

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (FRESHWATER
MUSSEL)TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: The study was conducted using a protocol based on procedures outlined in U.S. Environmental Protection Agency Series 850 – Ecological Effects Guidelines, OPPTS Number 850.1075 ; OECD 203: *Fish, Acute Toxicity Test*; and ASTM Standard E729-88a, *Standard Guide for Conducting Toxicity Tests with Fishes, Macroinvertebrates and Amphibians*.

Test type: Semi-static Renewal

GLP: Yes

Year Completed: Study completed 1999. Report completed 2000

Species: *Unio complanatus*

Analytical monitoring: Test substance concentrations measured by LCMS at 0, 48, 96-hours

Statistical methods: LC₅₀ values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test organism source: Obtained from Carolina Biological Supply Company, Burlington, North Carolina. Carolina collected from the wild.

Test organism age at study initiation: Unknown

Test conditions

Dilution water: 0.45 µm filtered well water

Dilution water chemistry (during the 4-week period immediately preceding the test):

hardness:	126 (120-132) mg/L as CaCO ₃
alkalinity:	174 (170-178) mg/L as CaCO ₃
pH:	8.3 (8.1-8.5)
TOC:	< 1.0 mg/L
Conductivity:	321 (310-330) µmhos/cm
Ca/Mg ratio:	35/13.5

Na/K ratio: 21.3/6.62

Lighting: Colortone® 50 fluorescent lights, intensity approximately 369 lux. Photoperiod of 16-hours light, 8-hours dark with a 30-minute transition period.

Stock and test solutions preparation: A primary stock solution was prepared in dilution water at 91 mg/L. It was mixed for approximately 24 hours prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless.

Exposure vessels: 25 liter polyethylene aquaria containing approximately 20 L of test solution. The approximate depth of test solution was 23.2 cm.

Number of replicates: two

Number of test organisms per replicate: ten

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

5.8 – 8.5 mg/L (control exposure)

5.0 – 8.6 mg/L (79 mg/L exposure)

pH range (0 – 96 hours)

8.0 – 8.4 (control exposure)

7.9 – 8.5 (79 mg/L exposure)

Test temperature range (0 – 96 hours)

21.4– 21.8°C (control exposure)

21.8 – 23.7°C (79 mg/L exposure)

Element Basis: Mortality. Mussels with open shells and not responding to gentle prodding were considered dead. The number of individuals exhibiting clinical signs of toxicity or abnormal behavior also were evaluated.

Method of calculating mean measured concentrations:
arithmetic mean

RESULTS

Nominal concentrations: <LOQ, 5.7, 11, 23, 46, 91 mg/L

Measured concentrations: <LOQ, 5.3, 12, 20, 41, 79 mg/L

Element value: 96-hour LC₅₀ = 59 mg/L (51-68 mg/L)

Statistical Evaluation: The LC₅₀ values and 95% confidence intervals were calculated when possible by probit analysis, the moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

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Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7%. Samples collected at test initiation had measured values from 73.7% to 96.0% of nominal. Measured values for samples taken at 48-hours ranged from 81.2 to 98.9% of nominal. Measured values for samples taken at 96-hours ranged from 88.5 to 130% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Duplicated Values at 0, 48, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
5.7	5.47, 4.93, 5.18, 5.70, 5.24, 5.26	5.3	93
11	11.4, 10.1, 11.2, 10.5, 10.9, 15.4	12	109
23	19.0, 16.8, 18.7, 18.7, 22.9, 22.4	20	87
46	37.2, 40.6, 37.1, 39.5, 48.2, 40.5	41	89
91	69.0, 74.7, 81.3, 77.6, 88.2, 85.7	79	87

Biological observations after 96-hours: Mussels in the negative control, the 5.3, 12 and the 20 mg/L treatments appeared healthy and normal throughout the test with no mortality or overt clinical signs of toxicity. Five percent mortality was observed at 96-hours in the 41 mg/L treatment and 90% mortality was observed in the 79 mg/L treatment. No abnormal behavior was noted in these concentrations.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	24 Hours	48 Hours	72 Hours	96 Hours
Neg. Control	0	0	0	0
5.3	0	0	0	0
12	0	0	0	0
20	0	0	0	0
41	0	0	0	5
79	30	40	50	90

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Control response: Satisfactory

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC50 for the Freshwater Mussel, *Unio complamatus* was determined to be 59 mg/L with a 95% confidence interval of 51-68 mg/L. The 96-hour no mortality concentration was 20 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 1.

REFERENCES

This study was conducted at Wildlife International, Ltd. Easton, MD at the request of the 3M Company.

OTHER

Last changed: 5/3/00

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