PFOS: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)

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 |
|---|---|---|
| $MgCl_2 \cdot 6H_2O$ | 12.16 | mg/L |
| $CaCl_2 \cdot 2H_2O$ | 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 |
| $FeCl_2 \cdot 6H_2O$ | 0.1598 | mg/L |
| $CoCl_2 \cdot 6H_2O$ | 1.428 | ug/L |
| $Na_2Mo0_4 \cdot 2H_2O$ | 7.26 | ug/L |
| $CuCl_2 \cdot 2H_2O$ | 0.012 | ug/L |
| $Na_2EDTA \cdot 2H_2O$ | 0.300 | mg/L |
| $NaNO_3$ | 25.50 | mg/L |
| $MgSO_4 \cdot 7H_2O$ | 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**: $1.0 \times 10^4$ 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

PFOS:
A 96-HOUR TOXICITY TEST WITH THE
FRESHWATER ALGA (*Selenastrum capricornutum*)

FINAL REPORT

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-103A

3M LAB REQUEST NO. U2723

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.5400
and
OECD Guideline 201

AUTHORS:

Kurt R. Drottar
Henry O. Krueger, Ph.D.

STUDY INITIATION DATE: December 4, 1998

STUDY COMPLETION DATE: June 4, 1999

AMENDED REPORT DATE: April 26, 2000

Submitted to

3M Corporation
Environmental Laboratory
935 Bush Avenue
St. Paul, Minnesota 55144

*Wildlife International Ltd.*
8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600



AMENDED

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-103A

STUDY COMPLETION: June 4, 1999

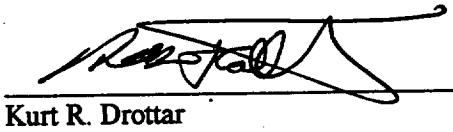
REPORT AMENDED: April 26, 2000

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice, OCDE/GD (92) 32, Environment Monograph No. 45, Paris 1992 and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984 with the following exceptions:

The test substance was not characterized in accordance with full GLP compliance; however, the characterization was performed according to 3M Standard Operating Procedures and Methods, and all raw data are being maintained in the 3M archives. The test substance is being recharacterized in accordance with GLP.

The stability of the test substance under conditions of storage at the test site was not determined in accordance with Good Laboratory Practice Standards.

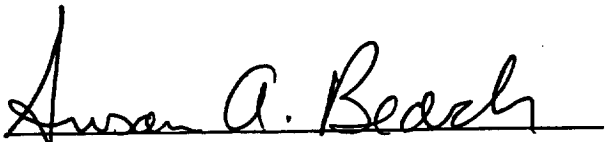
STUDY DIRECTOR:



Kurt R. Drottar
Senior Biologist

4/26/00
DATE

SPONSOR:



Sponsor

4/27/00
DATE

AMENDED

## QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice, OCDE/GD (92) 32, Environment Monograph No. 45, Paris 1992; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

| | | DATE REPORTED TO: | |
|---|---|---|---|
| ACTIVITY: | DATE CONDUCTED: | STUDY DIRECTOR: | MANAGEMENT: |
| Initial Trial 454A-103 | | | |
| Test Substance Preparation | February 18, 1999 | February 18, 1999 | February 19, 1999 |
| Instrument Set-up | February 22 and 23, 1999 | February 23, 1999 | February 24, 1999 |
| Draft Report and Data | March 29, 1999 | March 29, 1999 | March 30, 1999 |
| Definitive Trial 454A-103A | | | |
| Test Substance Preparation | April 9, 1999 | April 9, 1999 | April 12, 1999 |
| Draft Report and Data | May 10 and 11, 1999 | May 11, 1999 | May 12, 1999 |
| Analytical Data and Draft Report | May 10 and 11, 1999 | May 11, 1999 | May 11, 1999 |
| Final Report | June 4, 1999 | June 4, 1999 | June 4, 1999 |
| Amended Report | April 19 and 20, 2000 | April 20, 2000 | April 24, 2000 |

Timothy A. Springer, Ph.D.
Manager, Regulatory and Technical Support

4/24/00
DATE

AMENDED

**REPORT APPROVAL**

SPONSOR: 3M Corporation

TITLE: PFOS: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-103A

STUDY DIRECTOR:

Kurt R. Drottar
Senior Biologist

4/26/00
DATE

MANAGEMENT:

Henry O. Krueger, Ph.D.
Director, Aquatic Toxicology
and Non-Target Plants

4/26/00
DATE

AMENDED

## TABLE OF CONTENTS



## TABLES



AMENDED

## TABLE OF CONTENTS

**-Continued-**



### FIGURES



### APPENDICES



AMENDED

## SUMMARY

| | |
|---|---|
| SPONSOR: | 3M Corporation |
| SPONSOR'S REPRESENTATIVE: | Ms. Susan A. Beach |
| LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT: | Wildlife International Ltd.<br>Easton, MD 21601 |

| | |
|---|---|
| WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: | 454A-103A |
| TEST SUBSTANCE: | PFOS (Perfluoroocatane Sulfonic Acid Potassium Salt) |
| STUDY: | PFOS: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*) |
| MEAN MEASURED TEST CONCENTRATIONS: | Negative Control, 5.5, 11, 21, 44, 86 and 179 mg a.i./L |
| TEST DATES: | Experimental Start - April 12, 1999<br>Exposure Termination - April 16, 1999<br>Experimental Termination -April 21, 1999 |
| LENGTH OF EXPOSURE: | 96 Hours |

| | |
|---|---|
| TEST ORGANISM: | Freshwater Alga (*Selenastrum capricornutum*) |
| SOURCE OF TEST ORGANISMS: | Wildlife International Ltd.<br>Easton, Maryland 21601 |

| | |
|---|---|
| CELL DENSITY | |
| 72-HOUR EC50: | 70 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 44 and 78 mg a.i./L |
| 96-HOUR EC10: | 49 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 43 and 50 mg a.i./L |
| 96-HOUR EC50: | 71 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 66 and 73 mg a.i./L |
| 96-HOUR EC90: | 137 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 105 and 153 mg a.i./L |
| 72-HOUR NOAEC: | 44 mg a.i./L |
| 96-HOUR NOAEC: | 44 mg a.i./L |

AMENDED

**SUMMARY (Continued)**

| | |
|---|---|
| AREA UNDER THE GROWTH CURVE | |
| 72-HOUR EC50: | 74 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 55 and 82 mg a.i./L |
| 96-HOUR EC10: | 49 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 40 and 50 mg a.i./L |
| 96-HOUR EC50: | 71 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 67 and 74 mg a.i./L |
| 96-HOUR EC90: | 145 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 125 and 155 mg a.i./L |
| 72-HOUR NOAEC: | 44 mg a.i./L |
| 96-HOUR NOAEC: | 44 mg a.i./L |

| | |
|---|---|
| GROWTH RATE | |
| 72-HOUR EC50: | 120 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 103 and 132 mg a.i./L |
| 96-HOUR EC10: | 59 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 54 and 63 mg a.i./L |
| 96-HOUR EC50: | 126 mg a.i./L |
| 95% CONFIDENCE LIMITS: | 115 and 138 mg a.i./L |
| 96-HOUR EC90: | >179 mg a.i./L |
| 95% CONFIDENCE LIMITS: | Not Calculable |
| 72-HOUR NOAEC: | 44 mg a.i./L |
| 96-HOUR NOAEC: | 44 mg a.i./L |

AMENDED

## INTRODUCTION

This study was conducted by Wildlife International Ltd. for 3M Corporation at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The in-life phase of the test was conducted from April 12, 1999 to April 21, 1999. Raw data generated by Wildlife International Ltd and a copy of the final report are filed under Project Number 454A-103A in archives located on the Wildlife International Ltd. site.

## OBJECTIVE

The objective of the study was to evaluate the toxicity of PFOS (Perfluorooctane Sulfonic Acid Potassium Salt) to the growth of the freshwater alga, *Selenastrum capricornutum*, during a 96-hour exposure period.

## EXPERIMENTAL DESIGN

The freshwater alga, *Selenastrum capricornutum*, was exposed to a geometric series of six test concentrations and a negative (culture medium) control under static conditions for 96 hours. Three replicate test chambers were maintained for each treatment and control group. One additional replicate was also maintained for analytical sampling on Day 3 of the test. In addition, one "abiotic" replicate (test solution without algae) was prepared for the highest test concentration. Nominal test concentrations were selected in consultation with the Sponsor and were based upon the results of range finding and definitive tests. The nominal test concentrations selected were 5.7, 11, 23, 46, 91 and 183 mg active ingredient (a.i.)/L. Mean measured test concentrations were determined from samples of test medium collected from each treatment and the control group at test initiation, at approximately 72 hours, and at test termination.

At test initiation, an inoculum of the algal cells was prepared at a concentration of approximately $1.0 \times 10^6$ cells/mL. The concentration of algal cells in the inoculum was verified and 1.0 mL was added to each test chamber to achieve a nominal concentration of approximately $1.0 \times 10^4$ cells/mL. Samples were

collected from each replicate test chamber at approximately 24-hour intervals during the test to determine cell densities. Cell densities were measured for each replicate and were used to calculate areas under the growth curve and growth rates. Percent inhibition values relative to the control were calculated for each parameter over the 96-hour exposure period. EC50 values based upon cell densities, areas under the growth curve and growth rates were calculated for each 24 hour interval. EC10 and EC90 values were calculated, if possible, for the 72 and 96 hour intervals. The no-observed-adverse-effect-concentration (NOAEC) was determined based upon statistical evaluation of the 72-hour and 96-hour results. At the end of the 96-hour exposure, algistatic effects were differentiated from algicidal effects.

## MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "PFOS: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)". The protocol was based on procedures outlined in the U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines, OPPTS Number 850.5400: *Algal Toxicity Tiers I and II* (draft)(1) and OECD Guidelines for Testing of Chemicals, 201 *Algal, Growth Inhibition Test* (2).

### Test Substance

The test substance was received from 3M Corporation on October 29, 1998 and was assigned Wildlife International Ltd. identification number 4675. The test substance was described as a white powder. It was identified as FC-95 from lot number 217 (T-6295). Information provided by the Sponsor indicated a purity of 98.9% and an expiration date of 2008. The test substance was reanalyzed by the Sponsor and the Certificate of Analysis dated March 9, 2000 indicated a purity of 90.49%. The test substance was stored at ambient room temperature.

### Preparation of Test Concentrations

Nominal test concentrations were 5.7, 11, 23, 46, 91 and 183 mg a.i./L, based on a test substance purity of 90.49%. All materials which came into contact with the test substance during preparation of test concentrations were constructed of plastic or stainless steel. A primary stock solution was prepared in algal

medium at a concentration of 183 mg a.i./L. The primary stock solution was stirred with a magnetic stir plate for approximately 24 hours to aid in the solubilization of the test substance. 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.

Test Organism

The freshwater alga, *Selenastrum capricornutum*, was selected as the test species for this study. The species is representative of an important group of freshwater algae, and was selected for use in the test based upon a past history of use and ease of culturing in the laboratory. Original algal cultures were obtained from UTEX - The Culture Collection of Algae at the University of Texas at Austin and have been maintained in culture medium at Wildlife International Ltd., Easton, Maryland. Algal cells used in this test were obtained from Wildlife International Ltd. cultures that had been actively growing in culture medium for at least two weeks prior to test initiation. The negative control organisms were expected to exhibit exponential growth over the 96-hour exposure period. Exponential growth, defined as the period of growth where the algal cells are dividing at a constant rate, is indicated by the linear section of the growth curve (Figure 1). OECD guidelines recommend that control cell growth should have increased by a factor of at least 16 within 3 days.

Culture Medium

The algal cells were cultured and tested in freshwater algal medium (3). Stock nutrient solutions were prepared by adding reagent-grade chemicals to 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 (Appendix I). The pH of the medium was adjusted to 7.5 ± 0.1 using 10% HCl and the medium was sterilized by filtration (0.22 $\mu$m) prior to use. Analyses were performed at least once annually to determine the concentrations of selected organic and inorganic constituents in the well water. The results of analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International Ltd. are presented in Appendix II.

Test Apparatus

Test chambers were sterile, 250-mL polycarbonate Erlenmeyer flasks plugged with foam stoppers and contained 100 mL of test or control algal medium. The test chambers were labeled with the project number, concentration and replicate, and were indiscriminately positioned on a mechanical shaker table in an environmental chamber designed to maintain the desired test temperature throughout the test. The test chambers were shaken continuously at 100 rpm.

