

AR 226-3770

ACUTE TOXICITY TO FROG TADPOLES (Spring Peeper)

TEST SUBSTANCE

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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-NMR, 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: *Pseudacris crucifer*

Supplier: Carolina Biological Supply Company, Burlington, North Carolina

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 all LC50 values. The NOEC determined by visual interpretation of mortality and observation data.

Test organism age: Juveniles

Average wet weight at test initiation: at Day 0 = 0.030 (0.010-0.044) g; 0.040 (0.023-0.063) g at test termination

Loading: 0.12 g tadpole/L at Day 0, 0.16 g tadpole/L at test termination

Pretreatment: None

Acclimation: Prior to the test, tadpoles were held for 5 days in water from the same sources and at approximately the same temperature as used during the test. Temperatures ranged from 20.4 to 21.4°C. pH ranged from 7.8 to 8.2. Dissolved Oxygen ranged from 7.0 to 9.0 mg/L. Tadpoles were fed a commercially-prepared food daily.

Test conditions:

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

CONTAINS NO CBI

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

Specific Conductance:	291 (290-295) $\mu\text{mhos/cm}$
Hardness:	136 (132-140) mg/L as CaCO_3
Alkalinity:	183 (182-184) mg/L as CaCO_3
pH:	8.1 (8.1-8.1)

Stock and test solution preparation: A primary stock solution was prepared at a nominal concentration of 50 mg active ingredient per liter using a calculated amount of PFOS, and stirred overnight using a top-down electric mixer. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare each test concentration. Each test solution was stirred with a whisk. 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, renewal, 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: Approximately 3L plastic vessel containing 2.5 L of test solution; water depth approximately 9.3 cm.

Number of replicates: two

Number of tadpoles per replicate: ten

Number of concentrations: Five plus a negative control

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

Feeding schedule: Tadpoles were fed at test initiation on day 0 and at test renewal on day 2 with 25 mg ground commercially supplied tadpole food.

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

7.6 – 8.6 mg/L (negative control exposure)

7.2 – 8.7 mg/L (all test substance exposures)

pH range (0 – 96 hours)

8.3 – 8.5 (negative control exposure)

8.3 – 8.6 (all test substance exposures)

Test temperature range (0 – 96 hours)

21.5 – 22.6°C (negative control exposure)

21.2 – 22.7°C (all test substance exposures)

RESULTS

Nominal concentrations: Bk control, 3.2, 6.3, 13, 25, and 50 mg/L

Measured concentrations: <LOQ, 3.6, 7.0, 14, 27, 51 mg/L
Element value*: 24-hour LC₅₀ = >51 mg/L (C.I. not calculable)
 48-hour LC₅₀ = >27 mg/L (C.I. not calculable)
 72-hour LC₅₀ = 47 mg/L (C.I. not calculable)
 96-hour LC₅₀ = 38 mg/L (27 – 51 mg/L)
 96-hour NOEC = 3.6 mg/L

*All element values based on mean measured concentrations

Statistical Evaluation of Mortality: Binomial probability with nonlinear interpolation

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 1.00 mg/L in this study. The measured concentrations of matrix fortifications analyzed concurrently during sample analysis were 99.4-106% of nominal. Samples collected in exposure vessels at test initiation had measured values from 104 to 118% of nominal. New samples collected at 48 hours had measured concentrations from 105 to 131% of nominal. Old samples collected at 48 hours had measured concentrations ranging from 99.2 to 107% of nominal. Measured concentrations of PFOS in samples collected at test termination were 96.8 to 101% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values of New and Old Test Solutions Respectively, mg/L ⁽¹⁾	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ ⁽²⁾	<LOQ	-
3.2	3.77, 3.17, 4.18, 3.24	3.6	113
6.3	7.15, 6.62, 8.28, 6.10	7.0	111
13	13.8, 13.4, 15.0, 12.9	14	108
25	28.1, 26.8, 28.0, 25.2	27	108
50	51.9, 51.7, 52.3, 49.2	51	102

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

(2) LOQ = Limit of Quantitation = 1.00 mg/L as the active ingredient

Biological observations after 96-hours: All tadpoles in the negative control and the 3.6 mg/L appeared healthy and normal throughout the exposure period with no mortalities or overt signs of toxicity observed. Tadpoles that survived to test termination in the 7.0 mg/L treatment appeared normal. At test termination, mortality in the 7.0, 14, 27 and 51 mg/L treatment group was 15, 15, 15, and 80%, respectively. Signs of

toxicity observed in the affected tadpoles from the 14, 27 and 50 mg/L treatment groups included lethargy and curved tail and spine.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	0	0
3.6	0	0	0	0
7.0	0	0	0	15
14	5	5	10	15
27	0	0	0	15
51	10	45	60	80

Lowest concentration causing 100% mortality: > 51 mg/L

Mortality of controls: None

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC₅₀ for spring peeper tadpole was determined to be 38 mg/L. The 96-hour no mortality concentration was 3.6 mg/L and no observed effects concentration was 3.6 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

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Last changed: 06/26/07