

ACUTE TOXICITY TO *CHIRONOMUS TENTANS* USING SPIKED SEDIMENT

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. All results are reported corrected for purity.

METHOD

Method: Based on OPPTS 850.1735 and ASTM E1706-00

Test type: Flow-through

GLP: Yes

Year Completed: 2007

Species: *Chironomus tentans*

Supplier: Environmental Consulting and Testing, Superior, WI

Exposure period: 10-Days

Analytical monitoring: PFOS measured in stock solutions prior to Day 0 and in pore water and sediment samples on Day 0 and at test termination at Day 10.

Statistical methods: Survival data was analyzed using the computer software of C.E. Stephan and TOXSTAT version 3.5. Ash-free dry weight data were analyzed using TOXSTAT version 3.5. The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analysis of the survival and mean individual ash-free dry weight data. There were no statistically significant ($p = 0.05$) differences between the negative and solvent control groups for survival or ash-free dry weight, therefore both control groups were pooled for comparison to test groups.

Test organism age at study initiation: 10 days

Test conditions:

Test sediment: Formulated sediment consisting of approximately < 1% humic acid and dolomite, 5% alpha-cellulose, 14% silt and kaolin clay and 80% industrial quartz sand dry mixed for approximately 20-minutes. The final pH was 6.5.

Dilution water: 0.45 µm filtered moderately-hard well water

Dilution water chemistry (means during the 4-week period prior to the study):

Hardness: 135 (132 – 140) mg/L as CaCO₃

Alkalinity: 182 (180 – 182) mg/L as CaCO₃

pH: 8.1 (8.0 – 8.2)

Conductivity: 310 (305 – 315) µmhos/cm

Environmental conditions: Study conducted in a temperature-controlled water bath in a diluter unit. Fluorescent bulbs emitting wavelengths similar to

natural sunlight were on an automatic timer allowing 16 hours of light, 8 hours of darkness with a 30-minute transition period. Light intensity at test initiation was 206 lux at the surface of the water of one test chamber.

Stock and test solutions preparation: The test substance was administered to the midges in sediment. A primary stock solution of 0.600 mg/mL was prepared in acetone. Secondary stocks were prepared by serial dilution in acetone. A 15 mL volume of the appropriate stock was added to 150 grams of sediment, mixed by hand with a glass rod, then placed in fume hood for approximately one hour to allow the acetone to partially dissipate. The 150 gram premixes were then added to 600 grams of untreated sediment in plastic Nalgene bottles and mixed on a rotary mixer. Then 750 g of untreated sediment were added to the premixes to achieve a final weight of 1500 grams. The batch sediments were then mixed on a rotary mixer for approximately 15 hours.

Flow-through parameters: Diluter delivered 801 mL water per minute for four minutes to each splitting chamber two times per day. Resulting dilution was two volume additions per compartment per day.

Stability of the test chemical solutions: Extremely stable

Exposure vessels: 300 mL glass beakers with two stainless steel mesh-covered holes on opposite sides to allow for the flow of water through the compartment. Each vessel contained approximately 100 mL sediment (3.4 cm) and approximately 150 mL overlying water (6.0 cm).

Number of replicates: eight plus two for analytical sampling

Number of midges per replicate: ten

Number of concentrations: five plus a negative and a solvent control

Observations: Daily for signs of toxicity and abnormal behavior. On day 10, midges were removed and enumerated. Surviving midges were rinsed, dried and weighed as a group to determine ash-free dry weight.

Overlying water chemistry during the study:

Dissolved oxygen range (0 – 10 days):

5.3 – 7.8 mg/L (negative and solvent controls)

5.2 – 7.8 mg/L (6.0 mg/L exposure)

pH range (0 – 10 days):

8.1 – 8.3 (negative and solvent controls)

8.1 – 8.3 (6.0 mg/L exposure)

Temperature range (0 – 10 days):

22.8 – 23.4°C (negative and solvent controls)

22.9 – 23.4 °C (6.0 mg/L exposure)

Hardness range (0 – 10 days):

104 – 120 mg/L as CaCO_3 (negative and solvent controls)

120 – 122 mg/L as CaCO_3 (6.0 mg/L exposure)

Alkalinity range (0 – 10 days):

162 – 166 mg/L as CaCO_3 (negative and solvent controls)

162 – 166 mg/L as CaCO_3 (6.0 mg/L exposure)

Conductivity range (0 – 10 days) :

300 – 320 $\mu\text{mhos/cm}$ (negative and solvent controls)

310 – 320 $\mu\text{mhos/cm}$ (6.0 mg/L exposure)

Ammonia range (0 – 10 days):

<0.17 mg/L as NH₃ (negative and solvent controls)
<0.17 mg/L as NH₃ (6.0 mg/L exposure)

Element Basis: EC₅₀, NOEC, LOEC – survival and ash-free dry weight

Method of reporting test concentrations: nominal

RESULTS

Nominal concentrations: Bk control, solvent control, 0.10, 0.30, 1.0, 3.0, 6.0 mg/kg

Mean measured concentrations:

Sediment dry weight: <LOQ, <LOQ, 0.070, 0.202, 0.786, 2.08, 4.45 mg/kg

Pore water: < LOQ, <LOQ, 0.070, 0.202, 0.639, 1.80, 4.40 mg/L

Element values:

10-day EC₅₀ growth (ash-free dry weight) = 3.9 (3.2 – 4.3) mg/kg dry sediment

10-day LC₅₀ (survival) = > 6.0 mg/kg dry sediment

10-day NOEC (survival) = 1.0 mg/kg dry sediment

10-day LOEC (survival) = 3.0 mg/kg dry sediment

All element values were calculated using nominal concentrations.