Environmental Conditions

Test flasks were held in an environmental chamber at a temperature of 24±2°C. The temperature of a container of water adjacent to the test flasks in the environmental chamber was recorded twice daily during the test using a liquid-in-glass thermometer.

The algae were held under continuous cool-white fluorescent lighting throughout the test. The target light intensity was 4300 ± 430 lux. Light intensity was measured at the four corners and the middle of the shaker table daily. Light intensity was measured using an SPER Scientific Model 840006 light meter.

The pH of the medium prepared for each treatment and control group was measured at test initiation and termination using a Fisher Accumet Model 915 pH meter. Samples for pH measurement at test initiation were collected from the individual batches of test solution prepared for each treatment and control group. At test termination, samples of test solution were collected from pooled replicates of the treatment and control groups for pH measurement.

Algal Growth Measurements

Test medium samples were collected from the treatment and control groups for the determination of algal cell densities. Single samples were collected from each of the three "biological" replicates per treatment and control group at 24-hour intervals during the 96-hour exposure, and were counted immediately. Cell counts were conducted using a hemacytometer and microscope. Each sample was diluted using an electrolyte solution (Isoton®), as needed, to maintain counting accuracy. A small amount of each sample was loaded onto a hemacytometer and 10 grids were counted. The mean number of cells

per grid was estimated and this value was used to calculate the cell density of the sample. Using this technique, the minimum quantifiable cell density was $1.0 \times 10^3$ cells/mL.

All 96 hour cell count samples were examined microscopically for atypical cell morphology (e.g., changes in cell shape, size or color). Growth of cells in the replicate test chambers also was assessed for aggregations or flocculations of cells and adherence of the cells to the test chamber.

Statistical Analyses

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 (4). Area under the growth curve was calculated for the control and treatment group using the following formula:

$$A = ((N_1-N_o)/2)(t_1)+((N_1+N_2-2N_o)/2)(t_2-t_1)+((N_{n-1}+N_n-2N_o)/2)(t_n-t_{n-1})$$

where: $A$ = Area
$N_o$ = Nominal number of cells/mL at $t_o$
$N_1$ = Mean measured number of cells/mL at $t_1$
$N_2$ = Mean measured number of cells/mL at $t_2$
$N_n$ = Mean measured number of cells/mL at $t_n$
$t_1$ = time of first measurement after beginning of test (hours)
$t_2$ = time of second measurement after beginning of test (hours)
$t_n$ = time of $n^{th}$ measurement after beginning of test (hours)

Growth rates were calculated for the control and each treatment group using the following formula:

$$\text{Growth Rate} = \frac{\ln (N_n/N_o)}{t_n}$$

where: $N_o$ = Mean measured number of cells/mL at $t_o$
$N_n$ = Mean measured number of cells/mL at $t_n$
$t_n$ = Time of $n^{th}$ measurement after beginning of test (hours)

Percent inhibition values were calculated for each treatment group as the percent reduction in cell density, area under the growth curve and growth rate relative to the control replicates. The following formula was used:

$$\text{Percent Inhibition} = \frac{\text{Mean Cell Density}_{\text{Control}} - \text{Mean Cell Density}_{\text{Treatment}}}{\text{Mean Cell Density}_{\text{Control}}} \times 100$$

Cell densities, areas under the growth curve and growth rates were analyzed statistically to estimate the EC10, EC50 and EC90 values (i.e., the theoretical test concentrations that would produce a 10, 50 or 90% reduction in each parameter, respectively) and 95% confidence limits at 72 and 96 hours. EC50 values were also calculated for the 24 and 48 hour time intervals. The EC values and 95% confidence limits were calculated by linear interpolation using TOXSTAT Version 3.5 (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 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 NOAEC values.

Analytical Chemistry

Samples of test medium were collected from the negative control and each treatment group at test initiation, at approximately 72 hours and at test termination to measure concentrations of the test substance. Samples of test medium collected at test initiation were taken from the individual batches of test solution prepared for each treatment and the control group. Samples collected at 72 hours were collected from the additional "analytical" replicates. Samples collected at test termination were a composite of the remaining replicates for each treatment and the control group. The samples were placed in plastic (Nalgene®) bottles and were analyzed immediately without storage. Analytical procedures used in the analysis of the samples are presented in Appendix III.

## RESULTS AND DISCUSSION

Measurement of Test Concentrations

Results of analyses to measure concentrations of PFOS in the test solutions are presented in Table 1 and Appendix III. Nominal concentrations selected for use in this study were 5.7, 11, 23, 46, 91 and 183 mg a.i./L. Samples collected at the beginning of the test had measured concentrations that ranged from 82 to 98% of nominal. Samples collected at 72 hours and test termination had recoveries that ranged from

82 to 105% and 90 to 103% of nominal, respectively. When the values obtained for test initiation, at 72 hours and at test termination were averaged, the mean measured test concentrations were 5.5, 11, 21, 44, 86 and 179 mg a.i./L.

Observations and Measurements

Measurements of temperature and light intensity are presented in Tables 2 and 3, respectively. The temperatures ranged from 23.6 to 25.8°C and were within the range established for the test (24±2°C). The light intensity ranged from 3870 to 4540 lux and was within the desired range for the test (approximately 3870 to 4730 lux) (Table 3). Measurements of pH were 7.4 to 7.5 on Day 0 and ranged from 7.5 to 8.4 at 96 hours (Table 4). The pH of the "abiotic" replicate at test termination was 7.4.

The effect of PFOS upon *Selenastrum capricornutum* was determined by evaluating differences in cell densities, areas under the growth curve and growth rates. Mean values were used to calculate growth inhibition for each 24-hour period. Mean cell densities, areas under the growth curve and growth rates and their corresponding percent inhibition values are presented in Tables 5, 6 and 7, respectively. Cell density, area under the growth curve and growth rate values for each individual replicate are presented in Appendices IV, V and VI, respectively. EC10, EC50 and EC90 values and 95% confidence limits calculated for each 24-hour interval based on cell density, area under the growth curve and growth rate are presented in Tables 8, 9 and 10, respectively.

Changes in cell density indicated that exponential growth occurred in the negative control replicates (Figure 1). The coefficient of variation for control cell density was 4%. After 72 hours of exposure, cell density percent inhibition in the 5.5, 11, 21, 44, 86 and 179 mg a.i./L treatment groups was -5.2, -1.9, -5.5, 11, 72 and 97%, respectively. Dunnett's test showed that cell density was significantly reduced in the 86 and 179 mg a.i./L treatment groups in comparison to the negative control ($p \leq 0.05$). Consequently, the NOAEC for 72 hour cell density was 44 mg a.i./L. After 96 hours of exposure, cell density percent inhibition in the 5.5, 11, 21, 44, 86 and 179 mg a.i./L treatment groups was -11, -5.1, -18, -12, 77 and 99%, respectively. Dunnett's test showed that cell density was significantly reduced in the 86 and 179 mg a.i./L treatment groups ($p \leq 0.05$). Consequently, the NOAEC for 96 hour cell density was 44 mg a.i./L.

AMENDED

After 72 hours of exposure, area under the growth curve percent inhibition in the 5.5, 11, 21, 44, 86 and 179 mg a.i./L treatment groups was -2.9, 1.3, -3.8, 5.8, 68 and 94%, respectively. Dunnett's test showed that area under the growth curve was significantly reduced in the 86 and 179 mg a.i./L treatment groups ($p \leq 0.05$). Consequently, the NOAEC for 72 hour area under the growth curve was 44 mg a.i./L. After 96 hours of exposure, area under the growth curve percent inhibition in the 5.5, 11, 21, 44, 86 and 179 mg a.i./L treatment groups was -8.5, -3.3, -13, -5.3, 75 and 98%, respectively. Dunnett's test showed that area under the growth curve was significantly reduced in the 86 and 179 mg a.i./L treatment groups ($p \leq 0.05$). Consequently, the NOAEC for 96 hour area under the growth curve was 44 mg a.i./L.

After 72 hours of exposure, growth rate percent inhibition in the 5.5, 11, 21, 44, 86 and 179 mg a.i./L treatment groups was -1.1, -0.68, -0.72, 3.4, 31 and 82%, respectively. Dunnett's test showed that growth rate was significantly reduced in the 86 and 179 mg a.i./L treatment groups ($p \leq 0.05$). Consequently, the NOAEC for 72 hour growth rate was 44 mg a.i./L. After 96 hours of exposure, growth rate percent inhibition in the 5.5, 11, 21, 44, 86 and 179 mg a.i./L treatment groups was -1.9, -0.84, -3.0 -2.0, 27 and 79%, respectively. Dunnett's test showed that growth rate was significantly reduced in the 86 and 179 mg a.i./L treatment groups ($p \leq 0.05$). Consequently, the NOAEC for 96 hour growth rate was 44 mg a.i./L.

Visual and Microscopic Observations

After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the test flasks in the negative control or any PFOS 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 a.i./L treatment group.

Reversibility of Growth Inhibition

The 179 mg a.i./L treatment group was maximally inhibited at the end of the 96-hour exposure period. 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 algistatic. Cell densities for the recovery phase are presented in Table 11 and are illustrated graphically in Figure 3.

## CONCLUSIONS

The conclusions of this study were based on the most sensitive endpoint measured (i.e., cell density, area under the growth curve and/or growth rate). The 72-hour EC50, based on cell density, was 70 mg a.i./L with 95% confidence limits of 44 and 78 mg a.i./L. The 96-hour EC10, based on cell density, was 49 mg a.i./L with 95% confidence limits of 43 and 50 mg a.i./L. The 96-hour EC50, based on cell density, was 71 mg a.i./L with 95% confidence limits of 66 and 73 mg a.i./L. The 96-hour EC90, based on cell density, was 137 mg a.i./L with 95% confidence limits of 105 and 153 mg a.i./L The 72-hour and 96-hour NOAEC values, based on cell density, area under the growth curve and growth rate, were 44 mg a.i./L. Based on the presence of visible algal growth at recovery termination, PFOS was considered to be algistatic, rather than algicidal, at the concentrations tested.

## REFERENCES

1 **U.S. Environmental Protection Agency**. 1996. Series 850 - Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400: *Algal Toxicity, Tiers I and II.*

2 **Organization of Economic Cooperation and Development.** 1984. *Algal, Growth Inhibition Test.* OECD Guideline for Testing of Chemicals. Guideline 201. Paris.

3 **ASTM Standard Guide 1218-90E,** *Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae*. August 1990.

4 **The SAS System for Windows**. 1996. Release 6.12, TS Level 0020. SAS Institute Inc., Cary, North Carolina.

5 **West, Inc. and D.D. Gulley**. TOXSTAT Version 3.5. Copyright 1996. Western EcoSystems Technology, Inc., Cheyenne, Wyoming.

Table 1

Summary of Analytical Chemistry Data

| | |
|---|---|
| Sponsor: | 3M Corporation |
| Test Substance: | PFOS |
| Test Organism: | Freshwater Alga, *Selenastrum capricornutum* |
| Dilution Water: | Freshwater Algal Medium |

| Nominal Concentration (mg a.i./L) | Sampling Time (Hours) | Measured Concentration[1] (mg a.i./L) | Mean Measured Concentration (mg a.i./L) | Percent of Nominal |
|---|---|---|---|---|
| Negative Control | 0[2] | < LOQ | -- | -- |
| | 72[3] | < LOQ | | |
| | 96[4] | <LOQ | | |
| 5.7 | 0 | 4.73 | 5.5 | 96 |
| | 72 | 6.04 | | |
| | 96 | 5.84 | | |
| 11 | 0 | 10.7 | 11 | 100 |
| | 72 | 11.2 | | |
| | 96 | 12.1 | | |
| 23 | 0 | 19.8 | 21 | 91 |
| | 72 | 23.1 | | |
| | 96 | 20.7 | | |
| 46 | 0 | 42.7 | 44 | 96 |
| | 72 | 41.9 | | |
| | 96 | 46.3 | | |
| 91 | 0 | 83.3 | 86 | 95 |
| | 72 | 86.0 | | |
| | 96 | 88.3 | | |
| 183 | 0 | 179 | 179 | 98 |
| | 72 | 186 | | |
| | 96 | 172 | | |
| 183 | 72[5] | 150 | 169 | 92 |
| | 96[5] | 188 | | |

[1]Limit of Quantitation (LOQ) was 0.115 mg a.i./L.
[2]0-hour samples were collected from individual batches of test solution prepared for the treatment and control groups at test initiation.
[3]72-hour samples were collected from the additional (D) replicate (biotic).
[4]96-hour samples were composites of test solution collected from each of the three replicates per treatment and control group.
[5]72 and 96 hour sample collected from the additional (E) replicate (abiotic).

