AR226-3774 305965

ACUTE TOXICITY TO THE OLIGOCHAETE WORM (*Lumbriculus variegatus*)

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, ^{1}H-HMR, ^{19}F-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: *Lumbriculus variegatus*
Supplier: Wildlife International, Ltd. Cultures (originally from 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: Bionomial probability method used to calculate all LC50 values. The NOEC determined by visual interpretation of mortality and observation data.
Test organism age: Adult
Average wet weight at test initiation: Not provided
Loading: One worm per chamber
Pretreatment: None
Acclimation: Prior to the test, the test organisms were acclimated to test conditions for 2 weeks by holding them in a container with paper toweling and water from the same source and at approximately the same temperature that would be used in the test. Temperatures ranged from 23.3 to 24.4 °C. The pH ranged from 8.0 to 8.4. Dissolved oxygen ranged from 7.2 to 7.9 mg/L. Organisms were fed a mixture of yeast, cereal grass media, and trout chow during acclimation.

Test conditions:

CONTAINS NO CBI

93

Dilution water: Filtered, aerated, well water
Dilution water chemistry (during the 4-week period immediately preceding the test):

Specific Conductance:	291 (285 – 300) µmhos/cm
Hardness:	136 (132 – 140) mg/L as $CaCO_3$
Alkalinity:	182 (180 – 184) mg/L as $CaCO_3$
pH:	8.1 (7.9 - 8.1)

Stock and test solution preparation: A primary stock solution was prepared at a nominal concentration of 12 mg active ingredient per liter using a calculated amount of PFOS, and mixed by sonication for 15 minutes followed by inversion. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare each test concentration. Each test solution was mixed by inversion. 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: 30-mL Nalgene® polypropylene plastic beakers filled with approximately 20 mL of water; no substrate provided. Beakers covered with plastic wrap. Water depth was approximately 2.2 cm.
Number of replicates: Ten
Number of worms 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.
Feeding schedule: Test organisms were not fed during the test
Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):
8.0 – 8.3 mg/L (negative control exposure)
7.4 – 8.4 mg/L (all test substance exposures)

pH range (0 – 96 hours)
8.2 – 8.6 (negative control exposure)
8.2 – 8.6 (all test substance exposures)

Test temperature range (0 – 96 hours)
22.4 – 23.8°C (negative control exposure)
22.5 – 24.0°C (all test substance exposures)

RESULTS

Nominal concentrations: Bk control, 0.80, 1.5, 3.0, 6.0, and 12 mg/L

Measured concentrations: <LOQ, 0.71, 1.4, 2.8, 5.6, 11 mg/L
Element value: 24-hour LC_{50} = 11 (C.I. = >5.6) mg/L
48-hour LC_{50} = 7.9 (C.I. = >2.8) mg/L
72-hour LC_{50} = 6.5 (2.8 – 11) mg/L
96-hour LC_{50} = 5.6 (27 – 51) mg/L
96-hour NOEC = 2.8 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 0.200 mg/L in this study. The measured concentrations of matrix fortifications analyzed concurrently during sample analysis were 96.4-108% of nominal. Samples collected in exposure vessels at test initiation had measured values from 92 to 97% of nominal. New samples collected at 48 hours had measured concentrations from 85 to 94% of nominal. Old samples collected at 48 hours had measured concentrations ranging from 88 to 93% of nominal. Measured concentrations of PFOS in samples collected at test termination were 88 to 97% 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	-
0.80	0.749, 0.707, 0.683, 0.700	0.71	88.8
1.5	1.37, 1.35, 1.39, 1.36	1.4	93.3
3.0	2.87, 2.80, 2.73, 2.88	2.8	93.3
6.0	5.80, 5.33, 5.64, 5.80	5.6	93.3
12	11.4, 11.0, 11.3, 11.6	11	91.7

(1) Samples are listed in this order: Day 0 (new), 48-hours (old), 48-hours (new), 96-hours (old)
(2) LOQ = Limit of Quantitation = 0.200 mg/L active ingredient

Biological observations after 96-hours: All oligochaetes in the negative control and the 0.71, 1.4, and 2.8 mg/L treatments appeared healthy and normal throughout the exposure period with no mortalities or overt signs of toxicity observed. At test termination, mortality in the 5.6 and 11 mg/L treatment group was 50 and 100%, respectively. Signs of toxicity observed in the affected oligochaetes from the 5.6 and 11 mg/L treatment groups included pale appearance and occurrence of fragmentation.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	0	0
0.71	0	0	0	0
1.4	0	0	0	0
2.8	0	0	0	0
5.6	0	30	30	50
11	50	70	100	100

Lowest concentration causing 100% mortality: 11 mg/L
Mortality of controls: None

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC_{50} for the oligochaete worm *Lumbriculus variegatus* was determined to be 5.6 mg/L. The 96-hour no mortality concentration was 2.8 mg/L and no observed effects concentration was 2.8 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