TOXICITY TO AQUATIC PLANTS (Freshwater alga, *Anabaena flos-aquae*)

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 from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.5400
Test: Acute static
GLP: Yes
Year completed: 2001
Species: *Anabaena flos-aquae*
Source: Originally from UTEX – The Culture Collection of Algae at the university of Texas at Austin, and maintained in culture medium at Wildlife International Ltd., Easton, MD
Analytical monitoring: PFOS measured at 0, 72, 96-hours
Element basis: Reported three ways: number of cells/ml, area under the growth curve and growth rate
Exposure period: 96-hours
Start date: 1/28/00
End date: 6/5/00
Test organisms laboratory culture: Algae cultures had been actively growing in algal culture medium 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. Solutions were then diluted in purified well water to prepare final growth media.

Test Conditions:

Freshwater Algal medium

Compound	Nominal Concentration	Units
$MgCl_2 \cdot 6H_2O$	12.16	mg/L
$CaCl_2 \cdot 2H_2O$	4.40	mg/L
H_3BO_3	0.1856	mg/L
$MnCl_2 \cdot 4H_2O$	0.416	mg/L
$ZnCl_2$	3.28	µg/L
$FeCl_3 \cdot 6H_20$	0.1598	mg/L
$CoCl_2 \cdot 6H_20$	1.428	µg/L
$Na_2MoO_4 \cdot 2H_20$	7.26	µg/L
$CuCl_2 \cdot 2H_2O$	0.012	µg/L
$Na_2EDTA \cdot 2H_20$	0.300	mg/L
$NaNO_3$	25.5	mg/L
$MgSO_4 \cdot 7H_2O$	14.7	mg/L
K_2HPO_4	1.044	mg/L
$NaHCO_3$	15.0	mg/L

Dilution water source: The pH of the medium was adjusted to 7.5 $\pm$ 0.1 and it was sterilized by filtration (0.22µm) prior to use.
Test solution preparations: Individual test solutions were prepared in algal medium at each of the six nominal concentrations. The solutions were stirred with magnetic stir plates for approximately 18 hours. The final test solutions appeared clear and colorless.
Exposure vessels: Sterile 250 mL glass Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.
Agitation: Shaken continuously at 100 rpm
Number of replicates: six.
Initial algal cell loading: 1.0 X 10^4 cells/mL
Cell counts method: hemacytometer and microscope
Number of concentrations: six plus a negative control plus an abiotic control at the highest concentration tested
Water chemistry:

pH range (0 – 96 hours)

7.4 – 7.6 (control exposure)
7.4 – 7.4 (329 mg/L exposure)

Test temperature range (0 – 96 hours)

22.8 – 23.8ºC

Light levels: (0 – 96 hours)
1990 – 2310 lux from cool-white fluorescent lighting
Photoperiod: 24-hours light

Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

RESULTS

Nominal concentrations: Negative control, 37.9, 58.6, 88.8, 139, 216, 331 mg/L plus 331 abiotic replicate
Measured concentrations: <LOQ, 37.9, 63.9, 93.8, 143, 235, 329 mg/L; abiotic replicate = 349 mg/L
Element values (95% confidence interval):
24-hour EC_{50} (cell density) = 105 mg/L (C.I. not calculable)
24-hour E_bC_{50} (area under curve) = 90 (40 - 150) mg/L
24-hour E_rC_{50} (growth rate) = 94 (33 - 145) mg/L
48-hour EC_{50} (cell density) = 117 mg/L (C.I. not calculable)
48-hour E_bC_{50} (area under curve) = 103 mg/L (C.I. not calculable)
48-hour E_rC_{50} (growth rate) = 128 mg/L (C.I. not calculable)
72-hour EC_{10} (cell density) = 43 (34 – 84) mg/L
72-hour E_bC_{10} (area under curve) = <38 mg/L (C.I. not calculable)
72-hour E_rC_{10} (growth rate) = 82 (49 - 116) mg/L
72-hour EC_{50} (cell density) = 120 (92 - 139) mg/L
72-hour E_bC_{50} (area under curve) = 116 (49 - 142) mg/L
72-hour E_rC_{50} (growth rate) = 174 (146 - 208) mg/L
72-hour EC_{90} (cell density) = 224 (193 - 275) mg/L
72-hour E_bC_{90} (area under curve) = 204 (134 - 226) mg/L
72-hour E_rC_{90} (growth rate) = 275 (162 - 330) mg/L
96-hour EC_{10} (cell density) = 82 (29 - 123) mg/L
96-hour E_bC_{10} (area under curve) = 56 (26 - 107) mg/L
96-hour E_rC_{10} (growth rate) = 109 (84 - 125) mg/L
96-hour EC_{50} (cell density) = 131 (106 - 142) mg/L
96-hour E_bC_{50} (area under curve) = 124 (104 - 138) mg/L
96-hour E_rC_{50} (growth rate) = 176 (169 - 181) mg/L
96-hour EC_{90} (cell density) = 213 (203 - 219) mg/L
96-hour E_bC_{90} (area under curve) = 209 (197 - 218) mg/L
96-hour E_rC_{90} (growth rate) = 225 (220 - 235) mg/L

