed according to the procedures of the U.S. Environmental Protection Agency (1993). A summary of the test system is presented in Table 2.

A screening test was not conducted because nominal concentrations for the definitive test were provided by the sponsor. The definitive test was performed from April 11 to 15, 1996 with five concentrations of test substance and a dilution water control. Nominal concentrations of FC-1015 were 530, 830, 1,330, 2,100, and 3,330 mg/L. Appropriate amounts of test substance were added directly to dilution water in test vessels without the use of a solvent.

Twenty fathead minnows were indiscriminately and equally distributed among two replicates of each treatment. The test was performed in 20 liter glass aquaria that contained 15 liters of test solution (water depth was approximately 18 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) and they were loosely covered.

The test was conducted at $22 \pm 2^\circ\text{C}$. A 16 hour light and 8 hour dark photoperiod with a 15 minute transition period was automatically maintained with cool-white fluorescent lights that provided a light intensity of 30 footcandles.

Table 2. Test system summary for the toxicity test with FC-1015 and fathead minnows, *Pimephales promelas*.

Species:	<i>Pimephales promelas</i>
Age/Size of Test Organisms:	Juveniles/≈0.36 grams, wet weight
Test Duration:	96 hours
Test Vessels:	20 liter glass aquaria
Depth of Test Media:	18 cm
Number of Replicate Test Vessels:	2
Organisms per Replicate:	10
Temperature Range:	21.7-22.5°C
Minimum Dissolved Oxygen:	5.6 mg/L
Photoperiod:	16 hours light and 8 hours dark with a 15 minute transition period.
Light Intensity:	30 footcandles
Water Quality Measurements:	Dissolved oxygen, pH, temperature, and conductivity daily in each test vessel. Hardness and alkalinity in dilution water at the start of the test and in the controls at the end of the test. Temperature measured at least every hour in 1 test vessel. Acidity, and particulate matter in the dilution water. Dilution water analysis is summarized in Table 1.
End Points:	24, 48, 72, and 96 hour LC50; 96 hour NOEC.
Acceptability Criteria:	≤10% control mortality and acceptable temperature range.

The number of surviving organisms and the occurrence of sublethal effects (immobilization, loss of equilibrium, 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 1), pH (Beckman model pH 12 meter; instrument number 144), conductivity (Cole Farmer meter; instrument number 94), and temperature (Beckman model pH 12 meter; instrument number 144, probe PHT-5) were measured and recorded daily in each test chamber that contained live animals. The temperature in one test vessel was recorded at least hourly during the test.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate the median lethal concentrations (LC50s). All calculations were performed using nominal concentrations of test substance and the number of live fathead minnows. The no observed effect concentration is the highest concentration of test substance that did not cause any toxicant-related mortality or sublethal effects.

VII. RESULTS

Insoluble material was not noted during the test. Biological and water quality data generated by the acute toxicity test are presented in Table 3 and Appendix A, respectively. The measured concentrations of dissolved oxygen ranged from 5.6 to 9.2 mg/L, the temperature ranged from 21.7 to 22.5°C, conductivity ranged from 180 to 390 μ mhos/cm, and pH ranged from 7.2 to 7.9. One hundred percent survival occurred in the control exposure and these fathead minnows did not exhibit any sublethal effects.

The 24, 48, 72, and 96 hour LC50s for fathead minnows exposed to FC-1015 are presented in Table 4. The 96 hour LC50 for fathead minnows exposed to FC-1015 is 2,470 mg/L, with a 95% confidence interval of 2,100 to 3,330 mg/L. The slope of the 96 hour dose response curve is 10. The 96 hour NOEC is 830 mg/L.

Table 3. Survival and sublethal effect data from the toxicity test with fathead minnows, *Pimephales promelas*, and FC-1015.

Nominal Concentration of FC-1015 (mg/L)	Rep.	Number Alive					Number Affected				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
530	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
830	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
1,330	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	9	0	0	0	0	0
2,100	1	10	10	10	8	8	0	0	0	0	0
	2	10	10	10	9	9	0	0	0	0	0
3,330	1	10	10	2	0	0	0	4	0	0	-
	2	10	7	2	0	0	0	2	0	0	-

Note: At 24 hours six of the seventeen surviving fish exposed to 3,330 mg/L FC-1015 exhibited lethargy and a loss of equilibrium.

