

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

TEST SUBSTANCE

Identity: A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-203C and L-5440. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The 3M production lot number was not noted. The test sample is FC-203C and is referred to by the testing laboratory as "81-1/8". Current information indicates that FC-203C is a mixture of 1.47% PFOS, 60% water, 30% diethylene glycol butyl ether, 3% sodium octyl sulfate, 3% hydroxy foamer, 10% polyoxyethylene monooctylphenyl ether, 0.12% sodium lauryl sulfate, and 0.05% benzotriazol.

The following summary applies to a with incompletely characterized concentration of impurities. Data may not accurately reflect toxicity of the fluorochemical component of the test sample..

METHOD:

Method: EG&G, Bionomics protocol entitled "Methods for conducting flow-through toxicity tests with freshwater fish.

Test type: Flow-through acute

GLP: No

Year Completed: 1981

Species: *Pimephales promelas*

Supplier: Commercial supplier in Nebraska

Analytical monitoring: Temperature, pH, DO, and conductivity

Exposure period: 96-hours

Statistical methods: LC₅₀ values calculated using the computer program (created by Stephan, 1978) based on the moving average angle analysis, probit analysis, or binomial probability as appropriate.

Test fish age: Not noted.

Length and weight: Length = 33 – 54 mm with a mean of 41 mm
Weight = 0.25 – 1.53 g with a mean of 0.57 g

Loading: 0.38 g/L

Pretreatment: Not noted.

Test conditions:

Dilution water: Well water supplemented with Town of Wareham untreated and unchlorinated well water.

Dilution water chemistry:

Hardness: 22 - 24 mg/L (as CaCO₃)

Alkalinity: 18 – 24 mg/L (as CaCO₃)

Conductivity: 90 µmhos/cm

pH: 6.7 – 6.8

Lighting: Cool White® fluorescent lights provided a daily photoperiod of 16 hours light and 8 hours dark with an intensity of 30 – 70 footcandles.

Stock and test solution preparation: A primary stock solution of 780 mg/mL was prepared by adding 3120 grams of test sample to distilled water and diluting to 4000 mL. All test solutions were prepared by a diluter delivering five nominal concentrations of the test sample and control water.

Flow-through rate: Approximately 6.3 volume replacements of test water every 24 hours.

Stability of the test chemical solution: Not noted.

Exposure vessels: 39 x 20 x 25 cm glass tanks which maintained test solution volume of 15 liters.

Number of replicates: two

Number of fish per replicate: 10

Number of concentrations: 5 plus a blank control

Water chemistry during the study:

pH range (0-96 hours):

6.9 – 7.1 (control)

6.8 – 7.2 (2000 mg/L)

Temperature range (0-96 hours):

21 – 22°C (for all aquaria)

Dissolved oxygen range (0-96 hours):

8.2 – 9.1 mg/L (control)

6.4 – 8.4 mg/L (2000 mg/L)

RESULTS

Nominal concentrations: Bk control, 125, 250, 500, 1000, and 2000 mg/L

Element value: 96-hour LC₅₀ = >2,000 mg/L

Element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture as described in the test substance remarks field. The value reported applies to that mixture and not the fluorochemical portion alone.

CONCLUSIONS

The FC-203C 96-hour LC₅₀ for *Pimephales promelas* was determined to be >2,000 mg/L. The highest concentration tested, 2000 mg/L, induced only 5% mortality.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study meets all the criteria for quality testing. However, there is no analytical (or other) information to confirm the diluter was working properly. The study lacks information on the purity of the test substance and lacks actual measurements of the concentration of fluorochemical in the solution.

REFERENCES

This study was conducted by EG&G, of Wareham, MA at the request of the 3M Company, 3M Lab Request number 7038, 1981.

OTHER

Last changed: 6/26/00

EAI # 8/2/8

LR No. 7038

ACUTE TOXICITY OF 81-1/8 TO

FATHEAD MINNOW (Pimephales promelas).

FC-203C

TOXICITY TEST REPORT

SUBMITTED TO

3M

ST. PAUL, MINNESOTA.