AMENDED

Table 2

Temperature Measurements

| | |
|---|---|
| Sponsor: | 3M Corporation |
| Test Substance: | PFOS |
| Test Organism: | Freshwater Alga, *Selenastrum capricornutum* |
| Dilution Water: | Freshwater Algal Medium |

| Time (Day) | Temperature (°C) | |
|---|---|---|
| | Measurement 1 | Measurement 2 |
| 0 | 23.6 | 24.2 |
| 1 | 24.8 | 24.2 |
| 2 | 24.8 | 24.0 |
| 3 | 25.1 | 25.3 |
| 4 | 25.8 | 24.9 |
| 5 | 25.7 | 24.4 |
| 6 | 25.6 | 24.9 |
| 7 | 24.0 | 24.0 |
| 8 | 23.9 | 23.8 |
| 9 | 23.9 | 23.9 |

Table 3

Light Intensity Measurements

| | |
|---|---|
| Sponsor: | 3M Corporation |
| Test Substance: | PFOS |
| Test Organism: | Freshwater Alga, *Selenastrum capricornutum* |
| Dilution Water: | Freshwater Algal Medium |

| Test Day | Light Intensity Measurements (lux) | | | | |
|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 3 | No. 4 | No.5 |
| 0 | 3940 | 3880 | 4300 | 3900 | 3870 |
| 1 | 3910 | 4270 | 4440 | 4610 | 4010 |
| 2 | 4000 | 4290 | 4310 | 4540 | 4170 |
| 3 | 4120 | 4400 | 4350 | 4160 | 4240 |
| 4 | 4200 | 3990 | 3890 | 4070 | 4140 |
| 5 | 3910 | 4120 | 4180 | 4200 | 4340 |
| 6 | 4180 | 4200 | 4120 | 4200 | 4370 |
| 7 | 4300 | 4180 | 3950 | 4430 | 4080 |
| 8 | 4010 | 4230 | 4180 | 4230 | 4450 |
| 9 | 4150 | 4090 | 4240 | 4350 | 4270 |

Table 4

pH Measurements

| | |
|---|---|
| Sponsor: | 3M Corporation |
| Test Substance: | PFOS |
| Test Organism: | Freshwater Alga, *Selenastrum capricornutum* |
| Dilution Water: | Freshwater Algal Medium |

| Mean Measured Concentration (mg a.i./L) | pH Measurements | |
|---|---|---|
| | 0 Hours[1] | 96 Hours[2] |
| Negative Control | 7.5 | 8.1 |
| 5.5 | 7.4 | 8.2 |
| 11 | 7.5 | 8.2 |
| 21 | 7.5 | 8.4 |
| 44 | 7.4 | 8.3 |
| 86 | 7.4 | 7.8 |
| 179 | 7.4 | 7.5 |
| 169 (Abiotic) | -- | 7.4 |

[1] 0-hour samples were collected from the batches of test solution prepared for the treatment and control groups at test initiation.

[2] 96-hour samples were collected from the pooled replicates per treatment and control group.

AMENDED

Table 5

Mean Cell Densities and Percent Inhibition for Each 24-Hour Interval During the Test[1]

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Freshwater Alga, *Selenastrum capricornutum*
Dilution Water: Freshwater Algal Medium

| Mean Measured Concentration (mg a.i./L) | 24 Hours | | 48 Hours | | 72 Hours | | 96 Hours | |
|---|---|---|---|---|---|---|---|---|
| | Mean Cell Density | Percent Inhibition | Mean Cell Density | Percent Inhibition | Mean Cell Density | Percent Inhibition | Mean Cell Density | Percent Inhibition |
| Negative Control | 32,667 | -- | 129,000 | -- | 603,333 | -- | 2,740,000 | -- |
| 5.5 | 29,000 | 11 | 129,333 | -0.26 | 635,000 | -5.2 | 3,040,000 | -11 |
| 11 | 29,333 | 10 | 121,000 | 6.2 | 615,000 | -1.9 | 2,880,000 | -5.1 |
| 21 | 28,000 | 14 | 133,667 | -3.6 | 636,667 | -5.5 | 3,240,000 | -18 |
| 44 | 29,667 | 9.2 | 140,667 | -9.0 | 535,000 | 11 | 3,080,000 | -12 |
| 86 | 25,667 | 21 | 56,000 | 57 | 171,000[2] | 72 | 626,667[3] | 77 |
| 179 | 14,333 | 56 | 27,667 | 79 | 20,667[2] | 97 | 33,667[3] | 99 |

[1] Values calculated using SAS 6.12. Manual calculations may differ slightly
[2] Indicates a significant difference from the negative control at 72 hours using Dunnett's test ($p \leq 0.05$).
[3] Indicates a significant difference from the negative control at 96 hours using Dunnett's test ($p \leq 0.05$).

AMENDED

Table 6

Mean Areas Under the Growth Curve and Percent Inhibition for Each 24-Hour Interval During the Test[1]

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Freshwater Alga, *Selenastrum capricornutum*
Dilution Water: Freshwater Algal Medium

| Mean Measured Test Concentration (mg a.i./L) | 0 - 24 Hours | | 0 - 48 Hours | | 0 - 72 Hours | | 0 - 96 Hours | |
|---|---|---|---|---|---|---|---|---|
| | Mean Area | Percent Inhibition | Mean Area | Percent Inhibition | Mean Area | Percent Inhibition | Mean Area | Percent Inhibition |
| Negative Control | 272,000 | -- | 1,972,000 | -- | 10,520,000 | -- | 50,400,000 | -- |
| 5.5 | 228,000 | 16 | 1,888,000 | 4.3 | 10,820,000 | -2.9 | 54,680,000 | -8.5 |
| 11 | 232,000 | 15 | 1,796,000 | 8.9 | 10,388,000 | 1.3 | 52,088,000 | -3.3 |
| 21 | 216,000 | 21 | 1,916,000 | 2.8 | 10,920,000 | -3.8 | 57,200,000 | -13 |
| 44 | 236,000 | 13 | 2,040,000 | -3.4 | 9,908,000 | 5.8 | 53,048,000 | -5.3 |
| 86 | 188,000 | 31 | 928,000 | 53 | 3,412,000[2] | 68 | 12,7444,000[3] | 75 |
| 179 | 52,000 | 81 | 316,000 | 84 | 656,000[2] | 94 | 1,068,000[3] | 98 |

[1] Values calculated using SAS 6.12. Manual calculations may differ slightly
[2] Indicates a significant difference from the negative control at 72 hours using Dunnett's test ($p \leq 0.05$).
[3] Indicates a significant difference from the negative control at 96 hours using Dunnett's test ($p \leq 0.05$).

Table 7

Mean Growth Rates and Percent Inhibition for Each 24-Hour Interval During the Test[1]

| | |
|---|---|
| Sponsor: | 3M Corporation |
| Test Substance: | PFOS |
| Test Organism: | Freshwater Alga, *Selenastrum capricornutum* |
| Dilution Water: | Freshwater Algal Medium |

| Mean Measured Test Concentration (mg a.i./L) | 0 - 24 Hours | | 0 - 48 Hours | | 0 - 72 Hours | | 0 - 96 Hours | |
|---|---|---|---|---|---|---|---|---|
| | Mean Growth Rate | Percent Inhibition | Mean Growth Rate | Percent Inhibition | Mean Growth Rate | Percent Inhibition | Mean Growth Rate | Percent Inhibition |
| Negative Control | 0.0493 | -- | 0.0533 | -- | 0.0568 | -- | 0.0585 | -- |
| 5.5 | 0.0438 | 11 | 0.0533 | 0.0 | 0.0574 | -1.1 | 0.0595 | -1.9 |
| 11 | 0.0448 | 9.1 | 0.0518 | 2.7 | 0.0572 | -0.68 | 0.0590 | -0.84 |
| 21 | 0.0428 | 13 | 0.0540 | -1.4 | 0.0572 | -0.72 | 0.0602 | -3.0 |
| 44 | 0.0452 | 8.3 | 0.0551 | -3.3 | 0.0549 | 3.4 | 0.0597 | -2.0 |
| 86 | 0.0373 | 24 | 0.0357 | 33 | 0.0392[2] | 31 | 0.0429[3] | 27 |
| 179 | 0.0138 | 72 | 0.0208 | 61 | 0.0100[2] | 82 | 0.0124[3] | 79 |

[1] Values calculated using SAS 6.12. Manual calculations may differ slightly

[2] Indicates a significant difference from the negative control at 72 hours using Dunnett's test ($p \leq 0.05$).

[3] Indicates a significant difference from the negative control at 96 hours using Dunnett's test ($p \leq 0.05$).

Table 8

EC10, EC50 and EC90 Values Based on Cell Density Over the 96-Hour Exposure Period

| | |
|---|---|
| Sponsor: | 3M Corporation |
| Test Substance: | PFOS |
| Test Organism: | Freshwater Alga, *Selenastrum capricornutum* |
| Dilution Water: | Freshwater Algal Medium |

| Time | EC10 (mg a.i./L) | 95% Confidence Limits (mg a.i./L) | EC50 (mg a.i./L) | 95% Confidence Limits (mg a.i./L) | EC90 (mg a.i./L) | 95% Confidence Limits (mg a.i./L) |
|---|---|---|---|---|---|---|
| 24 Hours | Not Determined | -- | 163 | 74 and 191 | Not Determined | -- |
| 48 Hours | Not Determined | -- | 81 | 72 and 90 | Not Determined | -- |
| 72 Hours | 37 | <0.0 and 64 | 70 | 44 and 78 | 153 | 130 and 165 |
| 96 Hours | 49 | 43 and 50 | 71 | 66 and 73 | 137 | 105 and 153 |

AMENDED

Table 9

EC10, EC50 and EC90 Values Based on Area Under the Growth Curve Over the 96-Hour Exposure Period

| | |
|---|---|
| Sponsor: | 3M Corporation |
| Test Substance: | PFOS |
| Test Organism: | Freshwater Alga, *Selenastrum capricornutum* |
| Dilution Water: | Freshwater Algal Medium |

| Time | EC10 (mg a.i./L) | 95% Confidence Limits (mg a.i./L) | EC50 (mg a.i./L) | 95% Confidence Limits (mg a.i./L) | EC90 (mg a.i./L) | 95% Confidence Limits (mg a.i./L) |
|---|---|---|---|---|---|---|
| 24 Hours | Not Determined | -- | 122 | 19 and 176 | Not Determined | -- |
| 48 Hours | Not Determined | -- | 84 | 67 and 146 | Not Determined | -- |
| 72 Hours | 46 | <0.0 and 56 | 74 | 55 and 82 | 165 | 145 and 176 |
| 96 Hours | 49 | 40 and 50 | 71 | 67 and 74 | 145 | 125 and 155 |

AMENDED

Table 10

EC10, EC50 and EC90 Values Based on Growth Rate Over the 96-Hour Exposure Period

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Freshwater Alga, *Selenastrum capricornutum*
Dilution Water: Freshwater Algal Medium

| Time | EC10 (mg a.i./L) | 95% Confidence Limits (mg a.i./L) | EC50 (mg a.i./L) | 95% Confidence Limits (mg a.i./L) | EC90 (mg a.i./L) | 95% Confidence Limits (mg a.i./L) |
|---|---|---|---|---|---|---|
| 24 Hours | Not Determined | -- | 136 | 30 and 204 | Not Determined | -- |
| 48 Hours | Not Determined | -- | 142 | 107 and 185 | Not Determined | -- |
| 72 Hours | 53 | 23 and 64 | 120 | 103 and 132 | >179 | --[1] |
| 96 Hours | 59 | 54 and 63 | 126 | 115 and 138 | >179 | --[1] |

[1] Confidence limits could not be calculated with the data obtained.

