

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (EASTERN OYSTER)

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 not noted. 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: Standard Practice for Conducting Static Basic Acute Toxicity Tests with Larvae of Four Species of Bivalve Mollusks (ASTM, Draft No. 5).

Type: Acute static

GLP: No

Year completed: 1979

Species: *Crassostrea virginica*

Supplier: Induced spawning in the laboratory of field-collected mature adults from an estuary adjacent to Biloxi, Mississippi. Maintained at BMRL until testing.

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

Exposure period: 48-hours

Test organism age: Emryos, within 1 hour after fertilization.

Statistical method: Test concentrations converted to logarithm and corresponding percentage reduction of normal larvae was converted to a probit. EC₅₀ values then calculated using linear regression.

Test conditions:

Dilution water: Filtered natural seawater pumped from Big Lagoon, a Gulf of Mexico estuary adjacent to the laboratory, Pensacola, FL.

Dilution water chemistry:

Salinity: 22 ppt

Lighting: Not given.

Stock and test solution preparation: A primary stock solution was prepared by adding a weighed amount of test substance to filtered seawater. Exposure concentrations were then prepared by addition of the appropriate volume of stock solution.

Exposure vessels: 1 L glass beakers containing 900 mL of test solution.

Number of replicates: 3

Number of organisms per replicate: Approx. 23,400 embryos

Number of concentrations: five plus a blank control

Element basis: Number of normally developed larvae, counted with Sedgwick-Rafter cell

Water chemistry during the study:

Temperature range (0-48 hours): 20 ± 1 °C (Temperature controlled water bath)

Salinity range (0-48 hours): 22 ppt

pH range (0-48 hours): 8.0 –8.1

Dissolved oxygen range (0-48 hours): > 67% saturation

RESULTS

Nominal concentrations: Bk control, 0.6, 1.0, 3.2, 5.6, and 10 mg/L.

Element values: 48-hour EC_{50} = 3.5 (0.5 - 22) 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 substance 48-hour EC_{50} was determined to be 3.5 mg/L with a 95% Confidence Interval of 0.5 to 22 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, sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

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

OTHER

Last changed: 6/27/00

Acute toxicity of 3M Company's Sample
7902 to embryos-larvae of eastern
oysters (Crassostrea virginica)

FC 203 lot 1 EAI 79245
LR 4971 S

Toxicity Test Report

Submitted to

3M Company

St. Paul, Minnesota

Project Number H90-500

Report Number BP-79-8-123

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

A marine toxicity test was conducted at Bionomics Marine Research Laboratory (BMRL), Pensacola, Florida, to determine the effect of Sample 7902 on embryos-larvae of eastern oysters (Crassostrea virginica). The criterion for effect was reduction of the number of normal larvae (those which developed to the fully-shelled, straight-hinged veliger stage within 48 hours) in test concentrations as compared to the number of normal control larvae. Results of the test are expressed as a 48-hour EC50 (the concentration of Sample 7902 estimated to be effective in preventing normal development of 50% of the exposed embryos-larvae).

Data from the test are maintained 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 (PALAEMONETES 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

Oyster embryos were obtained by induced spawning of sexually mature adult oysters which had been collected from an estuary adjacent to Biloxi, Mississippi, on 20 July 1979 and maintained in flowing, unfiltered seawater at BMRL until testing began.

Test water

Water used for spawning and testing was natural seawater which was pumped from Big Lagoon, a Gulf of Mexico estuary adjacent to BMRL. The pump intake was about 85 meters (m) offshore at a depth of approximately 3 m..

Seawater was pumped by a #316 stainless steel pump through hard polyvinylchloride (PVC) pipes, through sandfilled fiberglass filters, and through 10-micrometers (μm) 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- μm 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 48-hour oyster embryo-larvae test were based on Standard Practice for Conducting Static Basic Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs (ASTM, Draft No. 5). Individual, sexually mature female oysters were induced to spawn by placing them in glass chambers containing 1 l of filtered (5- μm), 26 degrees Celsius ($^{\circ}\text{C}$) seawater and increasing the water temperature to 32 $^{\circ}\text{C}$ in the presence of viable sperm excised from the gonad of a sexually mature male oyster. Fertilization occurred upon release of the eggs into the spawning chambers and was confirmed microscopically. Fertilization success was estimated to be $\geq 90\%$. Density of the embryos was determined by a Sedgwick-Rafter count of a 1:10 dilution (1 ml embryo suspension:9 ml seawater) from the spawning chamber.

