

TOXICITY TO AQUATIC PLANTS (Marine Diatom, *Skeletonema costatum*)

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, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.5400

Test: Acute static

GLP: Yes

Year completed: 2001

Species: *Skeletonema costatum*

Source: Originally from The Culture Collection of Algae and Protozoa, Dunstaffnage marine Laboratory at Oban Argyll, Scotland, 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: 5/19/00

End date: 5/23/00

Analytical monitoring: Test concentrations measured at 0, 72, and 96-hours.

Test organisms laboratory culture: Algae cultures had been actively growing in saltwater 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 artificial saltwater to prepare final growth media.

Test Conditions:

Algal saltwater medium

Compound	Nominal Concentration	Units
FeCl ₃ ·6H ₂ O	0.72	mg/L
MnCl ₂ ·4H ₂ O	2.16	mg/L
ZnSO ₄ ·7H ₂ O	0.675	mg/L
CuSO ₄ ·5H ₂ O	2.36	µg/L
CoCl ₂ ·6H ₂ O	6.06	µg/L
H ₃ BO ₃	17.1	mg/L
Na ₂ EDTA·2H ₂ O	15.0	mg/L
K ₃ PO ₄	3.0	mg/L
NaNO ₃	50.0	mg/L
Na ₂ SiO ₃ ·9H ₂ O	20.0	mg/L
Thiamine Hydrochloride	0.25	mg/L
Biotin	0.05	µg/L
B ₁₂	0.5	µg/L

Dilution water source: The stock nutrient solutions were prepared by adding the appropriate volumes reagent-grade chemicals to Wildlife International Ltd. well water purified by reverse osmosis. The algal medium was prepared by adding appropriate volumes of the stock nutrient solutions to artificial saltwater at 30 ppt salinity. The pH of the medium was 8.1 and it was sterilized by filtration (0.22µm) prior to use.

Test solution preparation: A single test solution (3.46 mg/L) was prepared for this study in algal saltwater medium. The solution was sonicated for approximately 30 minutes and was stirred with a magnetic stir plate for approximately 43 hours. The final test solution 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: 7.7×10^4 cells/mL

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

Water chemistry:

pH range (0 – 96 hours)

8.0 – 8.4 (control exposure)

8.0 – 8.4 (3.20 mg/L exposure)
Test temperature range (0 – 96 hours)
20.2 – 21.4°C
Light levels: (0 – 96 hours)
3880 – 4710 lux from cool-white fluorescent lighting
Photoperiod: 14-hours light and 10 hours dark

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, 3.46 mg/L plus 3.46 mg/L abiotic control. This is apparently the highest concentration of PFOS attainable in this saltwater algal media.

Measured concentrations: <LOQ, 3.20 mg/L; abiotic control = 3.18 mg/L

Element value (95% confidence interval):

72 and 96-hour EC₁₀ via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

24, 48, 72, and 96-hour EC₅₀ via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

72 and 96-hour EC₉₀ via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

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

96-hour NOAEC (cell density, area under the curve, growth rate):
3.20 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 EC₁₀, EC₅₀, and EC₉₀ values and 95% confidence limits could not be calculated using statistical methods. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were evaluated for normality using the Shapiro-Wilk’s test and for equality of variance using an F-test. The treatment group were then compared to the control using ANOVA and a 2-sample t-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). The 72 and 96-hour samples were centrifuged approximately 10 minutes at approximately 2000 rpm 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 0.480 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 88.5% of nominal. The measured value for the sample taken at 72-hours was 92.2% of nominal. The measured value for the sample taken at 96-hours was 91.2% of nominal. For the abiotic control, the measured value for the sample taken at 72-hours was 97.1% of nominal and for the sample taken at 96-hours, 86.8% 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	-
3.46	3.26, 3.19, 3.15	3.20	92.5
3.46 (abiotic)	Not analyzed, 3.36, 3.00	3.18	91.9

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	2,481,667	-	-	-
3.20	2,601,667	-4.8	-7.3	-1.3

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

Reversibility of Growth Inhibition: After 96-hours of exposure, there was no significant inhibition of growth in the highest concentration tested (3.20 mg/L). Therefore, a recovery phase was not conducted.

CONCLUSIONS

A single concentration of potassium perfluorooctanesulfonate was evaluated for toxicity to *Skeletonema costatum*. This mean measured concentration, 3.20 mg/L, was the highest concentration attainable in this algal media. The 96-hour EC₅₀ and 95% confidence interval for *Skeletonema costatum*, as determined by cell density, area under the growth curve, and by growth rate was found to be > 3.20 mg/L. The 96-hour NOAEC was determined by ANOVA and a 2-sample t-test to be 3.20 mg/L calculated using cell density, area under the curve and growth rate. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls.

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