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 (F5919-1). 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 Molluscs (ASTM 1980).
Type: Acute static
GLP: No
Year completed: 1980
Species: *Crassostrea virginica*
Supplier: Induced spawning in laboratory-maintained mature adult oysters
Analytical monitoring: Temperature, pH, salinity, and DO.
Exposure period: 48-hours
Test organism age: Embryos, within 1 hour after fertilization.
Statistical method: Test concentrations converted to logarithm and corresponding percentage reduction of normal larvae was converted to a probit. EC_{50} 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: 20 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. 25,600 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): 21 ± 1 °C (temperature controlled environmental chamber).

Salinity range (0-48 hours): 20 ppt

pH range (0-48 hours): 7.7 –7.8

Dissolved oxygen range (0-48 hours): >74% saturation

RESULTS

Nominal concentrations: Bk control, 6, 10, 32, 56, and 100 mg/L.

Element values: 48-hour EC_{50} = 47 (10 - 234) 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 47 mg/L with a 95% Confidence Interval of 10 to 234 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, the 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, of Pensacola, FL at the request of the 3M Company, Lab Request number 4971, Sample 5729, 1980.

OTHER

Last changed: 6/27/00

Acute toxicity of Sample #5729 to embryos-larvae of eastern oysters (Crassostrea virginica)

FC-203 F 5919-1
Cc 805-28

Toxicity Test Report

Submitted to

3M Company

St. Paul, MN 55133

Project Number L55

Report Number BP-80-7-117

EG&G Bionomics
Marine Research Laboratory
10307 Gulf Beach Highway
Pensacola, Florida 32507
July 1980

A marine toxicity test was conducted at EG&G Bionomics Marine Research Laboratory (BMRL), Pensacola, Florida, to determine the effect of Sample #5729 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 test material 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, received at BMRL on 12 June 1980, was contained in a 0.9-liter (ℓ) clear glass bottle labeled "3M Company, Sample #5729." The sample was an orange liquid. Test concentrations are reported here as milligrams (mg) of whole 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 were 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 80 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 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 the spawning or 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, 1980). Individual, sexually mature female oysters were induced to spawn by placing them in glass chambers containing 1 ℓ of filtered seawater at 25 degrees Celsius (°C) and increasing the water temperature to approximately 30°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 ≥90%. Density of the embryos was determined by a Sedgwick-Rafter count of a 1:10 dilution (1 mℓ embryo suspension:9 mℓ of 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, natural seawater. A primary stock was prepared by adding a weighed amount of test material 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 25,600 embryos within 1 hour after fertilization and then maintained at 21±1°C in a temperature-controlled environmental chamber.

After 48 hours of exposure, the larvae from each container were collected in a 37-µm 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 larvae was determined as follows:

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

The test was conducted 1-3 July 1980.

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 #5729 in static, unaerated seawater was 47 ppm with 95% confidence limits of 10-234 ppm. No significant reduction of embryos-larvae which developed normally to the straight hinged veliger stage after 48 hours occurred in

6 or 10 ppm, but a significant reduction of normal larvae did occur in concentrations ≥32 ppm (Table 1).

Measured concentrations of dissolved oxygen remained ≥74% saturation and the pH was from 7.7-7.8 after 48 hours of exposure (Appendix B).

REFERENCES

ASTM Standard E 724-80, Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

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

TABLE 1. Toxicity of Sample #5729 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. The initial inoculum was 25,600 embryos. Salinity was 20 °/oo and temperature was 21°C.

Nominal concentration (mg/ℓ;ppm)	Number of normal larvae		Reduction of normal 48-hour larvae (%)
	Mean	SD[a]	
Control	21,612	2,478	---
6	22,657	2,151	---
10	22,373	2,010	---
32	16,530	2,707	24
56	11,258	1,054	48
100	380	142	98

[a]Standard deviation.

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APPENDIX A

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

Chemical Constituent	Concentration (mg/ℓ;ppm) 1979 Range[a]	1 April 1980
Arsenic	<0.001-0.006	0.013
Cadmium	<0.01-0.002	<0.01
Chromium	<0.01-0.008	<0.01
Copper	<0.01-0.02	<0.001
Mercury	<0.0005-0.0007	0.002
Nickel	<0.01-0.02	0.02
Zinc	<0.02-0.05	0.02
Lead	<0.02	0.05
Total Phosphate as P	<0.02	0.02
Ammonia Nitrogen as N	0.14-0.42	<0.01
Nitrate Nitrogen as N	<0.01	0.10
Nitrite Nitrogen as N	<0.01	<0.01
Total Petroleum Hydrocarbons	<5.0	<5.0
Sulfides	<1.0	<0.01
Pesticides	None detected[b]	None detected
Polychlorinated Biphenyls	None detected[c]	None detected

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

[a]Range of concentrations are based on two sampling periods.
[b]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;ppb.
[c]Polychlorinated Biphenyls: Aroclor® 1016, 1232, 1248, 1260, 1221, 1242, and 1254 all <0.05 µg/ℓ;ppb.

NO: BP-QAF-OE-001
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Revision: #1
Page:

APPENDIX B

QUALITY ASSURANCE FORM
OYSTER-EMBRYO TOXICITY TEST

BJECT: 48-HOUR STATIC DATA SHEET

CLIENT: 3M Company PROJECT NO: 455-

TEST MATERIAL: Sample #5729

TIME INOCULUM ADDED: 1045

TIME TEST MATERIAL ADDED: 1100

Temperature
24h 21°C
48h 21°C

0 HOUR MEASUREMENTS IN CONTROL (REPLICATE A)

SALINITY: 20

(See Table Below for Data by)

TEMPERATURE: 24

TEST HOUR	0 HOUR				48 HOUR			
DATE/TIME	~~1 July~~ ~~3 June~~ 3 July 80 / 1200				3 July 80 / 1100			
DATA BY								
MEASUREMENT	DISSOLVED OXYGEN (mg/l)			pH	DISSOLVED OXYGEN (mg/l)			pH
CONCENTRATION	REP A	REP B	REP C	REP A	REP A	REP B	REP C	REP A
control	7.1	7.0	7.0	~~6.9~~ 7.9	6.6	6.4	6.5	7.8
6 ppm	7.0	6.9	7.1	7.9	6.5	6.4	6.7	7.8
10 ppm	7.0	7.0	7.0	7.9	6.4	6.3	6.6	7.8
32 ppm	7.2	7.1	7.0	7.9	6.6	6.3	6.5	7.8
56 ppm	7.0	7.2	7.0	7.9	6.2	6.0	6.1	7.8
100 ppm	7.0	7.2	7.1	7.9	5.9	5.7	6.1	7.7

TIAL INOCULUM: Number of normal embryos found in 1 ml / number of fields x Sedgwick Rafter Factor x Dilution Factor = Total number of normally developed embryos per ml

CALCULATION: $\frac{180}{2} \times 11.4 \times 10 = 10,260$

2.5 ml added to each test Container

= ~ 25,600 / Container