ACUTE TOXICITY TO FISH (MUMMICHOG)

TEST SUBSTANCE

Identity: A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-780B. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot number was not noted. The test sample is FC-780B, identified by the laboratory as "7901". Current information indicates it is a mixture of 0.58% PFOS, 14% diethylene glycol monobutyl ether, 76.7% water, 5.5% urea, 1.17% Hydroxy foamer, 1.15% sodium octyl sulfate, 0.8% polyoxyethylene monooctylphenyl ether, 0.05% sodium lauryl sulfate, and 0.05% benzotriazole.

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

METHOD:

Method: DTNSRDC Standard Static Marine Bioassay Procedure for Shipboard Chemicals.
Type: Acute static
GLP: No
Year completed: 1979
Species: *Fundulus heteroclitus*
Supplier: Commerical supplier in MA.
Analytical monitoring: Dissolved oxygen, pH, salinity, and temperature.
Exposure period: 96-hours
Statistical methods: LC_{50} values and 95% confidence intervals calculated using a computer program by Stephan, et al. 1978.
Test fish age: Juveniles
Length and weight: Average length = 48 mm
Average weight = 1.16 g (wet)
Loading: Not given
Pretreatment: None.
Test conditions:
Dilution water: Instant Ocean® dissolved in deionized water.
Dilution water chemistry:
Salinity: 15 ppt
pH: 8.5

Lighting: Not given.
Stock and test solution preparation: The test material was added directly to the dilution water on a weight/volume basis and mixed by stirring with a glass rod.

Concentrations dosing rate: Once
Stability of the test chemical solutions: Not noted
Exposure vessels: 19.6 L glass jars containing 15 L of test solution.
Number of replicates: 2
Number of fish per replicate: 5
Number of concentrations: six plus a blank control
Water chemistry during the study:

pH range (0-96 hours):

7.1 – 8.4 (control exposure)
6.7 – 8.4 (10000 mg/L exposure)

Temperature range (0-96 hours): 18.5-20.0 °C

Salinity range (0-96 hours):

13 – 17 ppt (control exposure)
13 – 17 ppt (10000 mg/L exposure)

Dissolved oxygen range (0-96 hours):

6.8 – 7.8 mg/L (control exposure)
0.3 – 7.8 mg/L (10000 mg/L exposure)

RESULTS

Nominal concentrations: Blank control, 1900, 2800, 4100, 6000, 7700, and 10000 mg/L.

Element values: 96-hour LC_{50} = 3900 (3400 – 4600) mg/L
96-hour NOEC = <1900 mg/L

Element values based on nominal concentrations

Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical proportion alone.

CONCLUSIONS

The test sample 96-hour LC_{50} for mummichog was determined to be 3900 mg/L, with a 95% confidence interval of 3400 to 4600 mg/L. The 96-hour No Observed Effect Concentration (NOEC) was <1900 mg/L, the lowest concentration tested.

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

DATA QUALITY

Reliability: Klimisch ranking = 2. The study meets the criteria for quality testing. However, the sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

This study was conducted by EG&G, Bionomics, Wareham, MA at the request of the 3M Company, St. Paul, MN, 1979.

OTHER

Last changed: 6/28/00

780 B

ACUTE TOXICITY OF 7901 TO THE COMMON MUMMICHOG (*Fundulus heteroclitus*).

TOXICITY TEST REPORT

SUBMITTED TO

THE 3M COMPANY

ST. PAUL, MINNESOTA

REPORT #BW-79-4-416

E G & G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
April, 1979

INTRODUCTION

The purpose of this study was to estimate the acute toxicity of 7901 to the common mummichog (Fundulus heteroclitus) under static conditions. Preliminary range-finding tests and a subsequent 96-hour definitive test were conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts. The definitive test was conducted from 20-24 March 1979. All raw data generated are stored at the above location under the direction of Joyce Brundage.

MATERIALS AND METHODS

Procedures used in this acute toxicity test followed those described in "DTNSRDC Standard Static Marine Bioassay Procedure for Shipboard Chemicals" (Liberatore). All values are reported to various levels of significance depending on the measuring devices employed during any one process.

The 7901, a yellow liquid, was tested as received from the 3M Company, St. Paul, Minnesota. Nominal test concentrations are reported as milligrams of 7901 per liter of test solution (mg/ℓ).

The common mummichog (lot #78A59) used in this study were obtained from a commercial fish supplier in Massachusetts. Upon receipt at Bionomics, the fish were held in a 600-ℓ fiberglass

tank containing a solution of Instant Ocean^{R}. This water was characterized weekly, and had a pH range of 6.5-6.7, a salinity range of 14-18 parts per thousand (o/oo) and a temperature of 20 $\pm$ 1^{o}C. The water was recirculated through dolomite in a biologically active filter bed to preserve water quality during holding and acclimation. Dissolved oxygen (DO) was maintained between 91 and 99% of saturation by this system. The pH was measured with a Model #175 Instrumentation Laboratory pH meter and combination electrode, the temperature with a dial thermometer, the DO with a Model #57 YSI combination oxygen-temperature meter and probe, and the salinity with an American Optical refractometer. Experimental animals were maintained under these conditions for a minimum of 14 days. All fish were fed a dry pelleted food, ad libitum, daily except during the 48-hours prior to testing. There was 0.33% mortality during this 2 day period.

The definitive test was conducted in 19.6-ℓ glass jars which contained 15 ℓ of test solution. The dilution water used in this test was prepared by adding Instant Ocean^{R} to deionized water and mixing until dissolved. This water had a pH of 8.5 and a salinity of 15 o/oo. The 7901 was added directly to the dilution water and mixed by stirring with a glass rod.

