

ACUTE TOXICITY TO FISH (Sheepshead Minnow)

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-heptadecafluoro-, 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.

METHOD

Method: OPPTS 850.1075

Type: Static renewal

GLP: Yes

Date completed: Study completed 2001, report completed 2002

Species: *Cyprinodon variegatus*

Supplier: Aquatic BioSystems, Inc., Fort Collins, CO

Analytical monitoring: PFOS measured at initiation, prior to and after renewal at 24, 48, and 72 hours and at test termination (96-hours)

Exposure period: 96-hours

Statistical methods: The use of a single test concentration (at water solubility) precluded the statistical calculation of LC₅₀ values.

Test fish age: Juveniles

Average Total Length and weight: 3.0 (2.4–3.5) cm, 0.44 (0.21-0.66) g

Loading: 0.29 g fish/L

Pretreatment: None

Test conditions:

Dilution water: Natural seawater, filtered and diluted to a salinity of approximately 20 parts per thousand with well water

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

Salinity: 20 (20 – 20) parts per thousand

pH: 8.2 (8.1 – 8.3)

Stock and test solution preparation: Primary stock prepared in methanol at 40 mg/L, sonicated for approximately 20 minutes and inverted to mix prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the one test concentration. Each solution was stirred with a stainless steel

whisk for approximately one minute. All test solutions appeared clear and colorless.

Solvent: Methanol

Solvent concentration (treatment and solvent control groups):
0.5 mL/L

Concentrations dosing rate: Daily static renewal

Stability of the test chemical solutions: Extremely stable

Exposure vessels: 25L polyethylene aquaria containing approximately 15L of test solution; water depth approximately 17.1 cm.

Number of replicates: three (biotic), two (abiotic)

Number of fish per replicate: ten

Number of concentrations: One plus a negative and a solvent control, and an abiotic solution.

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

2.8 – 7.4 mg/L (negative control exposure)

1.7 – 7.6 mg/L (solvent control exposure)

1.6 – 7.6 mg/L (15 mg/L exposure)

pH range (0 – 96 hours)

7.9 – 8.3 (negative control exposure)

7.9 – 8.3 (solvent control exposure)

7.9 – 8.3 (15 mg/L exposure)

Test temperature range (0 – 96 hours)

21.9 – 22.6°C (negative control exposure)

22.1 – 22.9°C (solvent control exposure)

22.2 – 23.1°C (15 mg/L exposure)

- **Method of calculating mean measured concentrations:** arithmetic mean

RESULTS

Nominal concentrations: Bk control, solvent control, 20 mg/L (biotic), 20 mg/L (abiotic)

Measured concentrations: <LOQ, <LOQ, 15, 13 mg/L

Element value: 24-hour LC₅₀ = >15 mg/L (C.I. not calculable)

48-hour LC₅₀ = >15 mg/L (C.I. not calculable)

72-hour LC₅₀ = >15 mg/L (C.I. not calculable)

96-hour LC₅₀ = >15 mg/L (C.I. not calculable)

All element values based on mean measured concentrations

Statistical Evaluation of Mortality:

Element values and confidence interval could not be calculated due to lack of mortality.

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 5.00 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 99.2. Samples collected in exposure vessels at test initiation had measured values from 78.6 to 82.0% of nominal. New samples collected at Hours 24, 48 and 72 had measured concentrations from 74.9 to 81.2 and 86.9 to 92.9, and 82.9 to 87.1% of nominal, respectively. Old samples collected at Hours 24, 48, and 72 had measured concentrations ranging from 66.6 to 76.2, 56.4 to 66.9, and 75.8 to 89.9% of nominal, respectively. Mean measured concentrations of PFOS in samples collected at test termination were 55.6 to 68.4% of nominal. The measured concentrations of PFOS from the abiotic treatment group were slightly lower than those from the exposure treatment group. This may have been due to increased deposition of test substance at the limit of solubility, which could have resulted from the absence of the natural mixing action that was provided by the movement of the fish in the exposure treatment group.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Replicate Values of old and new Test Solutions Respectively, mg/L ⁽¹⁾	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
Solvent Control	All < LOQ	<LOQ	-
20 ⁽²⁾	16.4, 15.7, 16.0; 15.2, 13.3, 15.2; 16.2, 15.7, 15.0; 13.4, 11.3, 12.7; 17.4, 18.2, 18.6; 15.2, 18.0, 16.2; 17.0, 16.6, 17.4; 13.7, 11.1, 13.4	15	75
20 ⁽³⁾ (abiotic)	10.9, 9.22; 16.1, 16.6; 9.62, 9.69; 16.6, 17.5; 9.01, 9.25; 17.7, 15.9; 9.84, 9.22	13	65

(1) Replicate samples are listed in this order: Day 0 (new), 24-hours (old), 24-hours (new), 48-hours (old), 48-hours (new), 72-hours (old), 72-hours (new), 96-hours (old)

(2) Triplicate samples

(3) Day 0 not measured, Duplicate samples

Biological observations after 96-hours: Fish in the negative control and solvent control appeared healthy and normal throughout the exposure period. No mortalities appeared in the 15 mg/L treatment group during the study. However, upon transfer to the new test solution at approximately

48 and 72 hours, some fish were observed swimming erratically and turning a dark color. The fish appeared normal within approximately two hours after transfer, although one fish still appeared to be discolored at test termination.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	0	0
Solvent control	0	0	0	0
15	0	0	0	0

Lowest concentration causing 100% mortality: none – mortality limit apparently greater than solubility limit

Mortality of controls: None

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC₅₀ for sheepshead minnow was determined to be >15 mg/L, the limit of solubility in this study. The 96-hour no mortality concentration was 15 mg/L and no observed effects concentration was <15 mg/L (1 fish out of 30 was discolored at 96-hours).

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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: 1/24/02

