

## ACUTE TOXICITY TO AQUATIC INVERTEBRATES (GRASS SHRIMP)

### TEST SUBSTANCE

**Identity:** A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-203. (1-Octanesulfonic acid) (CAS # 2795-39-3).

**Remarks:** The 3M production lot number was 1. The test sample is FC-203. Current information indicates it is a mixture of 1.34% PFOS, 35% diethylene glycol butyl ether, 37.85% water, 20% ethylene glycol, 2.66 % Sultone foamer, 3% sodium octyl sulfate, 0.1% sodium lauryl sulfate, and 0.05% tolyltriazole.

***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:** Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians (U.S. EPA 1975).

**Type:** Acute static

**GLP:** No

**Year completed:** 1979

**Species:** *Palaemonetes pugio*

**Supplier:** Field collected from Big Lagoon, a Gulf of Mexico estuary adjacent to the laboratory, Pensacola, FL.

**Analytical monitoring:** Temperature, pH, salinity, and DO

**Exposure period:** 96-hours

**Test organism age:** 16-22 mm rostrum-telson length

**Statistical method:** EC<sub>50</sub> values calculated using the Stephan computer program, 1977 and 1978.

**Test conditions:**

**Dilution water:** Filtered natural seawater, from Big Lagoon, FL

**Dilution water chemistry:**

**Salinity:** 25 - 26 ppt

**Temperature:** 22 - 25 °C

**Lighting:** Not given.

**Stock and test solution preparation:** Exposure concentrations prepared by direct addition of test substance.

**Exposure vessels:** 19 L glass jars containing 15 L of test solution.

**Number of replicates:** 2

**Number of organisms per replicate:** 5

**Number of concentrations:** five plus a blank control

**Water chemistry during the study:**

**Salinity range (0-96 hours):** 26 ppt

**Temperature range (0-96 hours):** 22 °C

**Exhibit  
1215**

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

3M\_MN01656873

**pH range (0-96 hours):**

7.8 – 8.0 (control exposure)

7.5 – 8.1 (1000 mg/L exposure)\*

**Dissolved oxygen range (0-96 hours):**

4.8 – 6.4 mg/L (control exposure)

1.2 – 6.3 mg/L (1000 mg/L exposure)\*

\* 1000 mg/L (second highest concentration) exposure values used because the highest concentration resulted in total mortality by 72 hours.

**RESULTS**

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**Nominal concentrations:** Bk control, 220, 360, 600, 1000, and 1800 mg/L.

**Element values:** 96-hour EC<sub>50</sub> = 510 (360 - 710) 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**

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The test substance 96-hour EC<sub>50</sub> was determined to be 510 mg/L with a 95% confidence interval of 360 to 710 mg/L.

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

**DATA QUALITY**

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

**REFERENCES**

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Test was conducted by EG&G, Bionomics Marine Research Laboratory of Pensacola, FL at the request of the 3M Company, Lab Request number 4971S, Sample 7902, 1979.

**OTHER**

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**Last changed:** 6/27/00

Acute toxicity of 3M Company's  
sample 7902 to grass shrimp  
(Palaemonetes pugio)

FC-203 LA 1  
LR 4971 S  
EAI 79245

Toxicity Test Report

Submitted to

3M Company

St. Paul, Minnesota.

Project Number H90-500

Report Number BP-79-8-121

EG&G, Bionomics  
Marine Research Laboratory  
Route 6, Box 1002  
Pensacola, Florida 32507  
August 1979

3M\_MN01656875

1215.0003

A marine toxicity test was conducted at Bionomics Marine Research Laboratory (BMRL), Pensacola, Florida, to determine the acute effect of 3M Company's sample 7902 on grass shrimp (Palaemonetes pugio). The criterion for effect was death. Results of the test are expressed as 24-, 48-, 72-, and 96-hour LC50's (the concentrations of the test material estimated to be lethal to 50% of the test animals at the specified times).

All data related to this test are stored at BMRL.

#### MATERIALS AND METHODS

##### Test material

The sample was received at BMRL on 3 July 1979, and was contained in a 500-milliliter (mℓ) NALGENE® bottle labeled "3M SAMPLE 7902, BIOASSAYS: 96hr LC50-GRASS SHRIMP (PALAEONETES VULGARIS); 48hr LC50-ATLANTIC OYSTER LARVAE (CRASSOSTREA VIRGINICA).". The sample was a medium orange liquid.

