

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorodecanesulfonate, ammonium salt; may also be referred to as PFDS, FC-120, or 3M Sample J2905-1. (1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heneicosafuoro-, ammonium salt, CAS # 67906-42-7)

Remarks: Test sample is a mixture of the test substance in water and butyl cellosolve (approximately 25% test substance, 37.5% butyl cellosolve and 37.5% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample. Test sample was from 3M production Lot 164.

METHOD

Method: OECD 203

Type: Static acute

GLP: No

Year completed: 1992

Species: *Pimephales promelas*

Supplier: Aquatic Research Organisms, Hampton, New Hampshire

Analytical monitoring: pH, dissolved oxygen and conductivity and temperature only. Test substance concentration was not measured.

Exposure period: 96-hours

Statistical methods: Probit analysis.

Test fish age: Not noted other than "juvenile".

Length and weight: Average length = 26.5 mm.

Average weight = 0.14 g

Loading: 0.09 g fish / L

Pretreatment: None

Test conditions:

Dilution water: Aerated, filtered natural well water

Dilution water chemistry (from a representative sample):

hardness: 160 mg/L as CaCO₃

pH: 7.0

Conductivity: 640 µmhos/cm

Stock and test solution preparation: The test concentrations were prepared by transferring appropriate weights of FC-120

directly to the test chambers. Test concentrations were prepared based on total sample, not on percent concentration of the test substance in the test sample. All test vessels containing FC-120 were initially clear and remained clear throughout the study.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Not noted

Exposure vessels: 20 liter glass aquaria containing 15L of test solution, water depth ~ 18 cm.

Number of replicates: two

Number of fish per replicate: ten

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

6.8 - 8.5 mg/L (control exposure)

5.7 - 8.6 mg/L (15 mg/L exposure)

pH range (0 – 96 hours)

7.0 - 8.2 (control exposure)

7.0 - 8.0 (15 mg/L exposure)

Test temperature: Mean temperature stated as $23 \pm 2^{\circ}\text{C}$

RESULTS

Nominal concentrations: Bk control, 2.5, 4.0, 6.0, 10, 15mg/L

Element value: 24-hour $\text{LC}_{50} = > 15 \text{ mg/L}$

48-hour $\text{LC}_{50} = > 15 \text{ mg/L}$

72-hour $\text{LC}_{50} = 11 (9.7 - 12.5) \text{ mg/L}$

96-hour $\text{LC}_{50} = 4.8 (4.0 - 5.7) \text{ mg/L}$

All element values based on nominal concentrations

Statistical Evaluation of Mortality:

Probit analysis was used to calculate LC_{50} values and the corresponding confidence limits.

Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Neg. Control	0	0	0	0
2.5	0	0	0	10
4.0	0	0	0	25
6.0	0	0	0	85
10	0	0	40	85
15	0	20	85	100

CONCLUSIONS

The test sample 96-hour LC₅₀ for fathead minnow was determined to be 4.8 mg/L with a 95% confidence interval of 4.0 – 5.7 mg/L.

Testing was conducted on the "as-sold" mixture of the subject material in water (~25% subject material). The values reported apply to that mixture and not directly to the subject material.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for determination of the test substance concentration in the test solutions and determination of the sample purity. Because a mixture was tested, care must be taken when interpreting the results. The solvent in the mixture could have enhanced or restricted biological uptake and/or toxicity of the fluorochemical component.

REFERENCES

This study was conducted by EnviroSystems Division Resource Analysts, Incorporated, Hampton, New Hampshire on behalf of the 3M Company.

OTHER

Last changed: 5/18/00

J2904-1

Study Title

Static Acute Toxicity of FC-120 to the
Fathead Minnow, *Pimephales promelas*

Authors

Timothy J. Ward
Robert L. Boeri

Study Completed

March 1992

Performing Laboratory

EnviroSystems Division
Resource Analysts, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

