

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (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 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, Guideline 797.1300

Type: Static acute

GLP: No

Year completed: 1996

Species: *Daphnia magna*

Supplier: In-house cultures. Initial brood stock from Aquatic BioSystems, Fort Collins, Colorado

Analytical monitoring: Temperature, pH, conductivity and DO

Exposure period: 48-hours

Test organism age: <24 hours old

Statistical method: Probit analysis

Test conditions:

Dilution water: Deionized water adjusted to a hardness of 160-180 mg/L (as CaCO₃) and passed through a particulate filter, ultraviolet sterilizer, and activated carbon.

Dilution water chemistry:

Hardness: 176 mg/L as CaCO₃

Alkalinity: 108 mg/L as CaCO₃

TOC: <1 mg/L

Lighting: Cool-white fluorescent lights at 130 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 weights addition.

Exposure vessels: 300 mL glass beakers containing 250 mL test solution (9 cm depth).

Number of replicates: two

Number of daphnids per replicate: 10

Number of concentrations: five plus a blank control

Water chemistry during the study:

Conductivity range: (0-48 hours)

610 – 620 μ mhos/cm (control exposure)

710 – 720 μ mhos/cm (2000 mg/L exposure)*

pH range (0-48 hours):

8.2 – 8.3 (control exposure)

8.1 – 8.2 (2000 mg/L exposure)*

Temperature range (0-48 hours):

20.4 – 20.7 °C (control exposure)

20.3 – 20.8 °C (2000 mg/L exposure)*

Dissolved oxygen range (0-48 hours):

8.1 – 9.1 mg/L (control exposure)

8.1 – 9.1 mg/L (2000 mg/L exposure)*

* 2000 mg/L (second highest concentration) exposure values used because the highest concentration used resulted in total mortality by 48 hours.

RESULTS

Element values: 24-hour EC_{50} = 1790 (1550 - 2070) mg/L
48-hour EC_{50} = 1200 (730 - 2000) mg/L
48-hour NOEC = 730 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 FC-1015 48-hour EC_{50} was determined to be 1200 mg/L with a 95% confidence interval of 730 to 2070 mg/L. The 48-hour No Observed Effect Concentration was 730 mg/L

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

DATA QUALITY

Reliability: Klimisch ranking = 2. This study meets the criteria for quality testing. However, the study lacks information on purity of the test substance and actual measurements of the amount of test substance in solution.

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 -

P1624

Study Title

Acute Toxicity of PC-1015 to
the Daphnid, *Daphnia magna*

Data Requirement: Guideline Number

U.S. EPA-TSCA, Guideline 797.1300

Authors

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Study Completed

May 15, 1996

Sponsor

3M Company
Environmental Laboratory
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St. Paul, Minnesota 55144

Testing Facility

T.R. Wilbury Laboratories, Inc.
40 Doaks Lane
Marblehead, Massachusetts 01945

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III. SUMMARY

The acute toxicity of FC-1015 to the daphnid, *Daphnia magna*, is described in this final report. The test was conducted for 3M Company for 48 hours from April 16 to 18, 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 20.2 to 21.0°C. The dilution water was filtered deionized water collected at Marblehead, Massachusetts and adjusted to a hardness of 176 mg/L as CaCO₃.

Nominal concentrations of FC-1015 were 0 (control), 430, 730, 1,200, 2,000, and 3,330 mg/L. Daphnids used in the test were less than 24 hours old at the start of the test. They were produced at T.R. Wilbury Laboratories and acclimated under test conditions for their entire life (cultures that produced test specimens were maintained at test conditions for more than 7 days). After 48 hours of exposure the control daphnids had an average wet weight (blotted) of 0.35 mg. All daphnids were in good condition at the beginning of the study.

Exposure of daphnids to FC-1015 resulted in a 48 hour median lethal concentration (LC50) of 1,550 mg/L (95% confidence interval = 1,200 to 2,000 mg/L) and a 48 hour median effective concentration (EC50) of 1,200 mg/L (95% confidence interval = 730 to 2,000 mg/L). The 48 hour no observed effect concentration (NOEC) is 730 mg/L.

IV. GENERAL INFORMATION

Material Tested: PC-1015

Sponsor: 3M Company
Environmental Laboratory
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55144

Test Substance 3M Company
Shipped From: Environmental Laboratory
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55106

Reported Purity: 100%

Experimental Start Date: April 16, 1996

Experimental Completion Date: April 18, 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 and 48 hour LC50s and EC50s of the test substance to the daphnid, *Daphnia magna*, a freshwater invertebrate, 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, 4/2/48".

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 160-180 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 176 mg/L and an alkalinity of 108 mg/L as CaCO₃, and it contained <0.01 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 daphnids, *Daphnia magna*, 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 daphnids, *Daphnia magna*, employed as test organisms were less than 24 hours old at the start of the test. They were from a single source and were identified using an appropriate taxonomic key. Test specimens were produced by adult daphnids that were maintained under test conditions at the T.R. Wilbury facility for more than 7 days. The original culture was obtained on March 26, 1996 from Aquatic Biosystems, Fort Collins, Colorado. Measurements made during the 7 days before the start of the test indicated that the culture temperature ranged from 20.1 to 20.5°C and the dissolved oxygen concentration was always at least 6.9 mg/L. The culture produced young prior to day 12 and a subsample of adults produced at least 3 young/daphnid/day for 7 days before the start of the test.

