

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-206 or L-3243. (1-Octanesulfonic acid) (CAS # 2795-39-3).

**Remarks:** The 3M production lot number was not noted. The test sample is FC-206, identified by the laboratory as "Sample C". Current information indicates it is a mixture of 0.67% PFOS, 17.5% diethylene glycol butyl ether, 78.91% water, 1.33% Sultone foamer, 1% sodium octyl sulfate, 0.04% sodium lauryl sulfate, 0.5% polyoxyethylene monoctylphenyl ether, 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:** Measurement of Water Quality with the Pacific Oyster Bioassay, C.E. Woelke, 1967, ASTM Spec. Tech. Publ. 416

**Type:** Acute static

**GLP:** No

**Year completed:** 1974

**Species:** *Crassostrea virginica*

**Supplier:** Spawning in the laboratory of field-collected adults from Milford, CT harbor.

**Analytical monitoring:** Salinity.

**Exposure period:** 48-hours

**Test organism age:** Fertilized eggs

**Statistical method:** TL<sub>50</sub> (median tolerance limit) values calculated using a linear regression equation.

**Test conditions:**

**Dilution water:** Filtered seawater pumped from Milford, CT harbor and treated with ultraviolet light before use.

**Dilution water chemistry:**

**Salinity:** 26 – 28 ppt

**Lighting:** Not given.

**Stock and test solution preparation:** Direct addition based on weight/volume.

**Exposure vessels:** 500 mL beakers containing 300 mL of test solution.

**Number of replicates:** 3

**Number of organisms:** Approx 150,000 embryos/L

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

**Exhibit  
1720**

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

3M\_MN00437343

**Element basis:** Number of normally developed larvae (straight-hinged veliger stage)

**Water chemistry during the study:** Not given.

## RESULTS

**Nominal concentrations:** Blank control, 10, 24, 49, 75, 100, 240, 490, 750, and 1000 mg/L.

**Element values:** 48-hour  $TL_{50} = >100 <240$  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 FC-206 48-hour  $TL_{50}$  was determined to be  $>100$  and  $<240$  mg/L.

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

## DATA QUALITY

**Reliability:** Klimisch ranking 3. Testing lacks description and complete record of methodology used. 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 Bionomics, EG&G, Inc., Wareham, MA at the request of the 3M Company, St. Paul, MN, 1974.

## OTHER

**Last changed:** 6/28/00

BIOASSAY REPORT  
SUBMITTED TO  
3 M COMPANY  
ST. PAUL, MINNESOTA

ACUTE TOXICITY OF SAMPLE C TO ATLANTIC OYSTER  
(Crassostrea virginica).

Bionomics  
E G & G, Inc.  
Environmental Consultants  
790 Main Street  
Wareham, Massachusetts  
July, 1974

3M\_MN00437345

1720.0003

This investigation was performed at the aquatic toxicology laboratory of Bionomics, E G & G, Inc., in Wareham, Massachusetts through the cooperation of the U. S. Bureau of Commercial Fisheries Shellfish Research Laboratory in Milford, Connecticut. The susceptibility of the oysters to Sample C, a light brown liquid, which was tested as 100% active, was measured in terms of the 48-hour tolerance limit (TL<sub>50</sub>), the concentration of the chemical in water which causes 50 percent response under the test conditions during a 48-hour interval. The response observed in these studies was normal embryonic development. For observations on development of embryos, fertilized eggs were introduced into the test container soon after release and fertilization, usually when the eggs were in the two-cell stage of development. Quantitative samples were taken 48 hours later to determine the percentage of the fertilized eggs that had developed to a normal morphological stage (i.e. straight-hinged veliger larvae). The prediction of a TL<sub>50</sub> value, and its 95% confidence interval, was based on the conversion of the concentrations tested and the corresponding observed percent normal development to logs and probits respectively, and the subsequent mathematical calculation of a linear regression equation.

The test procedures used in these evaluations are those described by Woelke<sup>1</sup> for the measurement of water quality with the Pacific oyster embryo bioassay.

Sexually mature Atlantic oysters were collected from Milford harbor and held at the BCF Shellfish Laboratory in filtered sea water for 7 days at a temperature of 22°C. Several hours prior to starting a bioassay about ten (10) mature oysters are placed in a pyrex tray filled with ultraviolet-light-treated water. About 30 minutes before spawning is desired, the water temperature is raised to 30°C and a sperm suspension from a sexually mature, sacrificed male oyster is added to the water. The combination of increased temperature and sperm induces one or more of the female oysters to spawn. Eggs from a single female are selected for use in the bioassay and the number of eggs/unit volume are determined by sampling the sperm-egg suspension. The bioassay was conducted using 500 ml beakers containing 300 ml of filtered sea water (treated with ultraviolet light) having a salinity of 26-28 ‰; each unit was inoculated with a sufficient amount of egg suspension to give approximately 150,000 fertilized eggs per liter.

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<sup>1</sup>Woelke, C. E. - "Measurement of Water Quality with the Pacific Oyster Bioassay, Water Quality Criteria, ASTM Spec. Tech. Publ. 416, Am. Soc. Testing Mats, 1967, P. 112-120.

Sample C was tested at nominal concentrations ranging from 1000.0 to 10.0 mg/l, with triplicate cultures inoculated and incubated at each concentration. The cultures were incubated for a period of 48 hours at 25°C. At the end of this period cultures were poured through a 37  $\mu$  sieve to obtain samples containing about 200 larvae and preserved in 5% formalin for microscopic examination. The number of normal and abnormal larvae were counted in each sample with the values from the triplicates being averaged.

The predicted 48-hour TL<sub>50</sub> (i.e., the concentration which inhibited normal development of 50% of the developing oyster larvae) was >100.0 <240.0 mg/l of Sample C.

Table 1 presents the concentrations of Sample C tested and corresponding observed percent normal development after 48 hours of exposure. This data should be evaluated with the knowledge that errors involved in the above techniques for determining numbers of larvae developing from fertilized eggs to straight-hinge larvae have been found to be about  $\pm$  10 percent.<sup>2</sup>

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<sup>2</sup>Davis, H. C. and H. Hidu. 1969. Effects of pesticides on embryonic development of clams and oysters and on survival and growth of the larvae.

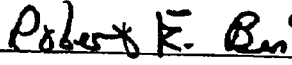
U. S. Dept. Interior, Bureau of Commercial Fisheries, Fishery Bulletin 67 (2): 393-404.

SUBMITTED BY:

Bionomics  
E G & G, Inc.  
Environmental Consultants  
790 Main Street  
Wareham, Massachusetts  
July, 1974

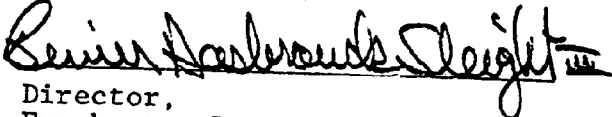
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Table 1 -- Concentrations tested and corresponding percent observed normal development for Atlantic oyster larvae (Crassostrea virginica) exposed to Sample C for 48 hours.

Concentration (mg active ingredient/liter)	Observed percent normal development <sup>a</sup> 48 hours
1000.0	0
750.0	0
490.0	0
240.0	0
100.0	> 90
75.0	> 90
49.0	> 90
24.0	> 90
10.0	> 90
control	> 90

<sup>a</sup>Each percent is an average of triplicate values from each concentration.