TOXICITY TO AQUATIC PLANTS (Duckweed, *Lemna gibba* G3)

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: Sample obtained from 3M production lot number 217. The test substance is a white powder. 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.

METHOD

Method: OPPTS 850.4400
Test: Static acute
GLP: Yes
Year completed: 2001
Species: *Lemna gibba G3*
Source: Originally from The United States Department of Agriculture. Maintained in culture medium at Wildlife International Ltd., Easton, MD
Analytical monitoring: Test concentrations measured at 0, 3, 5, and 7-days
Element basis: Number of fronds
Exposure period: 7-days
Start date: 3/3/00
End date: 3/10/00
Test organisms laboratory culture: Duckweed cultures had been actively growing in freshwater medium (20X AAP) for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

Test Conditions:

Test temperature range: 24.2 – 25.2ºC
Light levels: 5000 $\pm$ 750 lux from continuous warm-white fluorescent lighting

Growth medium: USEPA OPPTS 850.4400 20X AAP, 1996

Compound	Nominal Concentration	Units
$MgCl_2 \cdot 6H_20$	243.2	mg/L
$CaCl_2 \cdot 2H_20$	88.0	mg/L
H_3BO_3	3.712	mg/L
$MnCl_2 \cdot 4H20$	8.32	mg/L
$ZnCl_2$	65.6	ug/L
$FeCl_3 \cdot 6H_20$	3.196	mg/L
$CoCl_2 \cdot 6H_20$	28.56	ug/L
$Na_2Mo0_4 \cdot 2H_20$	145.2	ug/L
$CuCl_2 \cdot 2H_20$	0.240	ug/L
$Na_2EDTA \cdot 2H_20$	6.00	mg/L
$NaNO_3$	510	mg/L
$MgSO_4 \cdot 7H_20$	294	mg/L
K_2HPO_4	20.88	mg/L
$NaHCO_3$	300	mg/L

The pH of the medium was adjusted to 7.5 $\pm$ 0.1 using 10% HCl.

Dilution water source: Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water. The pH of the medium was adjusted to 7.5 $\pm$ 0.1 using 10% HCl and the medium was sterilized by filtration (0.22 µm) prior to use.

Stock and test solution preparation: A primary stock solution was prepared in duckweed medium at a concentration of 351 mg/L. The primary stock solution was stirred with a magnetic stir plate for approximately 24 hours. After mixing, the primary stock solution was proportionally diluted with duckweed medium to prepare the five additional test concentrations. All final test solutions appeared clear and colorless.

Exposure vessels: 250 mL plastic beakers containing 100 mL test solution, each covered with a disposable petri dish lid.

Agitation: None

Number of replicates: three plys 2 additional replicates for analytical sampling on Days 3 and 5

Initial loading: 5 plants/replicate, 15 fronds/replicate

Number of concentrations: six plus a negative control plus abiotic controls at the highest concentration tested

Water chemistry:

pH range (0 – 96 hours)

7.9 – 8.9 (control exposure)
8.4 – 8.7 (230 mg/L exposure)

Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at Days 0, 3, 5, and 7.

RESULTS

Nominal concentrations: Negative control, 11, 22, 43.9, 87.9, 176, and 351 mg/L plus 351 mg/L abiotic control.
Measured concentrations: <LOQ, 7.74, 15.1, 31.9, 62.5, 147, 230 mg/L; abiotic control = 231 mg/L
Element value and 95% confidence interval (based on frond number):
3-day IC_{10}: 101 mg/L (C.I. not calculable)
3-day IC_{50}: > 230 mg/L (C.I. not calculable)
3-day IC_{90}: > 230 mg/L (C.I. not calculable)
5-day IC_{10}: 30.7 mg/L (13.3 – 142 mg/L)
5-day IC_{50}: 182 mg/L (89.1 – 240 mg/L)
5-day IC_{90}: > 230 mg/L (C.I. not calculable)
7-day IC_{10}: 22.1 mg/L (13.3 – 26.0 mg/L)
7-day IC_{50}: 108 mg/L (45.7 – 144 mg/L)
7-day IC_{90}: > 230 mg/L (C.I. not calculable)

