

AR226-3776

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ACUTE TOXICITY TO THE MIDGE (*CHIRONOMUS TENTANS*)TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA, PFOA ammonium salt, Ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test substance is a solid. Sample was taken from 3M lot number 332. Sample was stored under frozen conditions prior to testing. Purity determined to be 95.0% by LC/MS, ¹⁹F-NMR, and elemental analyses techniques. The impurities were identified as: metals (0.002%); lower chain-length (C6 and C7) perfluorinated sulfonates (4.68%); NMR impurities (0.36).

METHOD

Method: ASTM E729-96

Type: Static renewal

GLP: Yes

Date completed: Study completed 2007

Species: *Chironomus tentans*

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

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

Exposure period: 96-hours

Statistical methods: The NOEC determined by visual interpretation of mortality, immobility and observation data.

Test organism age: 2nd to 3rd instar larvae (approximately 10 days old)

Average wet weight at test initiation: Not provided

Loading: 1 midge per test chamber

Pretreatment: None

Acclimation: Prior to the test, midges were held for 3 days with sand and water from the same sources and at approximately the same temperature as used during the test. Temperatures ranged from 22.0 to 22.8°C. pH ranged from 8.1 to 8.4. Dissolved Oxygen ranged from 7.2 to 8.6 mg/L. Midges were fed a 56 g/L suspension of Tetraamin® flake food daily during holding.

Test conditions:

Dilution water: Filtered, aerated well water

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

Specific Conductance:	293 (285-300) $\mu\text{mhos/cm}$
Hardness:	136 (132-140) mg/L as CaCO_3
Alkalinity:	183 (182-184) mg/L as CaCO_3
pH:	8.0 (7.9-8.1)

Stock and test solution preparation: A primary stock solution was prepared at a nominal concentration of 1000 mg active ingredient per liter using a calculated amount of PFOS, and sonicated for 5 minutes and mixed 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 in the test chambers 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 filled with approximately 20 mL of water. Beakers had approximately one-eighth teaspoon of sand on the bottom as substrate. Vessels were covered with plastic wrap.

Number of replicates: ten

Number of tadpoles per replicate: one

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: Midges were fed at test initiation on day 0 and at test renewal on day 2 with 4 g/L Tetramin® flake food

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

7.9 – 8.7 mg/L (negative control exposure)

7.6 – 8.7 mg/L (all test substance exposures)

pH range (0 – 96 hours)

8.2 – 8.5 (negative control exposure)

7.9 – 8.5 (all test substance exposures)

Test temperature range (0 – 96 hours)

22.0 – 22.6°C (negative control exposure)

21.7 – 23.0°C (all test substance exposures)

RESULTS

Nominal concentrations: Bk control, 63, 125, 250, 500, and 1000 mg/L

Measured concentrations: <LOQ, 74, 153, 277, 579, 1090mg/L

Element value*: 24-hour LC₅₀ = > 1090 mg/L (C.I. not calculable)

48-hour LC₅₀ = > 1090 mg/L (C.I. not calculable)

72-hour LC₅₀ = > 1090 mg/L (C.I. not calculable)

96-hour LC₅₀ = > 1090 mg/L (C.I. not calculable)

96-hour NOEC = 277 mg/L

*All element values based on mean measured concentrations

Statistical Evaluation of Mortality: Visual interpretation of observations and subsequent recorded data was utilized for assignment of NOEC.

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 10 mg/L in this study. The measured concentrations of matrix fortifications analyzed concurrently during sample analysis were 97.0-107% of nominal. Samples collected in exposure vessels at test initiation had measured values from 102 to 106% of nominal. New samples collected at 48 hours had measured concentrations from 105 to 104% of nominal. Old samples collected at 48 hours had measured concentrations ranging from 120 to 141% of nominal. Measured concentrations of PFOS in samples collected at test termination were 107 to 160% 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	-
63	65.4, 75.5, 65.1, 91.2	74	117
125	130, 154, 129, 200	153	122
250	259, 302, 259, 289	277	111
500	532, 707, 511, 566	579	116
1000	1020, 1260, 1020, 1070	1090	109

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

(2) LOQ = Limit of Quantitation = 10 mg/L active ingredient

Biological observations after 96-hours: A 10% mortality rate was observed in the negative control and the 74 mg/L treatment groups. No mortalities or signs of toxicity were observed among midges in the 153 and 277 treatment groups during the test. Midges that survived to test termination in the in all treatments appeared normal. At test termination,

mortality in the 579 and 1090 mg/L treatment group was 30 and 40%, respectively. The only sign of adverse effects observed in the affected midges from the 1090 mg/L treatment group was pale appearance.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	25-hours	48-hours	72-hours	96-hours
Negative Control	0	0	0	10
74	0	0	0	10
153	0	0	0	0
277	0	0	0	0
579	20	20	20	30
1090	20	20	30	40

Lowest concentration causing 100% mortality: > 1090 mg/L
Mortality of controls: 10%

CONCLUSIONS

The perfluorooctanoic acid, ammonium salt 96-hour LC₅₀ for *Chironomus tentans* was determined to be > 1090 mg/L. The 96-hour no mortality concentration was 3277mg/L and no observed effects concentration was 277 g/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