I. SUMMARY

The acute toxicity of FC-120 to the fathead minnow, *Pimephales promelas*, is described in this final report. The test was conducted for 3M Company for 96 hours during February 27 to March 2, 1992, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Ellen Stanford, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of $23 \pm 2^{\circ}\text{C}$. The dilution water was filtered natural well water collected at Hampton, New Hampshire. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of FC-120 were: 0 mg/L (control), 2.5 mg/L, 4.0 mg/L, 6.0 mg/L, 10.0 mg/L, and 15.0 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 more than 15 days. After 96 hours of exposure the control fish had an average total length of 26.5 mm and an average wet weight (blotted dry) of 0.14 g, resulting in a loading rate of approximately 0.09 g/L. 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 4.8 mg/L FC-120, with a 95% confidence interval of 4.0 to 5.7 mg/L. One of the eight surviving fish exposed to 15.0 mg/L at 48 hours and one of the three surviving fish exposed to 15.0 mg/L at 72 hours exhibited erratic swimming. The three surviving fish exposed to 10.0 mg/L at 96 hours exhibited a loss of equilibrium and erratic swimming. No other sublethal effects were observed in any test vessel during the 96 hour exposure.

III. INDEX OF TABLES

	PAGE
Table 1. Chemical characterization of a representative sample of natural well water used as dilution water for toxicity test	6
Table 2. Survival data from toxicity test	9
Table 3. Median lethal concentrations (LC50s) from toxicity test	10
Table A.1. Conductivity, pH, temperature, and dissolved oxygen concentration measured during toxicity test	13

IV. INTRODUCTION

This study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of FC-120 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.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

FC-120 (EnviroSystems Sample Number 5103E) was delivered to EnviroSystems on November 13, 1991. It was contained in a 250 ml plastic bottle that was labelled with the following information: "J2904-I, FC-120, Lot 164, 2-Butoxy Ethanol". FC-120 (a light brown 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 1.3 grams) will be retained 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 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 (the Aquatic Research Organisms Division of Resource Analysts, Inc., Hampton, New Hampshire) and acclimated at the EnviroSystems facility for more than 15 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 21.1° to 22.5°C, and the dissolved oxygen concentration was maintained above 8.0 mg/L. Fish were fed a commercial fish food (EnviroSystems lot number TM03) once or twice 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	--	7.0
Conductivity	umhos/cm	--	640
Hardness	mg/L as CaCO ₃	--	160
Organochlorine pesticides	ug/L	0.5	ND
Organophosphorus pesticides	ug/L	0.5	ND
Polychlorinated biphenyls	ug/L	0.5	ND

Notes: 1. ND = not detected at or above the reporting limit.
 2. pH, hardness, and conductivity were measured in dilution water collected from the control test vessel prior to introduction of organisms and pesticide and PCB data is collected during routine biannual water quality testing.

TOXICITY TESTING:

A screening test with the test substance was conducted during January 13 to 17, 1992. The test was conducted at 1, 10, 100, and 1,000 mg/L. After 96 hours there was 0% survival at 100 and 1,000 mg/L and 100% survival at all lower tested concentrations.

A definitive toxicity test was attempted during February 20 to 24, 1992. Nominal concentrations of the test material were: 0 mg/L, 15 mg/L, 25 mg/L, 40 mg/L, 60 mg/L, and 100 mg/L. The test was repeated because less than 50% survival occurred at all 5 concentrations after 96 hours.

The definitive toxicity test was performed during February 27 to March 2, 1992 according to procedures of the OECD (1984). The test was conducted at a target temperature of $23 \pm 2^\circ\text{C}$ with five concentrations of test substance and a dilution water control. No stock solution was prepared and 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), 2.5 mg/L, 4.0 mg/L, 6.0 mg/L, 10.0 mg/L, and 15.0 mg/L.

Twenty fish were equally distributed among two replicates of each treatment. The test was performed in 20 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 18 cm). Test vessels were randomly arranged in an incubator 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. Aeration was not employed 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), pH (Beckman model PHI 12 meter), conductivity (Cole Parmer model number 1481-60), and temperature (ASTM mercury thermometer) 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 24, 48, 72, and 96 hour LC50 values were calculated by the binomial, moving average, or probit method, when warranted. All calculations were performed by the author using nominal concentrations of the test substance.

VI. RESULTS

All test vessels containing FC-120 were initially clear and 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 26.5 mm, and an average wet weight (blotted dry) of 0.14 g at the end of the test. Loading rate during the toxicity test was approximately 0.09 g/L.