REPORT #BW-81-10-1030

EG&G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
October, 1981

INTRODUCTION

The purpose of this study was to estimate the acute toxicity of 81-1/8 to fathead minnow (Pimephales promelas) under flow-through conditions. Preliminary range-finding static tests and a subsequent 96-hour flow-through test were conducted at the Aquatic Toxicology Laboratory of EG&G, Bionomics, Wareham, Massachusetts. The acute toxicity of 81-1/8 was measured by estimating the LC50 value for the compound at each 24 hour interval of the test. The LC50 is defined as the concentration of the test compound in dilution water which caused mortality of 50% of the test animal population at the stated exposure interval. The flow-through test was conducted from 24-28 September 1981 with a two day pre-exposure period. All raw data generated are stored at the above location.

MATERIALS AND METHODS

Procedures used in this acute toxicity test closely followed those described in the EG&G, Bionomics protocol entitled "Methods for conducting flow-through toxicity tests with freshwater fish." Values are reported to different levels of significance depending on the accuracy of the measuring devices involved in any one process.

The 81-1/8, a brown colored liquid, was received from 3M, St. Paul, Minnesota on 12 August 1981. The test material was stored in the dark at ambient temperature (21°C). The material was tested as 100%

active ingredient. Nominal test concentrations are reported as milligrams of 81-1/8 per liter of test solution (mg/l).

The fathead minnow (Bionomics lot #81A17) were obtained from a commercial fish supplier in Nebraska and held in a 500-l fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. All fish were fed a dry, pelleted food, ad libitum, daily except during the 48 hours prior to testing. There was 1.0% mortality in the test fish population during this 2 day period (Daily Record of Fish Holding Conditions). The well water which flowed into this tank was characterized as having total hardness and alkalinity ranges as calcium carbonate (CaCO_3) of 22-24 mg/l and 18-21 mg/l, respectively and a specific conductance range of 80-90 micromhos per centimeter ($\mu\text{mhos/cm}$) (Weekly Gravity Feed Tank Water Quality Analysis Logbook). Other parameters monitored in the holding tank were a pH range of 6.5-6.7, a dissolved oxygen (DO) range of 94-99% of saturation and a flow rate of 18 tank volume replacements/day (Weekly Record of Fish Holding Water Characteristics). Test fish were maintained under these conditions for a minimum of 14 days. The temperature in the holding tank was 21°C during this 14 day period (Daily Record of Fish Holding Conditions). The specific conductance was measured with a YSI Model #33 salinity-conductivity-temperature meter and probe, the pH was measured with an Instrumentation Laboratory Model #175 pH meter and combination electrode, the DO was measured with a YSI Model #57 dissolved

oxygen meter and probe and the temperature was measured with a Brooklyn alcohol thermometer. Total hardness and alkalinity were measured according to APHA et al. (1975).

The exposure system used in this study was a modified, proportional diluter similar to that described by Mount and Brungs (1967) with a 0.50 dilution factor. The dilution water was well water which was pumped to an epoxy coated concrete reservoir where it was supplemented with Town of Wareham untreated and unchlorinated well water and aerated. This water was characterized as having total hardness and alkalinity ranges as CaCO_3 of 22-24 mg/l and 18-24 mg/l, respectively, a pH range of 6.7-6.8 and a specific conductance of 90 $\mu\text{mhos/cm}$ (Weekly Gravity Feed Tank Water Quality Analysis Logbook).

The diluter delivered five nominal concentrations of 81-1/8, 2000, 1000, 500, 250, 125 mg/l and control water containing no 81-1/8 to duplicate test aquaria. Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with 19.5 cm high drains which maintained water volumes of 15 l. The diluter delivered 0.5 l of test water to each aquarium at an average rate of 188 cycles per day. This is equivalent to 6.3 aquarium volume replacements per 24 hour period. Illumination was provided by Cool White^R fluorescent lights centrally located above the test aquaria. Sixteen hours of light at 30-70 footcandles at the water surface were

provided each day. Test temperatures were controlled by immersion coil heaters in a water bath where the test aquaria were located.

A 4-l glass Mariotte bottle in conjunction with a "dipping bird" delivery system (Lemke, et al. 1978) was utilized to deliver a stock solution to the mixing chamber of the diluter with every cycle. The stock solution of 780 mg/ml was prepared by adding 3120 grams of 81-1/8 to distilled water and diluting to 4000 ml.

The 81-1/8 stock solution was introduced into the diluter and cycling began on 22 September 1981. The test was initiated two days later when ten fathead minnow with a mean (range, N=30) wet weight and total length of 0.57(0.25-1.53) grams and 41(33-54) millimeters (Fish Weights and Lengths Log) were randomly distributed to each aquarium.