During acclimation daphnids were not treated for disease, they were free of apparent disease, injuries, and abnormalities at the beginning of the test and there was no mortality during the 48 hours preceding the start of the test. The culture was supplied with a yeast/trout chow mix (Lot# DC02) and the freshwater alga, *Selenastrum capricornutum*, daily during acclimation. Daphnids were not fed during the test. Control daphnids, blotted and weighed at the conclusion of the toxicity test, averaged 0.34 mg and the loading rate during the definitive toxicity test was approximately 14 mg/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 16 to 18, 1996 with five concentrations of test substance and a dilution water control. Nominal concentrations of FC-1015 were 430, 730, 1,200, 2,000, 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 daphnids were indiscriminately and equally distributed among two replicates of each treatment. The test was performed in 300 ml glass beakers that contained 250 ml of test solution (water depth was approximately 9 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) and they were loosely covered.

The test was conducted at $20 \pm 1^\circ\text{C}$. 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 130 footcandles.

Table 2. Test system summary for the toxicity test with VC-1015 and daphnids, *Daphnia magna*.

Species:	<i>Daphnia magna</i>
Age/Size of Test Organisms:	Juveniles/≈0.35 mg, wet weight
Test Duration:	48 hours
Test Vessels:	300 ml glass beakers
Depth of Test Media:	9 cm
Number of Replicate Test Vessels:	2
Organisms per Replicate:	10
Temperature Range:	20.2-21.0°C
Minimum Dissolved Oxygen:	7.9 mg/L
Photoperiod:	16 hours light and 8 hours dark with a 15 minute transition period.
Light Intensity:	130 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. Temperature measured at least every hour in a representative test vessel. Dilution water analysis is summarized in Table 1.
End Points:	24 and 48 hour LC50 and EC50; 48 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 and 48 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 Parmer 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 24 and 48 hour LC50s based on mortality and the EC50s based on immobilization. All calculations were performed using nominal concentrations of test substance. 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 7.9 to 9.2 mg/L, the temperature ranged from 20.2 to 21.7°C, conductivity ranged from 610 to 800 μ mhos/cm, and the pH ranged from 8.0 to 8.3. One hundred percent survival occurred in the control exposure and these daphnids did not exhibit any sublethal effects.

The 24 and 48 hour LC50s and EC50s for daphnids exposed to PC-1015 are presented in Table 4. The 48 hour LC50 for daphnids exposed to PC-1015 is 1,550 mg/L, with a 95% confidence interval of 1,200 to 2,000 mg/L and the 48 hour EC50 is 1,200 mg/L, with a 95% confidence interval of 730 to 2,000 mg/L. The 48 hour NOEC is 730 mg/L.

Table 3. Survival and sublethal effect data from the toxicity test with daphnids, *Daphnia magna*, and FC-1015.

Nominal Concentration of FC-1015 (mg/L)	Rep.	Number Alive			Number Affected		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
430	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
730	1	10	10	10	0	0	0
	2	10	10	10	0	0	0
1,200	1	10	10	10	0	2	3
	2	10	10	9	0	1	6
2,000	1	10	10	0	0	7	-
	2	10	10	1	0	4	1
3,330	1	10	2	0	0	2	-
	2	10	0	0	0	-	-

Note: Affected daphnids were immobilized.

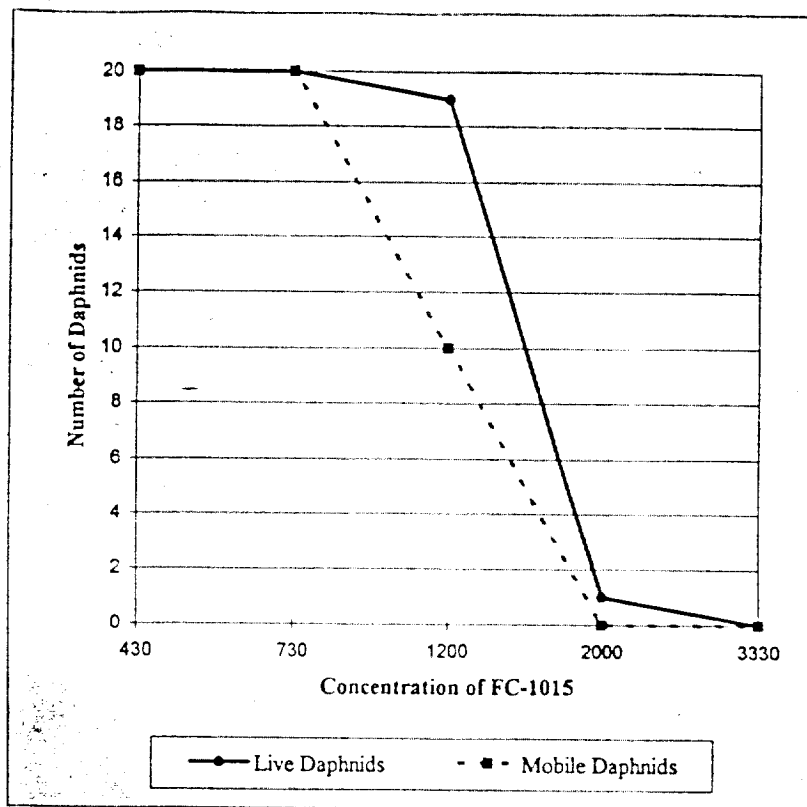


Figure 1. Survival and immobilization of daphnids, *Daphnia magna*, exposed to FC-1015 for 48 hours.