72-hour NOAEC (cell density, area under curve): 37.9 mg/L
72-hour NOAEC (growth rate): 93.8 mg/L
96-hour NOAEC (cell density, growth rate): 93.8 mg/L
96-hour NOAEC (area under curve): 63.9 mg/L
All element values based on mean measured concentrations

Statistical methods: Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using "The SAS System for Windows", Release 6.12. The EC_{10}, EC_{50}, and EC_{90} values and 95% confidence limits were calculated by linear interpolation with treatment response and exposure concentration data using TOXSTAT Version 3.5. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were evaluated for normality and homogeneity of variances using the Shapiro-Wilk's test and Levene's test, respectively. Where the data were normally distributed with equal variances, the treatment groups were compared to the control using Dunnett's test. In the one instance where data were not normally distributed, the non-parametric Kruskal-Wallis test was used. Results of the statistical analyses were used to determine the NOAEC values.

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). 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 4.80 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 103%. Samples collected at test initiation had measured values from 100 to 112% of nominal. The measured values for the samples taken at 72-hours were 99.0 - 110% of nominal. The measured values for the samples taken at 96-hours were 99.0 - 109% of nominal. For the abiotic replicate, the measured value for the sample taken at 72-hours was 103% of nominal and for the sample taken at 96-hours, 107% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at 0, 72, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
37.9	37.9, 38.2, 37.6	37.9	100
58.6	65.6, 62.7, 63.4	63.9	109
88.8	94.3, 97.4, 89.8	93.8	106
139	142, 146, 142	143	103
216	230, 238, 236	235	109
331	331, 328, 329	329	99.4
331 (abiotic)	Not analyzed, 342, 356	349	105

Biological observations after 96-hours:

Mean Measured Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Area Under the Curve	Percent Inhibition via Growth Rate
Negative Control	569,167	-	-	-
37.9	605,000	-6.3	3.0	-1.3
63.9	585,833	-2.9	13	-0.97
93.8	492,500	13	24*	3.6
143	228,833	60*	66*	22*
235	2,333	100*	99*	100*
329	8,500	99*	100*	96*

*Indicates a significant difference from the negative control using the appropriate statistical test (p <0.05)

Control response: satisfactory

Observations: After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the negative control or any treatment group. In addition, there were no noticeable changes in cell morphology when compared to the negative control.

Reversibility of Growth Inhibition: Aliquots of the 235 and 329 mg/L test solutions were diluted with algal medium and cultured for nine days after the exposure phase of the study concluded. Based on the increase in growth observed by Day 9 of the recovery phase, the effect on algal growth was algistatic at a concentration of 235 mg/L. However, no algal cells were detected during the recovery phase in the 329 mg/L treatment, indicating that PFOS was algicidal at that concentration.

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour EC50 and 95% confidence interval for *Anabaena flos-aquae* was determined using three calculation methods. By cell density, it was 131 (106 – 142) mg/L, by area under the growth curve it was 124 (104 – 138) mg/L and by growth rate 176 (169 – 181) mg/L. The 96-hour NOAEC values were determined to be 63.9 mg/L using the area under the growth curve, and 93.8 mg/L with the cell density and growth rate calculation method. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls. PFOS was determined to be algistatic at a concentration of 235 mg/L and algicidal at 329 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

Last changed: 6/12/01