AR226-0084
EE-2

# TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

## 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 90.49% by LC/MS, $^{1}$H-HMR, $^{19}$F-NMR and elemental analyses techniques.

## METHOD

**Method:** OECD 201, OPPTS 850.5400, ASTM 1218-90E
**Test:** Static acute
**GLP:** Yes
**Year completed:** Study completed 1999. Report completed 2000
**Species:** *Selenastrum capricornutum*
**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:** 4/12/99
**End date:** 4/16/99
**Analytical monitoring:** Test concentrations measured at 0, 72, and 96-hours.
**Test organisms laboratory culture:** Algae cultures had been actively growing in freshwater 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.

**Test Conditions:**

**Test temperature range:** 23.6 – 25.8°C

**Growth medium:** ASTM Standard Guide 1218-90E, 1990

| 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 |
| $MnCl_2 \cdot 4H20$ | 0.416 | mg/L |
| $ZnCl_2$ | 3.28 | ug/L |
| $FeCl_2 \cdot 6H_20$ | 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 |

**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 (0.22μm) prior to use.

**Stock and test solution preparation:** A primary stock solution was prepared in algal medium at a concentration of 183 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 algal medium to prepare the five additional test concentrations. All final test solutions appeared clear and colorless.

**Exposure vessels:** Sterile 250 mL polycarbonate 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 – 8.1 (control exposure)

7.4 – 7.5 (179 mg/L exposure)

**Test temperature range** (0 – 96 hours)

23.6 – 25.8°C

**Light levels:** (0 – 96 hours)

3870 – 4610 lux from continuous cool-white fluorescent lighting

**Method of calculating mean measured concentrations**: arithmetic mean

RESULTS

**Nominal concentrations:** Bk control, 5.7, 11, 23, 46, 91, 183 mg/L plus 183 mg/L abiotic control.

**Measured concentrations:** <LOQ, 5.5, 11, 21, 44, 86, 179, 169 mg/L

**Element value**:

24-hour $EC_{50}$ (cell density) = 163 (74-191) mg/L

24-hour $E_bC_{50}$ (area under curve) = 122 (19-176) mg/L

24-hour $E_rC_{50}$ (growth rate) = 136 (30 - 204) mg/L

48-hour $EC_{50}$ (cell density) = 81 (72 – 90) mg/L

48-hour $E_bC_{50}$ (area under curve) = 84 (67 – 146) mg/L

48-hour $E_rC_{50}$ (growth rate) = 142 (107 - 185) mg/L

72-hour $EC_{10}$ (cell density) = 37 (<0 – 64) mg/L

72-hour $E_bC_{10}$ (area under curve) = 46 (<0 – 56) mg/L

72-hour $E_rC_{10}$ (growth rate) = 53 (23 – 64) mg/L

72-hour $EC_{50}$ (cell density) = 70 (44 – 78) mg/L

72-hour $E_bC_{50}$ (area under curve) = 74 (55 – 82) mg/L

72-hour $E_rC_{50}$ (growth rate) = 120 (103 – 132) mg/L

72-hour $EC_{90}$ (cell density) = 153 (130 – 165) mg/L

72-hour $E_bC_{90}$ (area under curve) = 165 (145 – 176) mg/L

72-hour $E_rC_{90}$ (growth rate) = >179 mg/L (C.I. not calculable)

96-hour $EC_{10}$ (cell density) = 49 (43 – 50) mg/L

96-hour $E_bC_{10}$ (area under curve) = 49 (40 – 50) mg/L

96-hour $E_rC_{10}$ (growth rate) = 59 (54 – 63) mg/L

96-hour $EC_{50}$ (cell density) = 71 (66 – 73) mg/L

96-hour $E_bC_{50}$ (area under curve) = 71 (67 – 74) mg/L

96-hour $E_rC_{50}$ (growth rate) = 126 (115 – 138) mg/L

96-hour $EC_{90}$ (cell density) = 137 (105 – 153) mg/L

96-hour $E_bC_{90}$ (area under curve) = 145 (125 – 155) mg/L

96-hour $E_rC_{90}$ (growth rate) = >179 mg/L (C.I. not calculable)

72-hour NOEC (growth rate, cell density, area under the curve): 44 mg/L

96-hour NOEC (growth rate, cell density, area under the curve): 44 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 at 72 and 96. 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-Wilks's test and Bartlett'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 NOEC 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 0.115 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 99.1. Samples collected at test initiation had measured values from 82.0 to 98.1% of nominal. Measured values for samples taken at 72-hours ranged from 91.7 to 105% of nominal. Measured values for samples taken at 96-hours ranged from 90.3 to 102% of nominal. For the abiotic controls, measured values for samples taken at 72-hours ranged from 81.9 – 105% of nominal and for samples taken at 96-hours, 90.3 – 103% 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 | - |
| 5.7 | 4.73, 6.04, 5.84 | 5.5 | 96 |
| 11 | 10.7, 11.2, 12.1 | 11 | 100 |
| 23 | 19.8, 23.1, 20.7 | 21 | 91 |
| 46 | 42.7, 41.9, 46.3 | 44 | 96 |
| 91 | 83.3, 86.0, 88.3 | 86 | 95 |
| 183 | 179, 186, 172 | 179 | 98 |
| 183 (abiotic) | not analyzed, 150, 188 | 169 | 92 |

**Control response**: satisfactory

**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 | 2,740,000 | - | - | - |
| 5.5 | 3,040,000 | -11 | -8.5 | -1.9 |
| 11 | 2,880,000 | -5.1 | -3.3 | -0.84 |
| 21 | 3,240,000 | -18 | -13 | -3.0 |
| 44 | 3,080,000 | -12 | -5.3 | -2.0 |
| 86 | 626,667 | 77 | 75 | 27 |
| 179 | 33,667 | 99 | 98 | 79 |

**Observations:** After 96 hours of exposure, there were no signs of aggregation, flocculation 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 a few cells appeared enlarged in the 86 and 179 mg/L treatment groups.

**Reversibility of Growth Inhibition:** The 179 mg/L treatment group was maximally inhibited after 96-hours. Aliquots of the test solution were diluted with algal medium and cultured for five 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 *Selenastrum capricornutum* was determined using three calculation methods. By cell density, it was 71 (66-73) mg/L, by area under the growth curve it was 71 (67-74) mg/L and by growth rate 126 (115-138) mg/L. The 96-hour NOEC was determined by Dunnett's procedure ($p \leq 0.05$) to be 44 mg/L using all three methods. No signs of aggregation, flocculation, 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.

## OTHER

**Last changed:** 5/3/00