Table 4. Median lethal concentrations (LC50s) and median effective concentrations (EC50s) from the toxicity test with daphnids, *Daphnia magna*, and FC-1015.

Exposure period	LC50/EC50	95 percent confidence limit	Calculation method
LC50			
24 hours	2,730 mg/L	2,000 to 3,330 mg/L	Binomial
48 hours	1,550 mg/L	1,200 to 2,000 mg/L	Binomial
EC50			
24 hours	1,200 mg/L	730 to 2,000 mg/L	Binomial
48 hours	1,200 mg/L	730 to 2,000 mg/L	Binomial

→ This is an error. EC_{50} recalculated at 3M Env Lab by Probit Vers 1.4 :
1790 (1550 - 2070) mg/L

SAB 5/22/96

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING EC VALUES
Version 1.4

1524 24-hrs exp. Daphnia magna ug/L

sub 5/22/96

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
430.0000	20	0	0.0000	0.0000	0.0000
730.0000	20	0	0.0000	0.0000	0.0027
1200.0000	20	3	0.1500	0.1500	0.1079
2000.0000	20	11	0.5500	0.5500	0.4346
3330.0000	20	20	1.0000	1.0000	0.9726

Chi-Square Heterogeneity = 1.002

mu = 3.253773
sigma = 0.140232

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-10.195675	4.429512	-26.677518, 6.286168
Slope	7.131047	1.361221	4.463054, 9.800040

Theoretical Spontaneous Response Rate = 0.0000

P1624 24-hrs exp. Daphnia magna mg/L sab 5/22/96

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence	Upper Limits
EC 1.00	844.4060	524.6706	1066.0620
EC 5.00	1052.2174	737.6263	1264.8704
EC10.00	1183.1518	881.4062	1390.5376
EC15.00	1280.6676	991.5531	1485.9550
EC50.00	1789.6694	1552.8311	2026.2410
EC85.00	2500.9727	2152.0383	3246.6580
EC90.00	2707.0369	2298.9629	3653.5881
EC95.00	3043.9683	2526.5745	4367.1226
EC99.00	3773.0979	2996.8335	6141.5518

$$EC_{50} = 1790 (1550 - 2070) \text{ mg/L}$$

404 24-hrs exp. *Daphnia magna* mg/L - 5/22/96

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE

0.01

10+

9+

7+

5+

4+

3+

2+

1+

0+

0

0

0

0

0

0

0

0

0

0

0

0

0

0

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 797.1300.

IX. SIGNATURE PAGE

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Study Number: 1030-14

Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Dissolved oxygen concentration, conductivity, pH, and temperature measured during the toxicity test with daphnids, *Daphnia magna*, 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	0 hr	24 hr	48 hr
0 (control)	1	9.1	7.9	8.2	610	610	620
	2	9.1	8.1	8.3	610	610	620
430	1	9.1	8.2	8.3	630	630	630
	2	9.1	8.0	8.3	630	630	640
730	1	9.1	8.1	8.3	650	650	650
	2	9.1	8.5	8.3	650	650	660
1,200	1	9.1	8.3	8.4	680	670	680
	2	9.1	8.1	8.3	680	680	680
2,000	1	9.1	8.1	8.2	720	710	720
	2	9.1	8.3	8.2	720	710	710
3,330	1	9.2	8.6	8.3	800	780	780
	2	9.2	8.5	--	800	790	--

Table A.1. Continued.

Nominal Concentration of PC-1015 (mg/L)	Rep.	Temperature (°C)			pH		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
0 (control)	1	20.4	20.7	20.6	8.3	8.3	8.2
	2	20.4	20.7	20.5	8.3	8.3	8.3
430	1	20.3	20.6	20.8	8.3	8.3	8.3
	2	20.4	20.6	20.9	8.3	8.3	8.3
730	1	20.3	20.4	21.0	8.3	8.3	8.3
	2	20.2	20.3	21.0	8.3	8.3	8.3
1,200	1	20.2	20.6	20.7	8.2	8.2	8.3
	2	20.2	20.7	20.5	8.2	8.2	8.3
2,000	1	20.3	20.6	20.6	8.1	8.2	8.2
	2	20.3	20.8	20.6	8.1	8.2	8.2
3,330	1	20.4	20.4	20.8	8.0	8.2	8.2
	2	20.4	20.7	--	8.0	8.2	--