Concentrations are reported here as milligrams (mg) of whole test material per ℓ of seawater or as parts per million (ppm).

##### Test animals

Grass shrimp, 16-22 millimeters rostrum-telson length, were collected from Big Lagoon, a Gulf of Mexico estuary adjacent to BMRL. The shrimp were held in the laboratory in flowing, natural seawater. During the 48-hour period prior to testing, salinity was 25-26 parts per thousand (‰) and temperature, 22-25 degrees Celsius (°C). No mortality was observed during the holding period.

It should be noted that Palaemonetes pugio were tested instead of P. vulgaris based on approval of Dr. Eric Reiner, 3M Company.

### Test water

Water used for holding and testing was natural seawater which was pumped from Big Lagoon. The pump intake was 85 meters (m) offshore at a depth of approximately 3 m.

Test water was pumped by a #316 stainless steel pump through hard polyvinylchloride (PVC) pipes, through sandfilled fiberglass filters, and through 10-micrometers ( $\mu\text{m}$ ) pore size polypropylene core filters into an elevated fiberglass reservoir. Water was continuously and vigorously aerated in the reservoir and flowed by gravity through PVC pipes into the laboratory. There it was pumped through a 5- $\mu\text{m}$  pore size polypropylene core filter and distributed into test chambers.

The chemical composition of BMRL seawater is characterized in Appendix A.

### Test conditions

Methods for the 96-hour static test were those described in Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians (U.S. EPA, 1975). The test was conducted in 19- $\ell$  uncovered glass jars which contained 15  $\ell$  of filtered natural seawater. Salinity was 26 ‰ and temperature was maintained at 22°C. Five shrimp were tested per jar and all treatments were duplicated. There was no aeration. Dissolved oxygen (DO) concentrations were near 90% of saturation at initiation of the test.

Based on a range-finding test, shrimp were definitively tested at nominal concentrations of 220-1,800 ppm. Test concentrations were prepared by adding appropriate weighed amounts of sample 7902 directly into seawater in the test containers. No solvent/carrier was used. The seawater control received no test material.

Test animals were not fed during the definitive test which was conducted 8-12 August 1979.

### Statistical analyses

Based on the results of the test, 48-, 72-, and 96-hour LC50's and their 95% confidence limits were calculated. The computer program (Stephan, 1977 and 1978 personal communication) estimated LC50 values by using one of three statistical methods in the following order: moving average angle analysis, probit analysis, or binomial probability. The method selected was determined by the characteristics of the data, that is, the presence or absence of 0% and 100% mortality and the number of concentrations in which >0%<100% mortality occurred. The computer scanned the data, identified the most suited method, and performed the analyses. For this test, the moving average angle method was used to analyze the 72- and 96-hour data and the probit analysis method was used for the 48-hour data.

### RESULTS AND DISCUSSION

3M Company's sample 7902 was "practically nontoxic" to grass shrimp, based on EPA Hazardous Substances Guidelines, FEDERAL REGISTER, 30 December 1975. The calculated 96-hour LC50 was 510 ppm with 95% confidence limits of 360-710 ppm (Table 1). After 96 hours of exposure, mortality ranged from 0% in the control to 100% in 1,800 ppm (Table 2).

Low DO concentrations could have contributed to the toxicity of sample 7902. At 24 hours of testing and later, the DO concentrations generally decreased as the test concentrations increased and after 96 hours ranged from 16% of saturation in the 1,000 ppm test concentration to 66% of saturation in the control (Table 3). Loading of

test animals (0.013 g/l) was not sufficient to cause the low DO concentrations as evidenced by the 66% of saturation measurement in the control after 96 hours of testing. Apparently two factors, the inherent chemical nature of the test material and the increased oxygen demand of shrimp stressed by exposure to sample 7902, resulted in the low DO concentrations.

The pH in all treatments was within an acceptable range. After 96 hours, pH was from 7.5-7.8 (Table 3).