Control jars (A and B replicates) containing the same dilution water and maintained under the same conditions as test concentrations, but containing no 7901, were established. All test

solution temperatures were controlled by a system designed to maintain temperatures at 19 ± 1°C. All test solutions were gently aerated during the exposure period.

Ten common mummichog (five per replicate) with a mean (range N=30) wet weight and total length of 1.16 (0.64-2.17) grams and 48 (40-61) millimeters, respectively, were randomly distributed to each test concentration within 15 minutes after the solutions were prepared.

Mortalities were recorded and removed from each test jar every 24 hours during testing. 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, DO concentration and salinity of test solutions were measured at 0, 24, 48, 72 and 96 hours in the A and B replicates of the control, high, middle and low test concentrations. Temperature was also measured in each control jar replicate at the above mentioned time intervals.

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 LC50 is defined as the concentration (nominal or measured) of the test compound in dilution water

which caused 50% mortality in the test animal population at the stated exposure interval.

The computer program utilized (Stephan, 1978, personal communication) estimates 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 is determined by the characteristics of the data base (i.e. presence or absence of 100% mortality, number of partial mortalities, etc.). The computer program scans the data base, identifies the most preferred statistical method and performs the analysis.

RESULTS

The 96-hour LC50 for the common mummichog exposed to 7901, estimated by the moving average angle method, was 3,900 mg/ℓ

Table 1 summarizes the 24-, 48-, 72- and 96-hour LC50's and 95% confidence intervals and states the no discernible effect concentration through 96 hours. The no effect concentration is the highest concentration tested at which there were no mortalities or observed behavioral and physical abnormalities, e.g. loss of equilibrium, at surface, darkened pigmentation.

The pH, DO and salinity measured during the test are presented

in Table 2. The temperature of the control solutions ranged from 18.5-20.0°C during exposure.

Table 3 summarizes the nominal test concentrations and corresponding cumulative mortalities observed at 24, 48, 72 and 96 hours of exposure to each test solution.

Deviations from the protocol

- The common mummichog were ∿4.8 centimeters long
- Nineteen point six liter widemouth glass bottles were used as test containers
- Instant Ocean® solution was used for acclimation and testing
- The salinity for acclimation and testing was 15 o/oo
- Fish were acclimated in water with a pH range of 6.5 to 6.7 and tested in water having a pH of 8.5
- Ten fish were exposed to each concentration
- LC50's were calculated by Stephan, 1978, personal communication

It is our judgement that none of the above deviations have affected the integrity of this toxicity test.

LITERATURE CITED

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

Liberatore, Giacomo. David W. Taylor Research and Development Center, Standard Static Marine Bioassay Procedure for Shipboard Chemicals. Report TM-28-76-29.

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

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Table 1 -- The 24-, 48-, 72- and 96-hour LC50 values and 95% confidence intervals for the common mummichog (Fundulus heteroclitus) exposed to 7901.

LC50 (mg/ℓ)				No discernible effect concentration through 96 hours (mg/ℓ)
24 hour[a]	48 hour[b]	72 hour[b]	96 hour[b]	
5,600 (4,100-7,700)[c]	4,100 (3,500-3,700)	3,900 (3,400-4,600)	3,900 (3,400-4,600)	<1,900[d]

a Estimated by the binomial probability method.
b Estimated by the moving average angle method.
c 95% confidence intervals.
d Lowest concentration tested.

Table 2 -- The pH, DO and salinity measurements made during a 96-hour toxicity determination with 7901 and the common mummichog (Fundulus heteroclitus).

	Nominal concentration (mg/ℓ)	0 hour	24 hour	48 hour	72 hour	96 hour
pH[a]	10,000	8.4	8.0	7.7	6.7	6.7
	6,000	8.4	7.1	6.8	6.8	6.9
	1,900	8.4	7.2	6.9	7.2	7.4
	control	8.4	7.6	7.1	7.6	7.7
DO[a] (mg/ℓ)	10,000	7.8(98)[b]	0.3(3.8)	0.3(3.8)	0.2(2.5)	0.4(5.0)
	6,000	7.8(98)	0.6(7.5)	0.5(6.3)	0.3(3.8)	0.6(7.5)
	1,900	7.8(98)	3.6(45)	3.1(39)	6.3(79)	6.2(78)
	control	7.8(98)	6.8(85)	6.8(85)	7.2(90)	7.2(90)
salinity[a] (o/oo)	10,000	15	17	15	14	13
	6,000	15	17	15	14	13
	1,900	15	17	15	14	13
	control	15	17	15	14	13

[a] Measurements made in the A replicate solutions.

[b] % of saturation at 19°C.

Table 3 -- Concentrations tested and corresponding cumulative mortalities of the common mummichog (Fundulus heteroclitus) exposed to 7901 at 24, 48, 72 and 96 hours. Ten fish were exposed to each concentration, five fish per replicate.

Nominal[a] concentration (mg/ℓ)	Cumulative mortalities (A and B replicates) 24 hour	48 hour	72 hour	96 hour
10,000	10	10[b]	10[b]	10[b]
7,700	10	10[b]	10[b]	10[b]
6,000	4[c,d,e]	10[b]	10[b]	10[b]
4,100	0[c,d,e]	2[b,c,f]	2[b,c,e]	2[b,c,g]
2,800	0[c,e]	1[b,c]	2[b,e,g]	2[b,g]
1,900	0[c]	0[b]	0[b,c,e]	0[b]
control	0	0	0	0

[a] Solutions had foam on the surface throughout exposure.
[b] Solutions were cloudy.
[c] Some fish were at the surface.
[d] Some fish showed a loss of equilibrium.
[e] Fish were respiring rapidly.
[f] Some fish were gulping.
[g] Fish were lethargic.