Biological observations of the fish and observations of the physical characteristics of the test solutions were made and recorded at 0, 24, 48, 72 and 96 hours. The pH, temperature and dissolved oxygen (DO) concentrations were measured in both replicates of the control, high, middle and low concentrations at 24, 48, 72 and 96 hours and in both replicates of all concentrations at 0 hour of exposure.

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate 24-, 48-, 72- and 96-hour median lethal concentrations (LC50) and 95% confidence intervals. The computer program utilized (Stephan, 1978, personal communication) estimated LC50 values using one of three statistical methods in the following order of preference: moving average angle analysis, probit analysis, binomial probability. The method selected was determined by the characteristics of the data base (i.e. presence or absence of test concentrations causing mortality of 100% of the test animal population, test concentrations causing mortality of a partial number of animals in the population, etc.). The computer program scanned the data base, identified the most preferred statistical method and performed the analysis.

RESULTS

The test material 81-1/8, was not acutely toxic to fathead minnows at nominal concentrations as high as 2000 mg/l (Table 1). Although there appeared to be no 81-1/8 related mortality, fish exposed to 2000 mg/l 81-1/8 were lethargic after 48 hours exposure. Fish exposed to 1000, 500 and 250 mg/l 81-1/8 also appeared lethargic at 72 hours exposure. The estimated LC50 values after 24, 48, 72 and 96 hours of exposure was >2000 mg/l, the highest concentration tested.

The temperature in various aquaria ranged from 21-22°C during exposure. The pH and dissolved oxygen concentrations measured during the toxicity test are presented in Table 2.

LITERATURE CITED

APHA, AWWA, WPCF. 1975. Standard methods for the examination of water and wastewater. 14th Edition, Washington, D.C. 1193 pp.

Lemke, A.E., W.A. Brungs and B.J. Halligan. 1978. Manual for construction and operation of toxicity-testing proportional diluters. EPA-600/3-78-072.

Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 20-29.

Stephan, Charles. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

Table 1. Concentrations tested, corresponding percentage mortalities and behavioral observations of fathead minnow (Pimephales promelas) exposed to 81-1/8 under flow-through conditions. Twenty fish were exposed to each concentration.

Nominal concentration (mg/l)	Percentage mortality			
	24 hour	48 hour	72 hour	96 hour
2000	0 ^a	0 ^{a,b}	0 ^{a,b,c}	5 ^{a,b}
1000	0 ^a	0 ^a	0 ^{a,b}	0 ^a
500	0 ^a	0 ^a	0 ^{a,b}	0 ^a
250	0	5 ^a	10 ^{a,b}	10
125	0	0 ^a	5 ^a	5
control	0	0	0	5

^a Suds formed on the surface of the test solution.

^b Some fish were lethargic.

^c One fish was at the surface of the test solution.

Table 2. The pH and DO concentrations measured during the 96-hour flow-through toxicity test with fathead minnow (Pimephales promelas) and 81-1/8. The range of measurements made from replicate aquaria are reported.

	Nominal concentration (mg/l)	0 hour	24 hour	48 hour	72 hour	96 hour
pH	2000	7.2	7.2	6.8	6.8	6.8
	1000	7.1	_a	_a	_a	_a
	500	7.1	7.0	6.6-6.8	6.4-6.9	6.7-6.8
	250	7.0	_a	_a	_a	_a
	125	7.1	6.9	6.7-6.9	6.9	6.9
	control	7.1	7.0	6.9-7.0	7.0	7.0
DO (mg/l)	2000	8.3-8.4	7.6-7.9	7.9	7.0-7.4	6.4-6.9
	1000	8.2-8.3	_a	_a	_a	_a
	500	8.2-8.4	7.4-7.6	7.1-8.1	5.1-7.8	5.9-6.9
	250	8.7	_a	_a	_a	_a
	125	8.7-8.8	7.6-7.7	8.1-8.5	7.7-7.8	7.8-7.9
	control	9.0-9.1	8.2-8.4	8.3-8.5	8.2-8.4	8.2-8.3

a
No measurement made.

SUBMITTED BY:

EG&G, Bionomics
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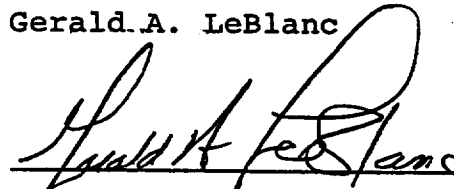
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