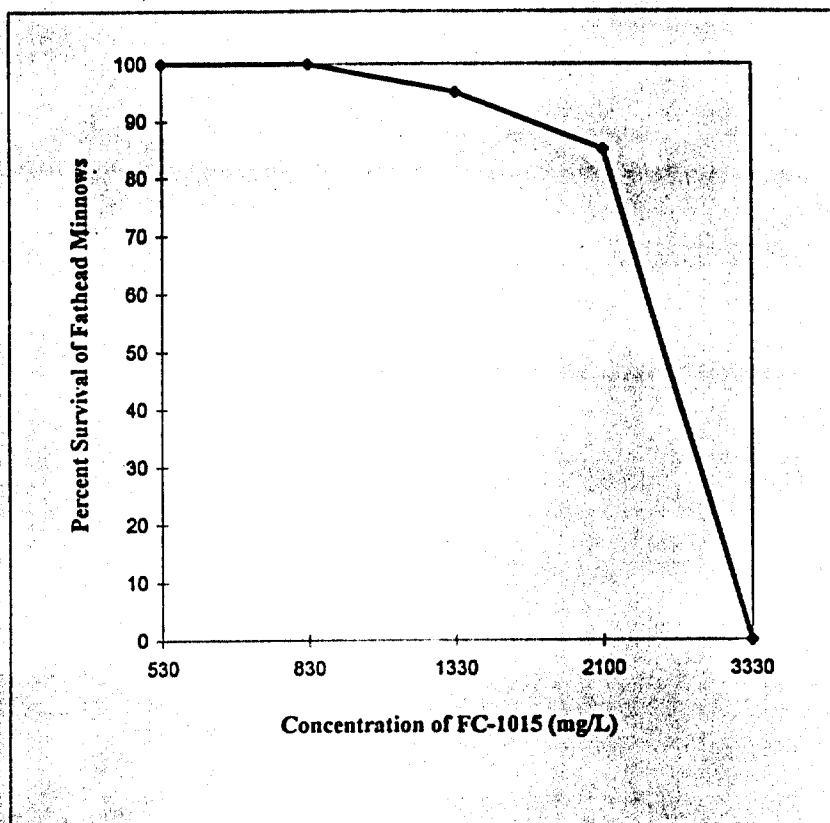


Figure 1. Survival of fathead minnows, *Pimephales promelas*, exposed to FC-1015 for 96 hours.

Table 4. Median lethal concentrations (LC50s) from the toxicity test with fathead minnows, *Pimephales promelas*, and FC-1015.

Exposure period	LC50	95 percent confidence limit	Calculation method
24 hours	>3,330 mg/L	-	-
48 hours	2,890 mg/L	2,100 to 3,330 mg/L	Binomial
72 hours	2,470 mg/L	2,100 to 3,330 mg/L	Binomial
96 hours	2,470 mg/L	2,100 to 3,330 mg/L	Binomial

VIII. REFERENCES

ASTM. 1986. Standard Practice for Conducting Acute Toxicity Tests with Fishes , Macroinvertebrates, and Amphibians. E-729-80. In Annual Book of Standards.

Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.

U.S. EPA. 1993. Toxic Substances Control Act Test Guidelines; Final Rules. Section 797.1400.

IX. SIGNATURE PAGE

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Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Dissolved oxygen concentration, conductivity, pH, and temperature measured during the toxicity test with fathead minnows, *Pimephales promelas*, and FC-1015.

Nominal Concentration of FC-1015 (mg/L)	Rep.	Dissolved Oxygen (mg/L)					Conductivity (μ mhos/cm)				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	9.2	7.0	5.8	6.0	6.0	180	180	180	180	190
	2	9.2	7.1	6.2	6.2	6.4	180	180	180	180	190
530	1	9.2	6.5	6.2	6.2	5.9	210	210	200	200	220
	2	9.2	6.8	6.1	6.0	6.1	210	210	200	200	220
830	1	9.2	6.9	6.1	6.0	5.8	220	230	230	230	230
	2	9.2	6.8	6.1	6.0	5.9	220	230	230	230	230
1,330	1	9.2	6.7	6.0	6.0	5.8	250	250	250	250	270
	2	9.2	6.8	5.6	5.7	5.6	250	250	250	250	270
2,100	1	9.2	6.5	6.0	5.9	5.8	320	300	300	300	310
	2	9.2	6.6	6.0	5.9	5.7	320	300	300	300	320
3,330	1	9.2	7.2	5.7	5.6	--	390	390	390	390	--
	2	9.2	7.0	5.8	5.6	--	370	380	390	390	--

Table A.1. Continued.

Nominal Concentration of FC-1015 (mg/L)	Rep.	pH					Temperature (°C)				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	7.9	7.5	7.3	7.4	7.4	22.1	22.4	22.0	22.0	22.0
	2	7.9	7.5	7.3	7.4	7.4	22.1	22.3	22.0	22.0	22.0
530	1	7.9	7.5	7.3	7.4	7.4	22.1	22.5	22.0	22.0	22.0
	2	7.8	7.4	7.3	7.4	7.4	21.9	22.4	21.9	22.0	21.9
830	1	7.8	7.4	7.2	7.3	7.4	21.9	22.4	21.9	22.0	21.9
	2	7.8	7.4	7.3	7.3	7.4	21.9	22.4	22.0	22.0	21.9
1,330	1	7.8	7.4	7.3	7.3	7.4	21.7	22.4	21.9	21.9	21.8
	2	7.7	7.4	7.3	7.3	7.4	21.9	22.4	21.9	21.9	21.9
2,100	1	7.7	7.4	7.2	7.3	7.4	21.8	22.4	21.9	21.9	21.8
	2	7.7	7.4	7.2	7.3	7.4	22.0	22.5	21.9	22.0	22.0
3,330	1	7.6	7.5	7.3	7.3	--	21.7	22.3	21.8	21.9	--
	2	7.6	7.4	7.2	7.3	--	21.9	22.4	21.8	21.9	--