

AR226-3772 305965

ACUTE TOXICITY TO THE FRESHWATER AMPHIPOD (*Hyalella azteca*)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 86.9% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques. The impurities were identified as: metals (1.45%); lower chain-length (C4-C7) perfluorinated sulfonates (8.41%); inorganic fluoride (0.59%); NMR impurities (1.905%); perfluorooctanoate anion (0.33%); and the total of tri-, penta-, hepta-, and nonapentafluoropropanoic acid (0.38%).

METHOD

Method: ASTM E729-96

Type: Static renewal

GLP: Yes

Date completed: Study completed 2007

Species: *Hyalella azteca*

Supplier: Environmental Consulting and Testing (ECT), Superior, WI

Analytical monitoring: PFOS measured at initiation, prior to and after renewal at 48 hours and at test termination (96-hours)

Exposure period: 96-hours

Statistical methods: Binomial probability method used to calculate the 24-hour LC50 values and probit analysis to calculate the 48, 72, and 96-hour LC50 values. The NOEC determined by visual interpretation of mortality and observation data.

Test organism age: 12-days old

Average wet weight at test initiation: Not measured

Loading: 1 amphipod per chamber

Pretreatment: None

Feeding: Fed YCT plus Tetramin® flake food at Days 0 and 2

Acclimation: Prior to the test, the test organisms were acclimated to test conditions for 6 days by holding them in a container with gauze and water from the same source and at approximately the same temperature that would be used in the test. Temperatures ranged from 21.8 to 23.4 °C. pH ranged from 8.1 to 8.5. Dissolved oxygen ranged from 6.4 to 8.0 mg/L. No signs of disease or stress were observed during the holding period. No information was provided on the characteristics of the "gauze" or its suitability for use in this application.

Test conditions:

CONTAINS NO CB

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Dilution water: Filtered, aerated, UV-sterilized well water

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

Specific Conductance:	290 (285-295) $\mu\text{mhos/cm}$
Hardness:	136 (132-140) mg/L as CaCO_3
Alkalinity:	182 (180-184) mg/L as CaCO_3
pH:	8.1 (8.1-8.1)

Stock and test solution preparation: A primary stock was prepared at a nominal concentration of 200 mg active ingredient per liter using a calculated amount of PFOS, and stirred overnight. For Day 0 solutions, the stock solution was sonicated for approximately 5 to 10 minutes the following day. The Day 2 stock solution was sonicated for only 5 minutes the following day. After mixing, primary stock solution was proportionally diluted with dilution water to prepare each test concentration. All test solutions were adjusted to 100% active ingredient during preparation, based on test substance purity. All test solutions appeared clear and colorless at test initiation and termination.

Solvent: none

Concentrations dosing rate: Static renewal after 48-hours

Stability of the test chemical solutions: under test conditions: Stable as demonstrated by analytical monitoring.

Exposure vessels: 30-mL Nalgene® plastic beakers containing approximately 20 mL test solution. Water depth was 1.9 cm. A piece of Nitex® screen was placed in each vessel as a substrate for the amphipods.

Number of replicates: 10 (test substance), and 20 (negative controls)

Number of amphipods per replicate: one

Number of concentrations: Five plus a negative control

Photo period: 16 hours light and 8 hours dark with a 30-minute transition in simulated natural sunlight from fluorescent bulbs.

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

8.2 – 8.5 mg/L (negative control exposure)

8.1 – 8.5 mg/L (all test substance exposures)

pH range (0 – 96 hours)

8.2 – 8.6 (negative control exposure)

8.1 – 8.6 (all test substance exposures)

Test temperature range (0 – 96 hours)

22.4 – 23.7°C (negative control exposure)

22.2 – 23.8°C (all test substance exposures)

RESULTS

Nominal concentrations: Bk control, 13, 25, 50, 100, and 200 mg/L

Measured concentrations: <LOQ, 13, 25, 51, 102, and 199 mg/L

Element value: 24-hour LC₅₀ = >161 mg/L (C.I. > 102 mg/L)

48-hour LC₅₀ = 93 (70 – 125) mg/L

72-hour LC₅₀ = 29 (20 – 40) mg/L

96-hour LC₅₀ = 15 (4.5 – 24) mg/L

96-hour NOEC = < 13 mg/L

All element values based on mean measured concentrations

Statistical Evaluation of Mortality: Binomial probability with nonlinear interpolation or probit analysis

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. The method consisted of dilution of the samples 1:1, v/v with acetonitrile, followed by secondary dilution using acetonitrile: HPLC-grade bottled water (50:50, v/v) and analysis by direct injection high performance liquid chromatography with mass spectrometric (LC/MS/MS) detection. The LOQ (limit of quantitation) was 2.00 mg/L in this study. The measured concentrations of matrix fortifications analyzed concurrently during sample analysis were 98.4-114% of nominal. Samples collected in exposure vessels at test initiation had measured values from 100 to 105% of nominal. New samples collected at 48 hours had measured concentrations from 95 to 100% of nominal. Old samples collected at 48 hours had measured concentrations ranging from 96.4 to 103% of nominal. Measured concentrations of PFOS in samples collected at test termination were 100 to 102% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values of New and Old Test Solutions, mg/L ⁽¹⁾	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ ⁽²⁾	<LOQ	-
13	13.4, 13.5, 12.3, 13.0	13	100
25	25.1, 24.9, 24.3, 25.4	25	100
50	52.6, 50.2, 49.4, 50.8	51	102
100	102, 103, 100, 101	102	102
200	204, na ⁽³⁾ , 193, na ⁽³⁾	199	99.5

(1) Samples are listed in this order: Day 0 (new), 48-hours (old), 48-hours (new), 96-hours (old)

(2) LOQ = Limit of Quantitation = 2.00 mg/L

(3) Not analyzed due to 100% mortality. Value not used in statistical calculations

Biological observations after 96-hours: All but two amphipods in the negative control appeared healthy and normal throughout the exposure period with no mortalities or overt signs of toxicity observed. Two control amphipods exhibited lethargy that resulted in mortality. At test termination, mortality in the 13, 25, and 51 mg/L treatment groups was 40, 80, and 80%, respectively. Signs of toxicity observed in the affected

amphipods from all treatment groups included lethargy. All surviving amphipods at test termination appeared normal.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	5	10
13	0	0	10	40
25	0	0	40	80
51	0	10	80	80
102	0	50	100	100
199	10	80	100	100

Lowest concentration causing 100% mortality: 102 mg/L

Mortality of controls: 10%

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC₅₀ for *Hyalella azteca* was determined to be 15 mg/L. The 96-hour no mortality concentration was < 13 mg/L and no observed effects concentration was < 13 mg/L.

Submitter: 3M Environmental Laboratory, Maplewood, Minnesota, 55144

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

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

OTHER

Last changed: 07/10/07