## REFERENCES

- Stephan, C.E. 1977. Methods for calculating an LC50. ASTM, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. F.L. Mayer and J.L. Hamelink, eds. pp. 65-84.
- U.S. Environmental Protection Agency. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. EPA-600/3-75-009. 61 p.



TABLE 1. Calculated LC50's for grass shrimp (Palaemonetes pugio) exposed to 3M Company's sample 7902 in static, unaerated seawater. Salinity was 26 ‰ and temperature, 22°C. Values were calculated based on nominal concentrations of whole material.

Hour	LC50 (mg/ℓ; ppm)	95% confidence limits (mg/ℓ)
24	>1,800	---
48	2,400	---
72	570	410-790
96	510	360-710

TABLE 2. Mortality of grass shrimp (Palaemonetes pugio) exposed to 3M Company's sample 7902 in static, unaerated seawater. Salinity was 26 ‰ and temperature, 22°C. Test concentrations are given as whole material.

Nominal concentration (mg/ℓ; ppm)	Mortality (%)			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
220	0	10	10	10
360	0	0	40	40
600	0	0	30	40
1,000	0	20	80	90
1,800	0	50	100	100

TABLE 3. Measured concentrations of dissolved oxygen and pH during a 96-hour exposure of grass shrimp (*Palaemonetes pugio*) to 3M Company's sample 7902. The test was conducted in static, unaerated seawater. Salinity was 26 ‰ and temperature, 22°C. Test concentrations are given as whole material. Dissolved oxygen concentrations are the average of measurements in Replicates A and B. The pH was measured in Replicate A only.

Nominal concentration (mg/l; ppm)	Dissolved oxygen mg/l (% of saturation)					pH	
	0 h	24 h	48 h	72 h	96 h	0 h	96 h
Control	6.4 (88)	6.0 (82)	6.0 (82)	5.8 (79)	4.8 (66)	8.0	7.8
220	6.2 (85)	5.6 (77)	5.0 (68)	3.8 (52)	2.8 (38)	8.1	7.7
360	6.3 (86)	5.2 (71)	3.8 (52)	2.2 (30)	1.6 (22)	8.0	7.6
600	6.4 (88)	5.4 (73)	3.6 (50)	2.2 (30)	1.6 (22)	8.1	7.6
1,000	6.3 (86)	5.0 (68)	2.4 (33)	1.4 (19)	1.2 (16)	8.1	7.5
1,800	6.4 (88)	4.8 (66)	1.8 (25)	1.0 (14)	- <sup>a</sup>	8.1	- <sup>a</sup>

<sup>a</sup>No measurement because 100% mortality had occurred.

APPENDIX A

Results of Chemical Analyses for Routine  
Characterization of Selected Chemical  
Constituents in Bionomics Marine Research  
Laboratory Seawater

Constituent	Concentration (mg/l; ppm)	
	30 January 1979	14 June 1979
Arsenic	<0.001	0.006
Cadmium	0.002	<0.01
Chromium	0.0075	<0.01
Copper	0.023	<0.01
Mercury	0.0007	<0.0005
Nickel	0.02	<0.01
Zinc	0.05	<0.02
Lead	<0.001	<0.02
Total Phosphate as P	<0.02	<0.02
Ammonia Nitrogen as N	0.42	0.14
Nitrate Nitrogen as N	<0.01	<0.01
Nitrite Nitrogen as N	<0.01	<0.01
Total Petroleum Hydrocarbons	<5.0	<5.0 <sup>c</sup>
Sulfides	<1.0	<1.0
Pesticides	None detected <sup>a</sup>	None detected
Polychlorinated Biphenyls	None detected <sup>b</sup>	None detected

Water samples were collected from Bionomics Marine Research Laboratory seawater system after the mixing station in the wet lab.

<sup>a</sup>Pesticides: BHC, lindane, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, perthane, DDE, TDE (DDD), DDT, methoxychlor, endosulfan, strobane, toxaphene, kelthane, and chlordane all <0.005 µg/l.

<sup>b</sup>Polychlorinated Biphenyls: Aroclor® 1016, 1232, 1248, 1260, 1221, 1242, and 1254 all <0.05 µg/l.

<sup>c</sup>Petroleum hydrocarbon sample collected 10 July 1979.

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