The 24, 48, 72, and 96 hour LC50s for fish exposed to FC-120 are presented in Table 3. The 96 hour LC50 value (and associated 95% confidence limits) is 4.8 mg/L FC-120 (4.0 - 5.7 mg/L). One of the eight surviving fish exposed to 15.0 mg/L at 48 hours and one of the three surviving fish exposed to 15.0 mg/L at 72 hours exhibited erratic swimming. The three surviving fish exposed to 10.0 mg/L at 96 hours exhibited a loss of equilibrium and erratic swimming. No other sublethal effects were observed in any test vessel during the 96 hour exposure.

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
2.5	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	8	0	0	0	0	0
4.0	1	10	10	10	10	5	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
6.0	1	10	10	10	10	3	0	0	0	0	0
	2	10	10	10	10	0	0	0	0	0	0
10.0	1	10	10	10	6	2	0	0	0	0	2
	2	10	10	10	6	1	0	0	0	0	1
15.0	1	10	10	8	2	0	0	0	0	1	-
	2	10	10	8	1	0	0	0	1	0	-

Note: All affected fish exhibited erratic swimming and affected fish at 96 hours also exhibited a loss of equilibrium.

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

Exposure period	LC50	95 percent confidence limit	LC50 calculation method
24 hours	>15.0 mg/L	--	--
48 hours	>15.0 mg/L	--	--
72 hours	11.0 mg/L	9.7 - 12.5 mg/L	Probit
96 hours	4.8 mg/L	4.0 - 5.7 mg/L	Probit

VII. REFERENCES

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

Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. 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)					pH				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	640	640	650	650	670	7.0	7.8	7.3	8.0	8.1
	2	640	660	670	660	690	7.0	7.8	7.3	8.0	8.2
2.5	1	650	670	670	660	660	7.0	7.9	7.3	8.0	8.2
	2	650	670	670	660	670	7.0	7.9	7.3	8.0	8.2
4.0	1	660	670	670	660	670	7.0	7.9	7.3	7.9	8.2
	2	660	670	670	660	670	7.0	7.9	7.3	7.9	8.1
6.0	1	660	660	670	670	670	7.0	7.9	7.3	7.9	8.1
	2	660	660	670	660	670	7.0	7.9	7.3	7.9	8.1
10.0	1	660	670	670	670	660	7.0	7.9	7.3	7.9	8.0
	2	660	670	670	670	660	7.0	7.9	7.3	7.9	8.1
15.0	1	670	670	670	670	670	7.0	7.9	7.3	7.8	8.0
	2	670	670	670	670	670	7.0	7.9	7.3	7.8	8.0

Table A.1. Continued.

Nominal concentration (mg/L)	Rep.	Temperature (°C)					Dissolved Oxygen (mg/L)				
		0 hr	24 hr	48 hr	72 hr	96 hr	0 hr	24 hr	48 hr	72 hr	96 hr
0 (control)	1	21.5	23.0	21.5	21.5	22.5	8.5	8.5	7.7	7.4	6.8
	2	21.6	21.0	21.8	21.4	22.9	8.5	8.5	7.7	7.3	7.5
2.5	1	21.5	22.0	21.4	21.4	21.5	8.6	7.9	7.6	7.4	7.0
	2	21.5	22.9	21.5	21.3	22.2	8.7	8.0	7.5	7.3	7.0
4.0	1	21.4	22.5	21.6	21.5	22.5	8.6	7.5	7.6	7.2	6.7
	2	21.5	23.0	21.5	21.3	22.6	8.6	7.6	7.4	7.0	6.0
6.0	1	21.5	22.3	21.7	21.2	21.8	8.6	7.5	7.5	7.1	6.4
	2	21.5	22.3	21.4	21.5	22.3	8.6	7.5	7.3	7.1	5.6
10.0	1	21.5	22.4	21.5	21.2	22.3	8.6	7.6	7.3	6.9	5.8
	2	21.5	22.3	21.7	21.4	22.1	8.6	7.5	7.4	6.8	6.2
15.0	1	21.5	22.7	21.6	21.2	22.2	8.6	7.6	7.2	6.8	5.7
	2	21.5	22.9	21.8	21.3	22.1	8.6	7.6	7.3	7.0	6.0