TOXICITY TO AQUATIC PLANTS (Freshwater Diatom, *Navicula pelliculosa*)

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 850.5400
Test: Acute static
GLP: Yes
Year completed: 2001
Species: *Navicula pelliculosa*
Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, 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: 2/25/00
End date: 2/29/00
Test organisms laboratory culture: Algae cultures had been actively growing in freshwater algal culture medium with silica and selenium 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:

Growth medium

Compound	Nominal Concentration	Units
$MgCl_2 \cdot 6H_20$	12.16	mg/L
$CaCl_2 \cdot 2H_20$	4.40	mg/L
H_3BO_3	0.1856	mg/L
$MnCl_2 \cdot 4H_20$	0.416	mg/L
$ZnCl_2$	3.28	ug/L
$FeCl_3 \cdot 6H_20$	0.1598	mg/L
$CoCl_2 \cdot 6H_20$	1.428	ug/L
$Na_2Mo0_4 \cdot 2H_20$	7.26	ug/L
$CuCl_2 \cdot 2H_20$	0.012	ug/L
$Na_2EDTA \cdot 2H_20$	0.300	mg/L
$NaNO_3$	25.50	mg/L
$MgSO_4 \cdot 7H_20$	14.70	mg/L
K_2HPO_4	1.044	mg/L
$NaHCO_3$	15.0	mg/L
$Na_2SiO_3 \cdot 9H_20$	20.0	mg/L
$Na_2SeO_3 \cdot 5H_20$	0.010	mg/L

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 0.1 N NaOH. The medium was sterilized by filtration (0.22µm) prior to use.

Test solution preparation: A primary stock solution was not prepared for this study. Individual test solutions were prepared in algal medium at each of the seven nominal concentrations. The individual test solutions were stirred with a magnetic stir plate for approximately 24 hours. All final test solutions appeared clear and colorless.

Exposure vessels: Sterile 250 mL plastic Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

Agitation: Shaken continuously at ~100 rpm

Number of replicates: three.

Initial algal cell loading: 1.0 X 10^4 cells/mL

Number of concentrations: seven plus a negative control plus an abiotic control at the highest concentration tested

Water chemistry:

pH range (0 – 96 hours)

7.5 – 8.6 (control exposure)

7.5 – 7.7 (335 mg/L exposure)

Test temperature range (0 – 96 hours)

23.1 – 24.6ºC

Light levels: (0 – 96 hours)

3910 – 4510 lux from continuous cool-white fluorescent lighting

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, 61.5, 81.3, 110, 147, 198, 264, 347 mg/L plus 347 mg/L abiotic control.

Measured concentrations: <LOQ, 62.3, 83.2, 111, 150, 206, 266, 335 mg/L; abiotic control = 339 mg/L

Element value (95% confidence interval):

24-hour EC_{50} (cell density) = 281 (214 - 312) mg/L
24-hour E_bC_{50} (area under curve) = 262 (205 - 308) mg/L
24-hour E_rC_{50} (growth rate) = 279 (212 - 306) mg/L
48-hour EC_{50} (cell density) = 261 (219 - 306) mg/L
48-hour E_bC_{50} (area under curve) = 259 (227 - 303) mg/L
48-hour E_rC_{50} (growth rate) = 294 (271 - 307) mg/L
72-hour EC_{10} (cell density) = <62.3 (C.I. not calculable) mg/L
72-hour E_bC_{10} (area under curve) = <62.3 (C.I. not calculable) mg/L
72-hour E_rC_{10} (growth rate) = 221 (190 - 252) mg/L
72-hour EC_{50} (cell density) = 242 (200 - 276) mg/L
72-hour E_bC_{50} (area under curve) = 246 (210 - 277) mg/L
72-hour E_rC_{50} (growth rate) = 295 (288 - 305) mg/L
72-hour EC_{90} (cell density) = 317 (306 - 326) mg/L
72-hour E_bC_{90} (area under curve) = 318 (307 - 325) mg/L
72-hour E_rC_{90} (growth rate) = 335 (323 - 335) mg/L
96-hour EC_{10} (cell density) = <62.3 (C.I. not calculable) mg/L
96-hour E_bC_{10} (area under curve) = <62.3 (C.I. not calculable) mg/L
96-hour E_rC_{10} (growth rate) = 243 (209 - 295) mg/L
96-hour EC_{50} (cell density) = 263 (217 - 299) mg/L
96-hour E_bC_{50} (area under curve) = 252 (220 - 285) mg/L
96-hour E_rC_{50} (growth rate) = 305 (295 - 316) mg/L
96-hour EC_{90} (cell density) = 322 (310 - 328) mg/L
96-hour E_bC_{90} (area under curve) = 319 (308 - 326) mg/L

96-hour E_rC_{90} (growth rate) = >335 mg/L (C.I. not calculable)

72-hour NOAEC (cell density, area under the curve): <62.3 mg/L
72-hour NOAEC (growth rate): 206 mg/L

96-hour NOAEC (cell density): 150 mg/L
96-hour NOAEC (area under the curve): <62.3 mg/L
96-hour NOAEC (growth rate): 206 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. These values were then analyzed by linear interpolation using TOXSTAT Version 3.5 to estimate the EC_{10}, EC_{50}, and EC_{90} values and 95% confidence limits. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were also evaluated for normality and homogeneity of variances using the Shapiro-Wilkes's test and Levene's test, respectively. The treatment groups were then compared to the control using Dunnett's test. 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 4.39 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 108%. Samples collected at test initiation had measured values from 96.2 to 106% of nominal. Measured values for samples taken at 72-hours ranged from 98.5 to 106% of nominal. Measured values for samples taken at 96-hours ranged from 94.8 to 101% of nominal. For the abiotic controls, the measured value for the sample taken at 72-hours was 98.2% of nominal and for the sample taken at 96-hours, 96.8% 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	-
61.5	62.3, 63.6, 61.1	62.3	101
81.3	83.8, 84.7, 81.0	83.2	102
110	109, 113, 110	111	101
147	147, 154, 149	150	102
198	209, 209, 199	206	104
264	271, 268, 258	266	101
347	334, 342, 329	335	96.5
347 (abiotic)	Not analyzed, 341, 336	339	97.7

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	2,726,667	-	-	-
62.3	2,366,667	13	24*	2.5
83.2	2,366,667	13	22*	2.7
111	2,373,333	13	24*	2.5
150	2,473,333	9.3	16	1.7
206	2,093,333	23*	24*	4.7
266	1,330,000	51*	58*	13*
335	35,333	99*	99*	79*

*Indicates a significant difference from the negative control using Dunnett's test ($p \leq 0.05$)

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 test treatment group. In addition, there were no noticeable changes in cell color or morphology when compared to the negative

control, although at 72 and 96 hours of exposure a few cells in the 335 mg/L treatment group appeared small in comparison to the control. **Reversibility of Growth Inhibition:** The 335 mg/L treatment group was maximally inhibited after 96-hours. The treatment group was diluted to a concentration of the test substance that would not inhibit growth and exposed for 7 days. Based on the growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour EC_{50} and 95% confidence interval for *Navicula pelliculosa* was determined using three calculation methods. By cell density, it was 263 (217 - 299) mg/L, by area under the growth curve it was 252 (220 - 285) mg/L and by growth rate 305 (295 - 316) mg/L. The 96-hour NOAEC was determined by Dunnett's procedure ($p \leq 0.05$) to be 150 mg/L using cell density, <62.3 mg/L when using area under the curve and 206 mg/L by growth rate. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls. This test substance was determined to be algistatic.

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: 6/19/01