AMENDED

Table 11

Cell Densities for the Recovery Phase

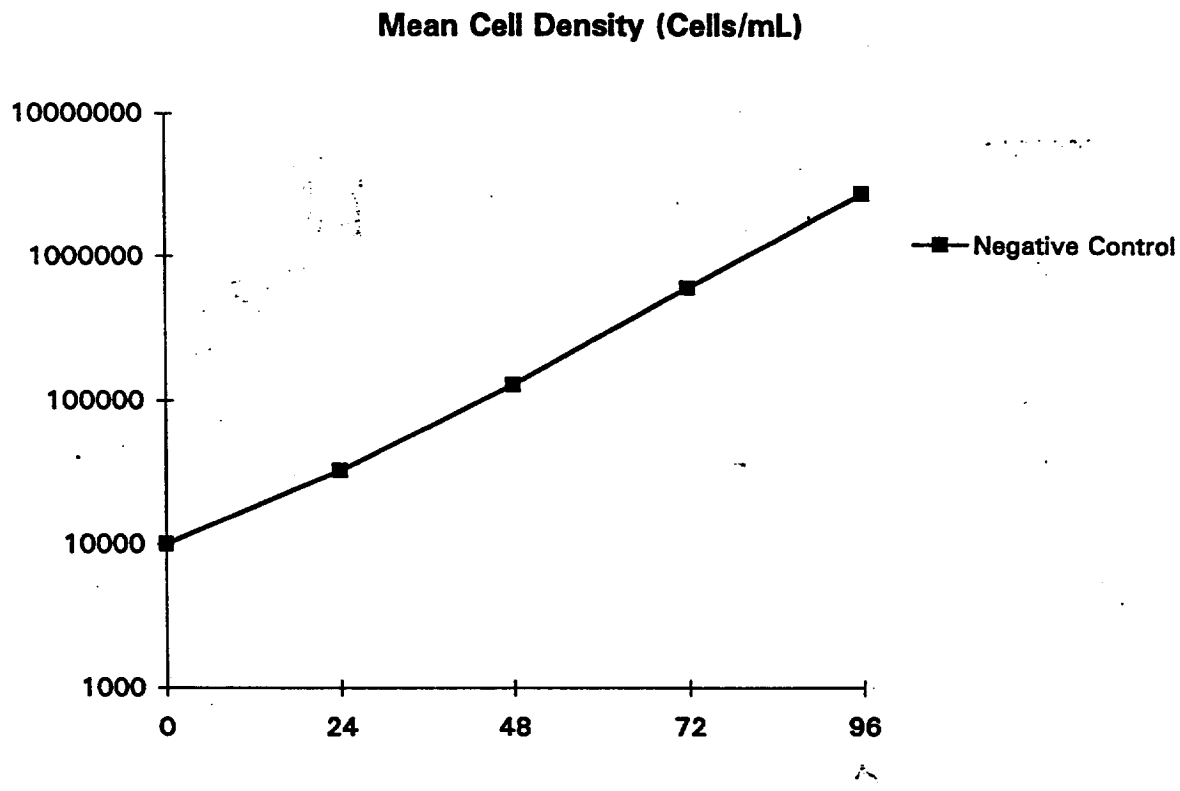
| | |
|---|---|
| Sponsor: | 3M Corporation |
| Test Substance: | PFOS |
| Test Organism: | Freshwater Alga, *Selenastrum capricornutum* |
| Dilution Water: | Freshwater Algal Medium |

| Mean Measured Test Concentration (mg a.i./L) | Cell Densities (Cells/mL[2]) | | |
|---|---|---|---|
| | Day 0 | Day 3 | Day 5 |
| Negative Control Rep. A | 10,000 | 1,220,000 | 6,420,000 |
| 179[1] | 1,000 | 35,000 | 1,180,000 |

[1] The treatment group was diluted to a concentration of the test substance that theoretically would not inhibit growth.

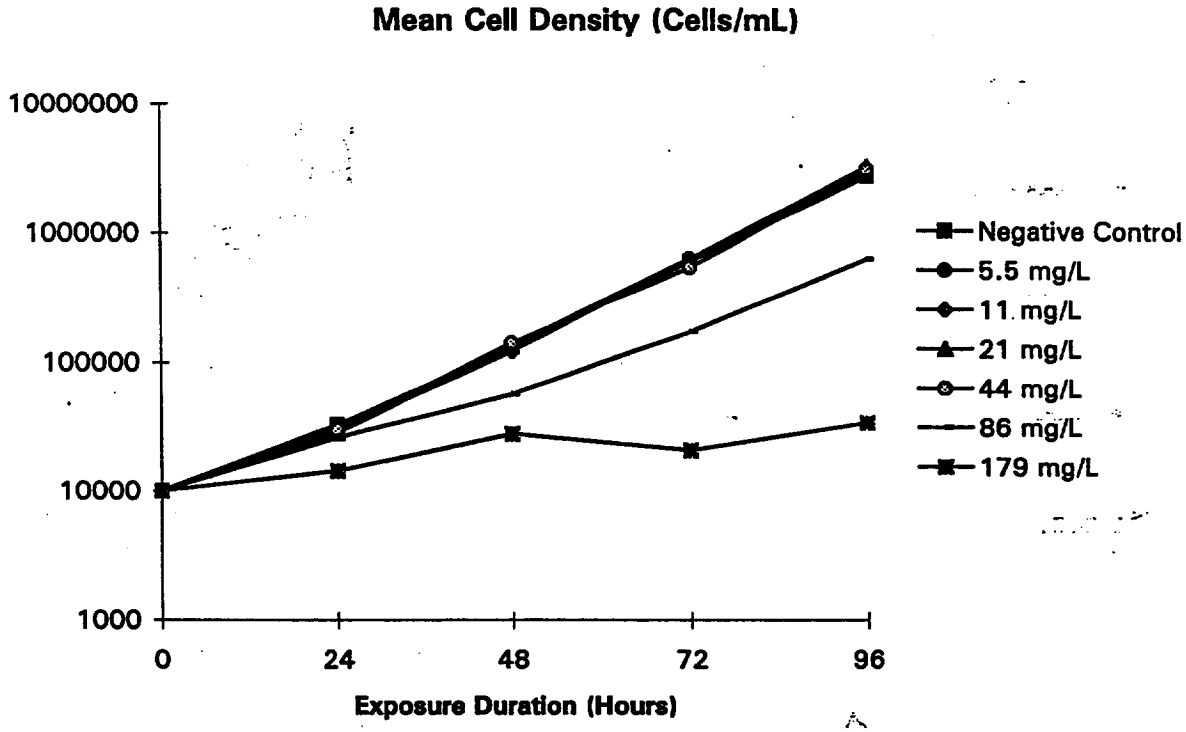
[2] Due to the method used to prepare recovery phase test solutions, initial cell densities were not equivalent throughout the treatment groups.

Figure 1. Negative Control Algal Growth, Expressed in Cell Density, During the 96-Hour Exposure.



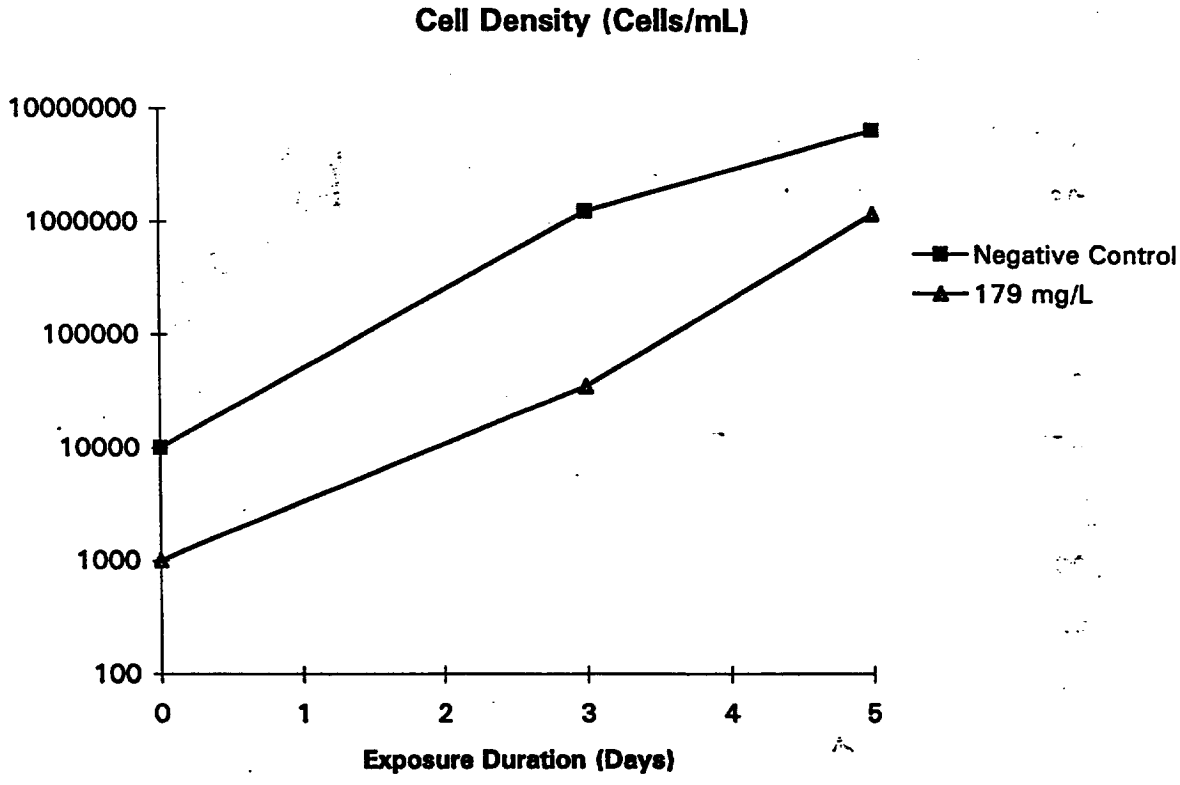

AMENDED

Figure 2. Concentration-Response Curve, Expressed in Cell Density




AMENDED

Figure 3 - Recovery Phase Response Curve, Expressed in Cell Density




AMENDED

# APPENDIX I

## Freshwater Algal Medium[1]

| | |
|---|---|
| Sponsor: | 3M Corporation |
| Test Substance: | PFOS |
| Test Organism: | Freshwater Alga, *Selenastrum capricornutum* |
| Dilution Water: | Freshwater Algal Medium |

| Compound | Nominal Concentration | |
|---|---|---|
| $MgCl_2 \bullet 6H_2O$ | 12.16 | mg/L |
| $CaCl_2 \bullet 2H_2O$ | 4.40 | mg/L |
| $H_3BO_3$ | 0.1856 | mg/L |
| $MnCl_2 \bullet 4H_2O$ | 0.416 | mg/L |
| $ZnCl_2$ | 3.28 | µg/L |
| $FeCl_3 \bullet 6H_2O$ | 0.1598 | mg/L |
| $CoCl_2 \bullet 6H_2O$ | 1.428 | µg/L |
| $Na_2MoO_4 \bullet 2H_2O$ | 7.26 | µg/L |
| $CuCl_2 \bullet 2H_2O$ | 0.012 | µg/L |
| $Na_2EDTA \bullet 2H_2O$ | 0.300 | mg/L |
| $NaNO_3$ | 25.50 | mg/L |
| $MgSO_4 \bullet 7H_2O$ | 14.70 | mg/L |
| $K_2HPO_4$ | 1.044 | mg/L |
| $NaHCO_3$ | 15.0 | mg/L |

[1] The pH was adjusted to 7.5 ± 0.1 using 10% HCl.