7-day NOAEC (number of fronds): 15.1 mg/L

All element values based on mean measured concentrations

Statistical methods: Mean plant and frond numbers, percent inhibition values and the percentages of necrotic, chlorotic and dead fronds were calculated using “Microsoft Excel Version 5.0”, while statistical analyses were conducted using “TOXSTAT Version 3.5”. Percent inhibition values were calculated for each treatment group as the percent reduction in mean frond number relative to mean frond number in the control replicates. The IC_{10}, IC_{50}, and IC_{90} values and 95% confidence intervals were determined, when possible, using linear interpolation with frond number and exposure concentration data. The percentages of dead, chlorotic and necrotic fronds also were calculated relative to the total number of fronds in each test chamber. The frond number data was evaluated for normality and homogeneity of variances ($p = 0.05$) using the Shapiro-Wilks’ and Levene’s tests, respectively. The data were normally distributed and the variances were homogeneous, thus statistically significant differences between the control and treatment groups were identified using ANOVA and Dunnett’s test. Results of the statistical

analyses, as well as an evaluation of the concentration-response pattern and other observations of effects were used in the determination of the no-observed-adverse-effect-concentration (NOAEC).

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Samples were centrifuged as necessary prior to analysis. 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 4.39 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 104%. Samples collected at test initiation had measured values from 64.2 to 82.6% of nominal. Measured values for samples taken at Day 3 ranged from 67.3 to 83.3% of nominal. Measured values for samples taken at Day 5 ranged from 65.4 to 85.4% of nominal. Samples collected at test termination (Day 7) ranged from 63.9 to 83.8% of nominal. For the abiotic controls, measured values for samples taken at Day 3, Day 5, and Day 7 ranged from 64.2 – 66.9% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at Days 0, 3, 5, and 7, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
11	7.57, 8.35, 7.47, 7.55	7.74	70
22	15.2, 15.4, 14.6, 15.2	15.1	69
43.9	32.2, 31.9, 31.8, 31.7	31.9	73
87.9	63.5, 63.1, 61.5, 61.8	62.5	71
176	145, 146, 150, 147	147	84
351	226, 237, 232, 224	230	66
351 (abiotic)	not analyzed, 225, 235, 232	231	66

Biological observations after 7-Days:

Counts

Mean Measured Concentration, mg/L	Mean Number of Plants	Mean Number of Fronds	Percent Inhibition via Frond Number
Negative Control	19	197	-
7.74	18	177	10
15.1	20	219	-11
31.9	14	151*	24
62.5	11	134*	32
147	15	69*	65
230	17	37*	81

*Statistically significant difference ($p < 0.05$) from the negative control using ANOVA and Dunnett's Test.

Effects

Mean Measured Concentration, mg/L	Mean Dead Fronds, %	Mean Chlorotic Fronds, %	Mean Necrotic Fronds, %
Negative Control	0	0	0
7.74	0	0	0
15.1	0	1.1	0
31.9	0	0	0.23
62.5	0	0.9	0.61
147	1.0	11	4.5
230	3.8	9.4	19

Control response: satisfactory. Plants appeared healthy and exhibited normal growth throughout the test with the exception of one necrotic frond observed on Day 3 and Day 5 of the test.

Observations: Duckweed exposed to 147 and 230 mg PFOS/L exhibited a dose-responsive increase in the incidence of dead, chlorotic or necrotic fronds during the test. By Day 7, all treatment groups $\geq$ 31.9 gm/L showed evidence of sublethal effects, including root destruction and/or a cupping of the plant downward on the water surface.

CONCLUSIONS

The potassium perfluorooctanesulfonate 7-Day IC_{50} and 95% confidence interval for duckweed was determined to be 108 (45.7 – 144) mg/L. The 7-Day NOAEC, based on the inhibition of frond production and evidence of sublethal effects, was 15.1 mg/L.

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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 the 3M Company.

OTHER

Last changed: 6/19/01