Statistical Evaluation:

An exact element value and confidence intervals could not be calculated for the 10-day survival endpoint due to insufficient mortality and an inversion in the concentration –effect. There were no significant differences between the blank and solvent controls for growth and survival therefore the groups were pooled.

Biological observations:

Nominal Sediment Concentration, mg/kg dry sediment	Survival %	Individual Ash-Free Dry weight mg
Neg. Control	98	1.52
Solvent Control	96	1.42
0.10	98	1.39
0.30	95	1.47
1.0	75	1.31
3.0	44*	0.94**
6.0	63*	0.27**

*Statistically significantly different from pooled control (p<0.05) using Dunn's Multiple Comparison Test

**Statistically significantly different from pooled control (p<0.05) using Bonferroni t-test

Control response: Satisfactory

Analytical Methodology: Analyses of test sediment and pore water were performed at Wildlife International Ltd. using high performance liquid chromatography with triple quadrupole mass spectrometric detection (HPLC/MS/MS). On Days 0 and 10, the entire test vessel from the analytical replicate of each test concentration was submitted for analysis of PFOS in the sediment and pore water compartments. The LOQ (limit of quantitation) in pore water analyses was 0.0400 mg PFOS/L. The LOW for sediment samples varied as it was calculated as the product of the concentration of the lowest calibration standard (0.0010 mg PFOS/L) and the dilution factor of the control samples (20.0) divided by the dry weight percent. No interferences were observed in the control samples at or above the LOQ during the sample analyses.

The recovery of matrix fortifications analyzed concurrently during sample analysis ranged from 96.3 - 102% in the sediment. Sediment samples collected at test initiation had measured values from 96.7 - 102% of nominal. Measured values for samples taken at test termination ranged from 96.3 – 99.1% of nominal.

Values reported as <LOQ were excluded from all calculations of mean concentration.

Summaries of Analytical Chemistry Data (All results reported as dry weight):

Sediment Concentrations, Day 0:

Nominal Test Concentration, mg/kg	Measured Concentration, mg/kg	Percent of Nominal
Negative Control	< LOQ ⁽¹⁾	-
Solvent Control	< LOQ ⁽¹⁾	-
0.10	0.085	84.7
0.30	0.228	75.9
1.0	0.790	79.0
3.0	2.18	72.6
6.0	4.60	76.6

⁽¹⁾LOQ = Limit of Quantitation and varied from 0.0256 – 0.0261 depending on the percent moisture

Sediment Concentrations, Day 10:

Nominal Test Concentration, mg/kg	Measured Concentration, mg/kg	Percent of Nominal
Negative Control	< LOQ ⁽¹⁾	-
Solvent Control	< LOQ ⁽¹⁾	
0.10	0.055	54.8
0.30	0.175	58.5
1.0	0.781	78.1
3.0	1.98	66.1
6.0	4.30	71.7

⁽¹⁾LOQ = Limit of Quantitation and varied from 0.0261 – 0.0263 depending on the percent moisture

Pore Water Concentrations, Day 0:

Nominal Test Concentration, mg/kg	Measured Concentration, mg/L
Negative Control	< LOQ ⁽¹⁾
Solvent Control	< LOQ ⁽¹⁾
0.10	0.0843
0.30	0.241
1.0	0.736
3.0	1.98
6.0	4.66

⁽¹⁾LOQ = Limit of Quantitation, = 0.0400 mg/L

Pore Water Concentrations, Day 10:

Nominal Test Concentration, mg/kg	Measured Concentration, mg/L
Negative Control	< LOQ ⁽¹⁾
Solvent Control	< LOQ ⁽¹⁾
0.10	0.0560
0.30	0.162
1.0	0.541
3.0	1.61
6.0	4.13

⁽¹⁾LOQ = LOQ = Limit of Quantitation, = 0.0400 mg/L

CONCLUSIONS

The potassium perfluorooctanesulfonate 10-day EC₅₀ was found to be:
3.9 (3.2 – 4.3) mg/kg dry sediment based on ash-free dry weight
> 6.0 mg/L dry sediment based on survival

The 10-day no observed effect concentration (NOEC) was found to be:

1.0 mg/kg based on survival

Submitter: 3M Corporation, Environmental Laboratory, St. Paul, Minnesota,
55144

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: 02/14/08