## APPENDIX II
Analyses of Pesticides, Organics, Metals and Other Inorganics
in Wildlife International Ltd. Well Water[1]

| ANALYSIS | MEASURED CONCENTRATION | | |
|---|---|---|---|
| **Miscellaneous Measurements** | | | |
| Total Dissolved Solids | | 286 | mg/L |
| Ammonia Nitrogen | < | 0.050 | mg/L |
| Total Organic Carbon[2] | < | 1.0 | mg/L |
| Total Cyanide | < | 10.0 | µg/L |
| **Organochlorines and PCBs** | | | |
| Aldrin | < | 0.005 | µg/L |
| Alpha BHC | < | 0.005 | µg/L |
| Beta BHC | < | 0.005 | µg/L |
| Delta BHC | < | 0.005 | µg/L |
| Gamma BHC (Lindane) | < | 0.006 | µg/L |
| Chlordane | < | 0.025 | µg/L |
| DDD, pp' | < | 0.006 | µg/L |
| DDE, pp' | < | 0.005 | µg/L |
| DDT, pp' | < | 0.008 | µg/L |
| Dieldrin | < | 0.005 | µg/L |
| Endosulfan, A | < | 0.005 | µg/L |
| Endosulfan, B | < | 0.005 | µg/L |
| Endosulfan Sulfate | < | 0.018 | µg/L |
| Endrin | < | 0.010 | µg/L |
| Endrin Aldehyde | < | 0.005 | µg/L |
| Heptachlor | < | 0.005 | µg/L |
| Methoxychlor | < | 0.007 | µg/L |
| Heptachlor Epoxide | < | 0.005 | µg/L |
| Toxaphene | < | 0.500 | µg/L |
| PCB-1016 | < | 0.260 | µg/L |
| PCB-1221 | < | 0.260 | µg/L |
| PCB-1232 | < | 0.260 | µg/L |
| PCB-1242 | < | 0.720 | µg/L |
| PCB-1248 | < | 0.720 | µg/L |
| PCB-1254 | < | 0.720 | µg/L |
| PCB-1260 | < | 0.720 | µg/L |
| **Metals and Other Inorganics** | | | |
| Aluminum[3] | < | 100 | µg/L |
| Arsenic[3] | < | 25.0 | µg/L |
| Beryllium[3] | < | 0.50 | µg/L |
| Cadmium[3] | < | 1.0 | µg/L |
| Calcium[3] | | 35.0 | mg/L |
| Chromium[3] | < | 2.0 | µg/L |
| Cobalt[3] | < | 1.0 | µg/L |
| Copper[3] | < | 20.0 | µg/L |
| Iron[3] | < | 100 | µg/L |
| Lead[3] | < | 10.0 | µg/L |
| Magnesium[3] | | 13.5 | mg/L |
| Manganese[3] | < | 1.0 | µg/L |
| Mercury | < | 0.20 | µg/L |
| Molybdenum[3] | < | 2.0 | µg/L |
| Nickel[3] | < | 2.0 | µg/L |
| Iron[3] | | 6.62 | mg/L |
| Selenium[3] | < | 25.0 | µg/L |
| Silver[3] | < | 1.0 | µg/L |
| Sodium[3] | | 21.3 | mg/L |
| Zinc[3] | < | 20.0 | µg/L |

[1] Analyses performed by QST Environmental, Gainesville, Florida for samples collected on November 3 through November 7, 1997.
[2] Analyses performed by Wildlife International Ltd. for the sample collected on November 5, 1997.
[3] Analyses performed by Wildlife International Ltd. for samples collected on November 5 through 7, 1997.

# APPENDIX III

## THE ANALYSIS OF PFOS IN FRESHWATER ALGAL MEDIUM

## IN SUPPORT OF

## WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454A-103A

## REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFOS: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454A-103A

PRINCIPAL INVESTIGATOR:

Raymond L. Van Hoven, Ph.D.
Scientist

4-24-00
DATE

MANAGEMENT:

Willard B. Nixon, Ph.D.
Manager, Analytical Chemistry

4/24/00
DATE

AMENDED

Introduction

Freshwater algal medium samples were collected from an acute toxicity study designed to determine the effects of PFOS (Perfluorooctane Sulfonic Acid Potassium Salt) to the freshwater alga (*Selenastrum capricornutum*). This study was conducted by Wildlife International Ltd. and identified as Project No.: 454A-103A. The analyses of these water samples were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Samples were received for analysis on April 12, 15 and 16, 1999 and were analyzed on each sample receipt day.

Test Substance and Internal Standard

The test substance used for the analytical portion of this study was Wildlife International Ltd. identification number 4675. The test substance was used to prepare calibration standards and matrix fortification samples.

The internal standard was received from 3M Corporation on July 2, 1998 and was assigned Wildlife International Ltd. identification number 4526 upon receipt. The internal standard, a granular material, was identified as: 1H, 1H, 2H, 2H Perfluorooctane Sulfonic Acid, Chemical Abstract Number: 27619-97-2. The standard was stored under ambient conditions.

Analytical Method

The method used for the analysis of the freshwater algal medium samples was developed at Wildlife International Ltd. and entitled "Analytical Method Validation for the Determination of PFOS in Freshwater, Saltwater, and Algal Media". This methodology was included as Appendix II of Wildlife International Ltd. protocol number 454/011299/MVAL/SUB454. It was based upon methodology provided by 3M Corporation.

Samples were diluted in a 50% methanol : 50% NANOpure® water solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v) so that they fell within the calibration range of the PFOS methodology.

Concentrations of PFOS in the standards and samples were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid

Chromatograph (HPLC) with a Perkin-Elmer API 100LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. HPLC separations were achieved using a Keystone Betasil $C_{18}$ analytical column (100 mm x 2 mm I.D., 3 µm particle size). The instrument parameters are summarized in Table 1. A method flowchart is provided in Figure 1.

Calibration Curve and Limit of Quantitation

Calibration standards of PFOS prepared in a 50% methanol : 50% NANOpure® water solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v), ranging in concentration from 0.00229 to 0.0457 mg a.i./L were analyzed with the samples. Linear regression equations were generated using peak area response ratios (PFOS : internal standard) versus the respective concentration ratios (PFOS : internal standard) of the calibration standards. A typical calibration curve is presented in Figure 2. The concentration of PFOS in the samples was determined by substituting the peak area response ratios into the applicable linear regression equation. Representative ion chromatograms of low and high calibration standards are presented in Figures 3 and 4, respectively.

The method limit of quantitation (LOQ) for these analyses was set at 0.115 mg a.i./L calculated as the product of the lowest calibration standard analyzed (0.00229 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

Matrix Blank and Fortification Samples

Three matrix blank samples were analyzed to determine possible interference. No interferences were observed at or above the LOQ during samples analyses (Table 2). A representative chromatogram of a matrix blank is presented in Figure 5.

Freshwater algal medium was fortified at 0.915, 45.7 and 220 mg a.i./L and analyzed concurrently with the samples to determine the mean procedural recovery (Table 3). Sample concentrations were not corrected for the mean procedural recovery of 99.1%. A representative chromatogram of a matrix fortification is presented in Figure 6.

Example Calculations

Sample number 454A-103A-2, nominal concentration of 5.7 mg a.i./L in freshwater algal medium.

Initial Volume: 0.100 mL

Final Volume: 25.0 mL

Dilution Factor: 250

PFOS Peak Area: 164427

Internal Standard Peak Area: 240533

Peak Area Ratio: 0.6836

Calibration curve equation.

Slope: 3.43926

Intercept: 0.03327

Curve is weighted (1/x).

$$\text{PFOS (µg a.i./L) at instrument} = \frac{(\text{Peak area ratio} - (\text{Y-intercept})) \times \text{I.S. Concentration}}{\text{Slope}}$$

$$= \frac{(0.6836 - 0.03327) \times 100 \text{ µg a.i./L}}{3.43926}$$

$$= 18.9 \text{ µg a.i./L}$$

Note: I.S. = internal standard.

$$\text{PFOS (mg a.i./L) in sample} = \frac{\text{PFOS (µg a.i./L) at instrument} \times \text{Dilution Factor}}{1000 \text{ µg/mg}}$$

$$= \frac{18.9 \times 250}{1000}$$

AMENDED

$$= 4.73 \text{ mg a.i./L}$$

$$\text{Percent of Nominal Concentration} = \frac{\text{PFOS (mg a.i./L) in sample}}{\text{PFOS (mg a.i./L) nominal}} \times 100$$

Calculated recovery: 82.0%

Note: manual calculation may differ.

## RESULTS

Sample Analysis

Freshwater algal medium samples were collected from the acute toxicity study with the freshwater alga (*Selenastrum capricornutum*) at test initiation, April 12, 1999 (Hour 0), on April 15, 1999 (Hour 72), and at test termination, April 16, 1999 (Hour 96). The measured concentrations of PFOS in the samples collected at initiation of exposure of the test organisms (Hour 0) ranged from 82.0 to 98.1% of the nominal concentrations. Samples collected at Hour 72 had a measured concentration range of 91.7 to 105% of nominal values. The Hour-72 abiotic sample was 81.9% of the nominal concentration. Samples collected at test termination (Hour 96) had a measured concentration range of 90.3 to 102% of nominal values. The Hour-96 abiotic sample was 103% of the nominal concentration (Table 4). A representative chromatogram of a test sample is shown in Figure 7.

Table 1

Typical LC/MS Operational Parameters

| | |
|---|---|
| INSTRUMENT: | Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 100LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in selective ion monitoring mode (SIM). |
| ANALYTICAL COLUMN: | Keystone Betasil $C_{18}$ column (100 mm x 2 mm I.D., 3 µm particle size) |
| OVEN TEMPERATURE: | 30°C |
| STOP TIME: | 10.0 minutes |
| FLOW RATE: | 0.220 mL/minute |
| MOBILE PHASE: | 72.0% Methanol : 28.0% NANOpure® Water containing 0.1% Formic Acid |
| INJECTION VOLUME: | 25.0 µL |
| PFOS RETENTION TIME: | Approximately 6.6 minutes |
| INTERNAL STANDARD RETENTION TIME: | Approximately 4.5 minutes |
| PFOS MONITORED MASS: | 498.6 amu |
| INTERNAL STANDARD MONITORED MASS: | 426.7 amu |

Table 2

Matrix Blanks Analyzed Concurrently During Sample Analysis

| Sample | | Measured Concentration of PFOS[1] (mg a.i./L) |
|---|---|---|
| Number (454A-103A-) | Type | |
| MAB-1 | Matrix Blank | < LOQ |
| MAB-2 | Matrix Blank | < LOQ |
| MAB-3 | Matrix Blank | < LOQ |

[1] The limit of quantitation (LOQ) was 0.115 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.00229 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

Table 3

Matrix Fortifications Analyzed Concurrently During Sample Analysis

| Sample Number (454A-103A-) | Concentrations of PFOS (mg a.i./L) | | Percent Recovered |
|---|---|---|---|
| | Fortified | Measured | |
| MAS-1 | 0.915 | 0.884 | 96.6 |
| MAS-4 | 0.915 | 1.10 | 120 |
| MAS-7 | 0.915 | 1.02 | 112 |
| MAS-2 | 45.7 | 41.3 | 90.2 |
| MAS-5 | 45.7 | 48.4 | 106 |
| MAS-8 | 45.7 | 41.4 | 90.6 |
| MAS-3A | 220 | 210 | 95.2 |
| MAS-3B | 220 | 201 | 91.7 |
| MAS-3C | 220 | 209 | 95.0 |
| MAS-6 | 220 | 221 | 101 |
| MAS-9 | 220 | 202 | 91.9 |
| | | Mean | = 99.1 |
| | | Standard Deviation | = 9.75 |
| | | CV | = 9.84 |
| | | N | = 11 |

Note: Results and corrections for new test substance purity were generated using MacQuan version 1.5 software and manual calculations. Values have been rounded for reporting purposes.

Table 4

Measured Concentrations of PFOS in Freshwater Algal Medium Samples from a Freshwater Alga Acute Toxicity Test

| Nominal Test Concentration (mg a.i./L) | Sample Number (454A-103A-) | Sampling Time (Hours) | PFOS Measured Concentration[1] (mg a.i./L) | Percent of Nominal |
|---|---|---|---|---|
| 0.0 | 1 | 0 | < LOQ | -- |
| | 8 | 72 | < LOQ | -- |
| | 15 | 96 | < LOQ | -- |
| 5.7 | 2 | 0 | 4.73 | 82.0 |
| | 9 | 72 | 6.04 | 105 |
| | 16 | 96 | 5.84 | 101 |
| 11 | 3 | 0 | 10.7 | 89.8 |
| | 10 | 72 | 11.2 | 93.6 |
| | 17 | 96 | 12.1 | 102 |
| 23 | 4 | 0 | 19.8 | 86.2 |
| | 11 | 72 | 23.1 | 101 |
| | 18 | 96 | 20.7 | 90.3 |
| 46 | 5 | 0 | 42.7 | 93.4 |
| | 12 | 72 | 41.9 | 91.7 |
| | 19 | 96 | 46.3 | 101 |
| 91 | 6 | 0 | 83.3 | 91.0 |
| | 13 | 72 | 86.0 | 94.0 |
| | 20 | 96 | 88.3 | 96.5 |
| 183 | 7 | 0 | 179 | 98.1 |
| | 14 | 72 | 186 | 102 |
| | 21 | 96 | 172 | 94.0 |
| 183 (Abiotic) | 14A 22 | 72 96 | 150 188 | 81.9 103 |

Note: Results and corrections for new test substance purity were generated using MacQuan version 1.5 software and manual calculations. Values have been rounded for reporting purposes.