All concentrations and the control were triplicated. Test containers were 1-l glass beakers, each of which contained 900 ml of filtered (5- μ m), natural seawater. A primary stock solution was prepared by adding a weighed amount of Sample 7902 to a known volume of filtered seawater and the appropriate volumes were added to each test container to obtain the desired test concentrations.

Each test container was inoculated with an estimated 23,400 embryos within 1 hour after fertilization and then maintained at $20 \pm 1^\circ\text{C}$ in a temperature-controlled water bath.

After 48 hours of exposure, the larvae from each container were collected in a 37- μ m mesh size sieve, rinsed into a plastic bottle with 24 ml of filtered seawater, and preserved with 1 ml of neutralized formalin. The number of normally developed 48-hour larvae was determined by a Sedgwick-Rafter count from each triplicate test and control container.

Percentage reduction of normal embryos was determined as follows:

$$\text{Percentage reduction} = \frac{\begin{array}{l} \text{Number of normal 48-hour control larvae} \\ \text{minus the number of normal 48-hour larvae} \\ \text{in each test concentration} \end{array}}{\text{Number of normal 48-hour control larvae}} \times 100$$

The test was conducted 1-3 August 1979.

Statistical analyses

Each test concentration was converted to a logarithm and the corresponding percentage reduction of normal larvae was converted to a probit (Finney, 1971). The 48-hour EC50 and 95% confidence limits were then calculated by linear regression.

RESULTS AND DISCUSSION

The calculated 48-hour EC50 for embryos-larvae of eastern oysters exposed to Sample 7902 in static, unaerated seawater was 3.5 ppm with 95% confidence limits of 0.5-22 ppm. Reduction of embryos-larvae which developed normally to the straight-hinged veliger stage after 48 hours was from 12% in 0.6 ppm to 74% in 10 ppm (Tables 1 and 2).

Measured concentrations of dissolved oxygen remained $\geq 67\%$ of saturation and the pH was from 8.0-8.1 after 48 hours of exposure.

REFERENCES

American Society for Testing and Materials Committee E-35 on
Pesticides. August 1978. Standard Practice for Conducting
Static Basic Acute Toxicity Tests with Larvae of Four
Species of Bivalve Molluscs. Draft No. 5.

Finney, D.J. 1971. Probit Analysis. Cambridge University
Press, London. 333 p.

TABLE 1. Toxicity of 3M Company's sample 7902 to embryos-larvae of eastern oysters (Crassostrea virginica) exposed for 48 hours in static, unaerated seawater. The criterion for effect was the reduction of the number of normal larvae in test concentrations as compared to the number of normal control larvae. Salinity was 22 ‰ and temperature, 20±1°C.

<u>Nominal concentration (mg/l; ppm)</u>	<u>Percentage reduction of normal 48-hour larvae^a</u>
Control	---
0.6	12
1.0	31
3.2	44
5.6	61
10	74

$$^a \text{Percentage reduction} = \frac{\text{Number of normal 48-hour control larvae} - \text{Number of normal 48-hour larvae in each test concentration}}{\text{Number of normal 48-hour control larvae}} \times 100$$

TABLE 2. Calculated number of normal eastern oysters (*Crassostrea virginica*) larvae following 48 hours of exposure to 3M Company's Sample 7902 in static, unaerated seawater. The numbers were based on Sedgwick-Rafter counts. Initial inoculum was 23,400. Salinity was 22 ‰ and temperature, 20±1°C.

Nominal concentration (mg/l; ppm)	Number of normal larvae				
	Rep A	Rep B	Rep C	Mean	SD ^a
Control	19,380	22,515	18,382	20,092	2,157
0.6	15,390	18,098	19,238	17,575	1,976
1.0	13,822	11,970	15,818	13,870	1,924
3.2	12,112	8,978	12,825	11,305	2,046
5.6	8,408	6,484	8,764	7,885	1,226
10	5,771	3,919	6,270	5,320	1,239

^aStandard deviation.

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 ^c
Sulfides	<1.0	<1.0
Pesticides	None detected ^a	None detected
Polychlorinated Biphenyls	None detected ^b	None detected

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

^aPesticides: 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.

^bPolychlorinated Biphenyls: Aroclor® 1016, 1232, 1248, 1260, 1221, 1242, and 1254 all <0.05 µg/l.

^cPetroleum hydrocarbon sample collected 10 July 1979.

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