[1]The limit of quantitation (LOQ) was 0.115 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.00229 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

AMENDED

**METHOD OUTLINE FOR THE ANALYSIS OF PFOS IN FRESHWATER ALGAL MEDIUM**

Prepare matrix fortification samples by spiking the requisite volume of PFOS stock solutions directly into freshwater algal medium using gas-tight syringes and Class A volumetric flasks.

↓

Dilute matrix fortification and test samples into the range of the calibration standards by partially filling Class A volumetric flasks with 50% methanol : 50% NANOpure® water solution containing 0.100 mg 4H PFOS (internal standard)/L and 0.05% formic acid (v/v). Add the appropriate volume of sample and bring the flask to volume with the dilution solvent. Process the matrix blank sample using the same dilution and aliquot volume as for the lowest fortification level. Mix well by several repeat inversions.

↓

Ampulate samples and submit for LC/MS analysis.

**Figure 1.** Analytical method flowchart for the analysis of PFOS in freshwater algal medium.

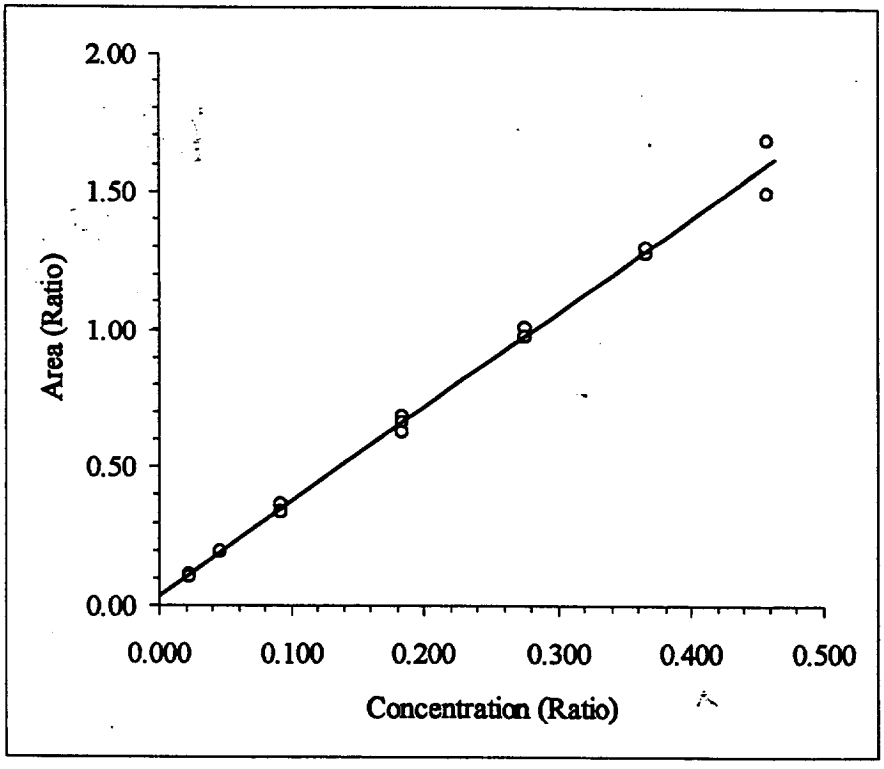



**Figure 2.** A typical calibration curve for PFOS. Slope = 3.43926; Intercept = 0.03327; r = 0.9986. Curve is weighted (1/x).

AMENDED

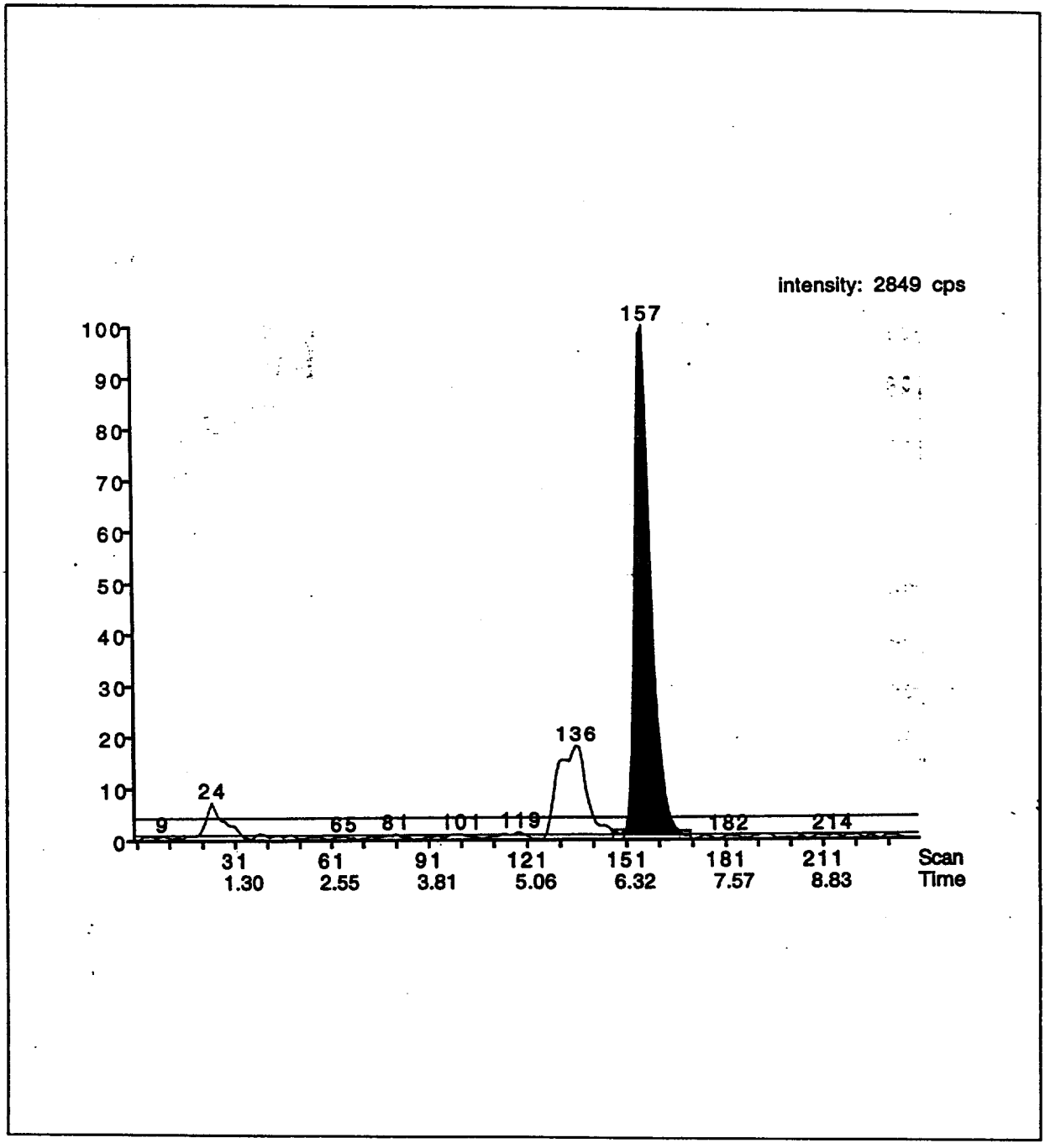



**Figure 3.** A representative ion chromatogram of a low-level (0.00229 mg a.i./L) PFOS standard.

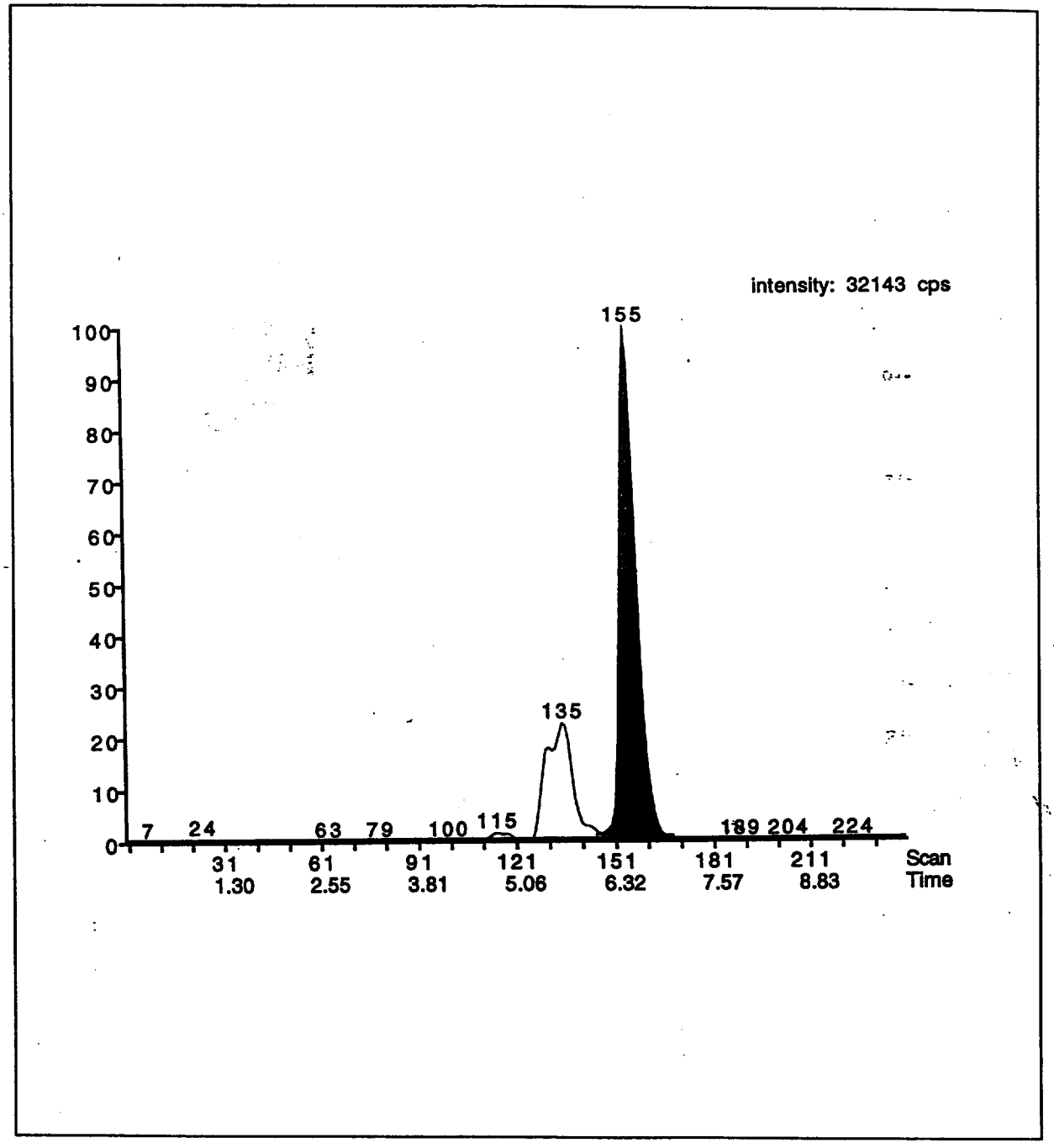



**Figure 4.** A representative ion chromatogram of a high-level (0.0457 mg a.i./L) PFOS standard.

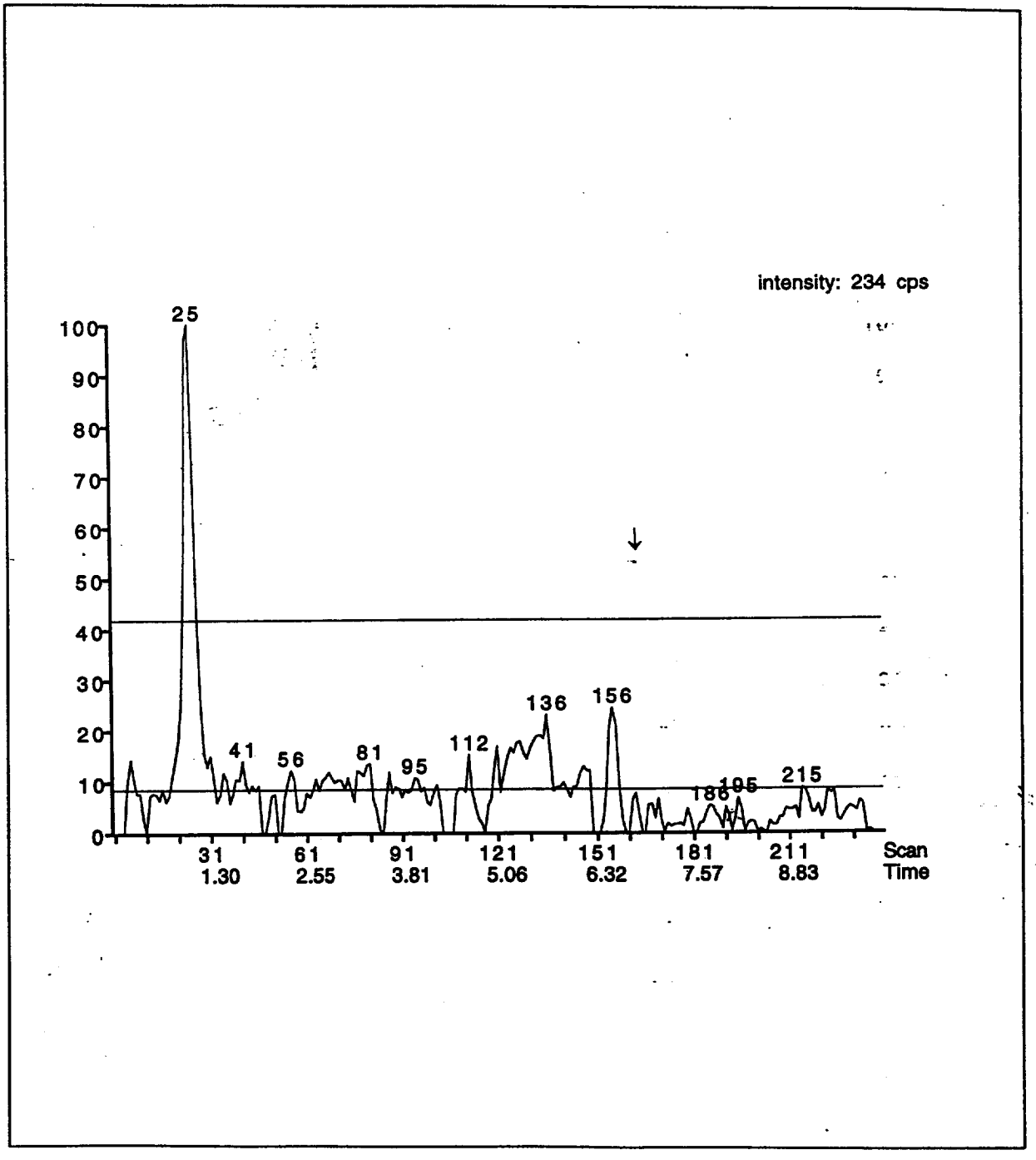



**Figure 5.** A representative chromatogram of a matrix blank sample (454A-103A-MAB-1). The arrow indicates the retention time of PFOS.

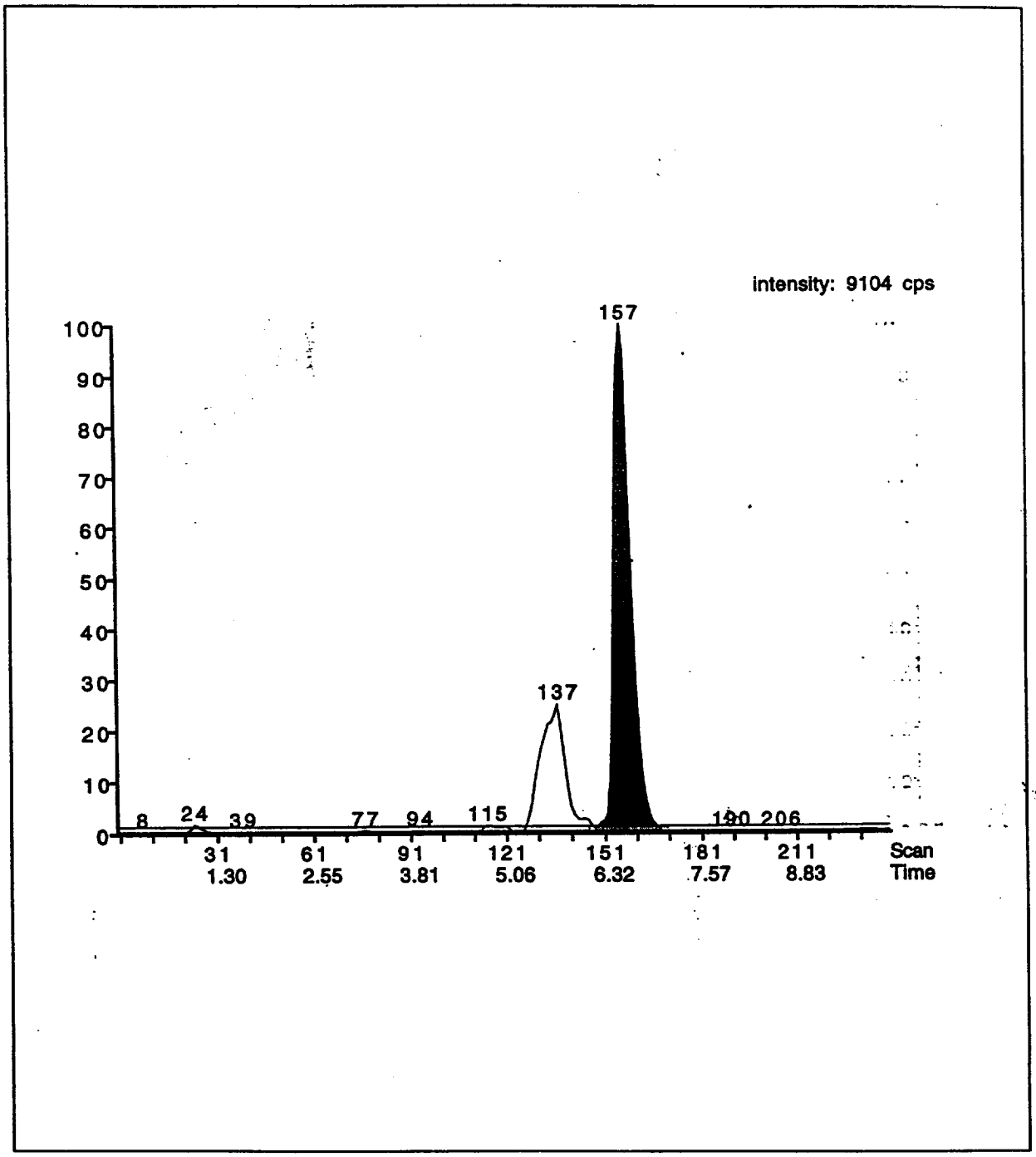



**Figure 6.** A representative chromatogram of a matrix fortification sample (454A-103A-MAS-1).

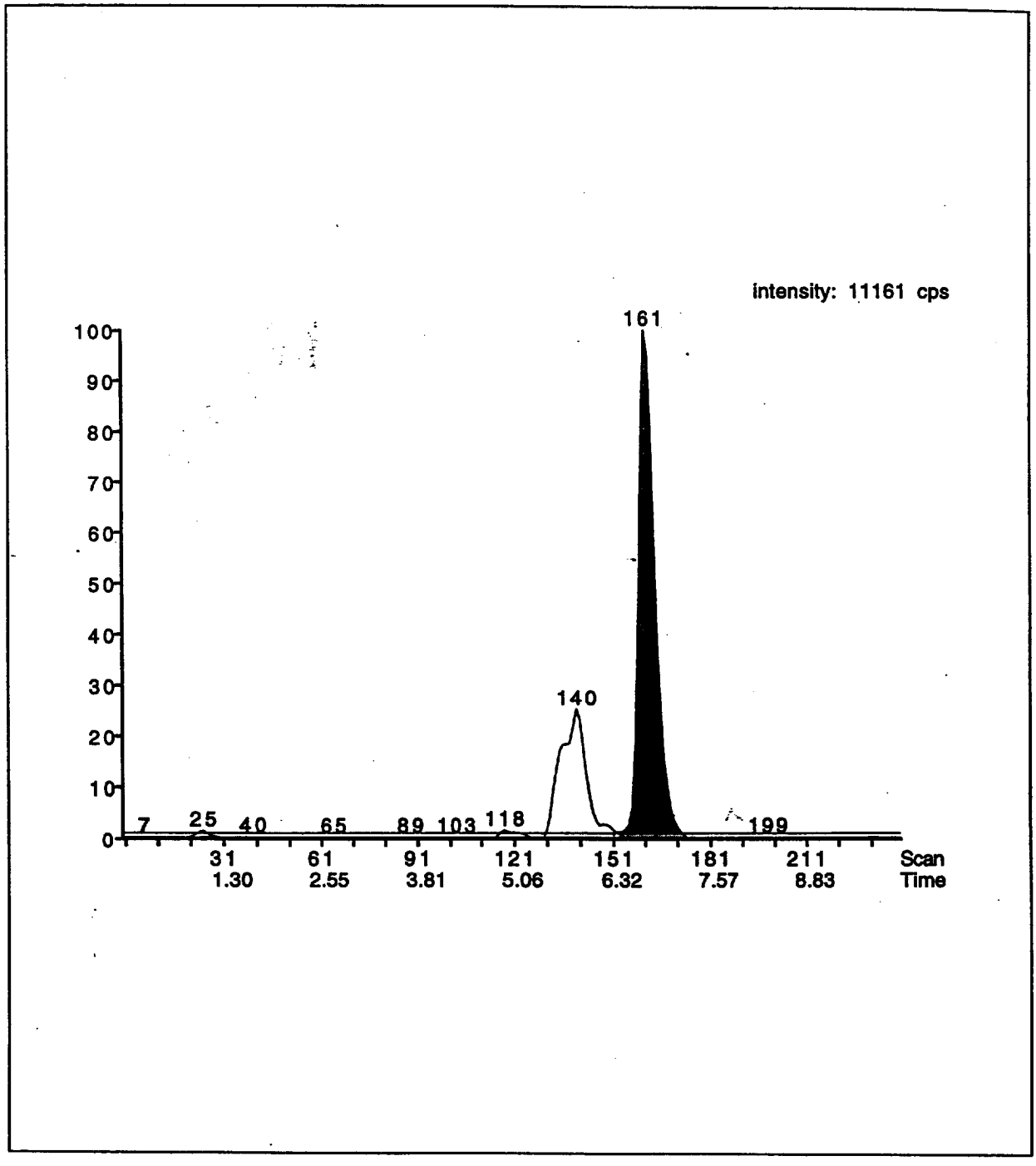



**Figure 7.** A representative chromatogram of a test sample (454A-103A-2).

## APPENDIX IV

### Cell Density for Each Replicate Per Treatment Over the 96-Hour Exposure Period

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Freshwater Alga, *Selenastrum capricornutum*
Dilution Water: Freshwater Algal Medium

| Mean Measured Concentration (mg a.i./L) | Replicate | Cell Densities (Cells/mL)[1] 24 Hours | 48 Hours | 72 Hours | 96 Hours |
|---|---|---|---|---|---|
| Negative Control | A | 34,000 | 132,000 | 710,000 | 2,720,000 |
| | B | 33,000 | 130,000 | 605,000 | 2,860,000 |
| | C | 31,000 | 125,000 | 495,000 | 2,640,000 |
| 5.5 | A | 29,000 | 136,000 | 780,000 | 3,000,000 |
| | B | 23,000 | 118,000 | 500,000 | 3,180,000 |
| | C | 35,000 | 134,000 | 625,000 | 2,940,000 |
| 11 | A | 30,000 | 105,000 | 560,000 | 2,680,000 |
| | B | 27,000 | 124,000 | 660,000 | 2,740,000 |
| | C | 31,000 | 134,000 | 625,000 | 3,220,000 |
| 21 | A | 30,000 | 141,000 | 860,000 | 3,340,000 |
| | B | 26,000 | 132,000 | 450,000 | 3,080,000 |
| | C | 28,000 | 128,000 | 600,000 | 3,300,000 |
| 44 | A | 30,000 | 136,000 | 515,000 | 2,800,000 |
| | B | 32,000 | 151,000 | 700,000 | 3,020,000 |
| | C | 27,000 | 135,000 | 390,000 | 3,420,000 |
| 86 | A | 37,000 | 65,000 | 215,000 | 650,000 |
| | B | 18,000 | 57,000 | 136,000 | 760,000 |
| | C | 22,000 | 46,000 | 162,000 | 470,000 |
| 179 | A | 15,000 | 35,000 | 21,000 | 45,000 |
| | B | 18,000 | 22,000 | 18,000 | 30,000 |
| | C | 10,000 | 26,000 | 23,000 | 26,000 |

[1] The initial cell density of the stock culture was determined and an inoculum volume was administered to each test chamber to yield a cell density of approximately 10,000 cells/mL at test initiation (0 hours).

AMENDED

# APPENDIX V

## Area Under the Growth Curve for Each Replicate Per Treatment Over the 96-Hour Exposure Period

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Freshwater Alga, *Selenastrum capricornutum*
Dilution Water: Freshwater Algal Medium

| Mean Measured Concentration (mg a.i./L) | Replicate | Cumulative Area Under the Growth Curve | | | |
|---|---|---|---|---|---|
| | | 0 – 24 Hours | 0 - 48 Hours | 0 - 72 Hours | 0 - 96 Hours |
| Negative Control | A | 288,000 | 2,040,000 | 11,904,000 | 52,824,000 |
| | B | 276,000 | 1,992,000 | 10,572,000 | 51,912,000 |
| | C | 252,000 | 1,884,000 | 9,084,000 | 46,464,000 |
| 5.5 | A | 228,000 | 1,968,000 | 12,720,000 | 57,840,000 |
| | B | 156,000 | 1,608,000 | 8,784,000 | 52,704,000 |
| | C | 300,000 | 2,088,000 | 10,956,000 | 53,496,000 |
| 11 | A | 240,000 | 1,620,000 | 9,360,000 | 48,000,000 |
| | B | 204,000 | 1,776,000 | 10,944,000 | 51,504,000 |
| | C | 252,000 | 1,992,000 | 10,860,000 | 56,760,000 |
| 21 | A | 240,000 | 2,052,000 | 13,824,000 | 63,984,000 |
| | B | 192,000 | 1,848,000 | 8,592,000 | 50,712,000 |
| | C | 216,000 | 1,848,000 | 10,344,000 | 56,904,000 |
| 44 | A | 240,000 | 1,992,000 | 9,564,000 | 49,104,000 |
| | B | 264,000 | 2,220,000 | 12,192,000 | 56,592,000 |
| | C | 204,000 | 1,908,000 | 7,968,000 | 53,448,000 |
| 86 | A | 324,000 | 1,308,000 | 4,428,000 | 14,568,000 |
| | B | 96,000 | 756,000 | 2,832,000 | 13,344,000 |
| | C | 144,000 | 720,000 | 2,976,000 | 10,320,000 |
| 179 | A | 60,000 | 420,000 | 852,000 | 1,404,000 |
| | B | 96,000 | 336,000 | 576,000 | 912,000 |
| | C | 0 | 192,000 | 540,000 | 888,000 |

AMENDED

## APPENDIX VI

### Growth Rate for Each Replicate Per Treatment Over the 96-Hour Exposure Period

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Freshwater Alga, *Selenastrum capricornutum*
Dilution Water: Freshwater Algal Medium

| Mean Measured Concentration (mg a.i./L) | Replicate | Growth Rate | | | |
|---|---|---|---|---|---|
| | | 0 – 24 Hours | 0 – 48 Hours | 0 - 72 Hours | 0 - 96 Hours |
| Negative Control | A | 0.0510 | 0.0538 | 0.0592 | 0.0584 |
| | B | 0.0497 | 0.0534 | 0.0570 | 0.0589 |
| | C | 0.0471 | 0.0526 | 0.0542 | 0.0581 |
| 5.5 | A | 0.0444 | 0.0544 | 0.0605 | 0.0594 |
| | B | 0.0347 | 0.0514 | 0.0543 | 0.0600 |
| | C | 0.0522 | 0.0541 | 0.0574 | 0.0592 |
| 11 | A | 0.0458 | 0.0490 | 0.0559 | 0.0582 |
| | B | 0.0414 | 0.0525 | 0.0582 | 0.0585 |
| | C | 0.0471 | 0.0541 | 0.0574 | 0.0602 |
| 21 | A | 0.0458 | 0.0551 | 0.0619 | 0.0605 |
| | B | 0.0398 | 0.0538 | 0.0529 | 0.0597 |
| | C | 0.0429 | 0.0531 | 0.0569 | 0.0604 |
| 44 | A | 0.0458 | 0.0544 | 0.0547 | 0.0587 |
| | B | 0.0485 | 0.0566 | 0.0590 | 0.0595 |
| | C | 0.0414 | 0.0542 | 0.0509 | 0.0608 |
| 86 | A | 0.0545 | 0.0390 | 0.0426 | 0.0435 |
| | B | 0.0245 | 0.0363 | 0.0363 | 0.0451 |
| | C | 0.0329 | 0.0318 | 0.0387 | 0.0401 |
| 179 | A | 0.0169 | 0.0261 | 0.0103 | 0.0157 |
| | B | 0.0245 | 0.0164 | 0.0082 | 0.0114 |
| | C | 0.0000 | 0.0199 | 0.0116 | 0.0010 |

AMENDED

## APPENDIX VII

### Changes to Protocol

This study was conducted in accordance with the approved Protocol with the following changes:

1. The protocol was amended to add the proposed experimental start and termination dates, test concentrations and test substance number.
2. The protocol was amended to specify the analytical method.
3. The protocol was amended to repeat the test with a higher test concentration.

## APPENDIX VIII

### Personnel Involved in the Study

The following key personnel were involved in the conduct or management of this study:

1. Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants
2. Willard B. Nixon, Ph.D., Manager, Analytical Chemistry
3. Raymond L. VanHoven, Ph.D., Scientist
4. Kurt R. Drottar, Senior Biologist
5. Mark A. Mank, Laboratory Supervisor

## APPENDIX IX

### Report Amendment

1. Original Report: Pages 1-4, 6 and 36

   Amendment: The pages were changed to include the amended report date, revised page numbers, and new signatures and dates due to the addition of the report amendment as Appendix IX.

   Reason: To reflect the issuing of an amended report.

2. Original Report: Page 2

   Amendment: The compliance statement was revised.

   Reason: To clarify how the test substance was characterized.

3. Original Report: Page 10

   Amendment: Information provided by the Sponsor reflecting the reanalysis of the test substance, including the reanalysis date and the purity, was added to the Test Substance section.

   Reason: To reflect the current test substance information provided by the Sponsor.

4. Original Report: Entire report

   Amendment: All test substance concentrations were changed to reflect the purity of the test substance as determined by the Sponsor in a reanalysis of the test substance (FC-95, Lot 217). Test concentrations originally were based on the reported purity of 98.9%. The certificate of analysis dated March 9, 2000 indicated a purity of 90.49%. Therefore, all test substance concentrations, including nominal concentrations, measured concentrations, EC values, and NOAEC values were recalculated and reported as mg a.i./L based on the 90.49% purity.

   Reason: To report the results of the test based on the test substance purity of 90.49% at the request of the Sponsor.

**AMENDMENT TO STUDY PROTOCOL**

**STUDY TITLE:** PFOS: A 96-HOUR TOXICITY TEST WITH THE FRESHWATER ALGA (*Selenastrum capricornutum*)

**PROTOCOL NO.:** 454/110998/SEL-96H1/SUB454 **AMENDMENT NO.:** 1

**SPONSOR:** 3M Corporation **PROJECT NO.:** 454A-103

**EFFECTIVE DATE:** January 4, 1999

**AMENDMENT:** Page 2

Add: Experimental Start Date: 2/19/99
Experimental Termination Date: 2/23/99
Test Concentrations: Negative Control, 6.3, 13, 25, 50 and 100 mg a.i./L
Test Substance No.: 4675

**REASON:** The above information was not known when the protocol was signed by the Study Director.

**AMENDMENT:** APPENDIX II, Page 16

Add: Liquid Chromatography Mass Spectrometry (LCMS) Method for the Determination of Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS) in Freshwater, Filtered Saltwater and Algal Medium.

**REASON:** To add the analytical method to be used in the study.

STUDY DIRECTOR — DATE 2/19/99

LABORATORY MANAGEMENT — DATE 2/9/99

SPONSOR'S REPRESENTATIVE — DATE 2/16/99

QA Reviewed by [illegible] 2/19/99

## AMENDMENT TO STUDY PROTOCOL

**STUDY TITLE:** PFOS: A 96-HOUR TOXICITY TEST WITH THE FRESHWATER ALGA (*Selenastrum capricornutum*)

**PROTOCOL NO.:** 454/110998/SEL-96H1/SUB454 **AMENDMENT NO.:** 2

**SPONSOR:** 3M Corporation **PROJECT NO.:** 454A-103

**EFFECTIVE DATE:** April 8, 1999

**AMENDMENT:** Page 2

Change: Experimental Start Date: 2/19/99
Experimental Termination Date: 2/23/99
Test Concentrations: Negative Control, 6.3, 13, 25, 50 and 100 mg a.i./L

To: Experimental Start Date: 4/12/99
Experimental Termination Date: 4/16/99
Test Concentrations: Negative Control, 6.3, 13, 25, 50, 100 and 200 mg a.i./L

**REASON:** The test will be repeated because cell counts were not made with a hemacytometer and a microscope. An additional test concentration was added in an attempt to obtain EC90 values.

**AMENDMENT: EXPERIMENTAL DESIGN, Page 3**

Change: Target concentrations will not exceed 100 mg/L or the solubility limit of the test substance in water.

To: Target concentrations will not exceed 1000 mg/L or the solubility limit of the test substance in water.

**REASON:** The new nominal concentrations exceed 100 mg a.i./L.

STUDY DIRECTOR — DATE 4/12/99

LABORATORY MANAGEMENT — DATE 4/12/99

SPONSOR'S REPRESENTATIVE — DATE 4/29/99

QA Review by JLB 8Apr99

*a454\103\amend2*

PROTOCOL

# PFOS: A 96-HOUR TOXICITY TEST WITH THE FRESHWATER ALGA (*Selenastrum capricornutum*)

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.5400

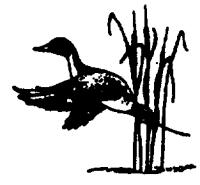
OECD Guideline 201

3M Lab Request No. U2723

Submitted to

3M Corporation
Environmental Laboratory
P.O. Box 33331
St. Paul, Minnesota 55133

**WILDLIFE INTERNATIONAL LTD.**



8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

November 9, 1998

PROTOCOL NO.: 454/110998/SEL-96H1/SUB454

3M LAB REQUEST NO. U2723

PFOS: A 96-HOUR TOXICITY TEST
WITH THE FRESHWATER ALGA (*Selenastrum capricornutum*)

| | |
|---|---|
| SPONSOR: | 3M Corporation<br>Environmental Laboratory<br>P.O. Box 33331<br>St. Paul, Minnesota 55133 |
| SPONSOR'S REPRESENTATIVE: | Ms. Susan A. Beach |
| TESTING FACILITY: | Wildlife International Ltd.<br>8598 Commerce Drive<br>Easton, Maryland 21601 |
| STUDY DIRECTOR: | Kurt Drottar<br>Senior Aquatic Biologist |
| LABORATORY MANAGEMENT: | Henry O. Krueger, Ph.D.<br>Director of Aquatic Toxicology & Non-Target Plants |

**FOR LABORATORY USE ONLY**

Proposed Dates:

Experimental Start Date: _____ Experimental Termination Date: _____

Project No.: 454A-103

Test Concentrations: _____

Test Substance No.: _____ Reference Substance No. (if applicable): _____

PROTOCOL APPROVAL

| | |
|---|---|
| _____ | 12/14/98 |
| STUDY DIRECTOR | DATE |
| _____ | 12/4/98 |
| LABORATORY MANAGEMENT | DATE |
| Susan A. Beach | 12/1/98 |
| SPONSOR'S REPRESENTATIVE | DATE |

## INTRODUCTION

Wildlife International Ltd. will conduct a 96-hour toxicity test with the freshwater alga, *Selenastrum capricornutum*, for the Sponsor at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.5400 (1) and OECD Guideline for Testing of Chemicals, 201: *Alga, Growth Inhibition Test* (2). Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report.

## PURPOSE

The purpose of this study is to determine the toxicity of a test substance to the freshwater green alga, *Selenastrum capricornutum*, during a 96-hour exposure period.

## EXPERIMENTAL DESIGN

The green alga, *Selenastrum capricornutum*, will be exposed to a geometric series of at least five test concentrations and a negative (culture medium) control for 96 hours. Target concentrations will not exceed 100 mg/L or the solubility limit of the test substance in water. Generally, the nominal concentration of each test substance used in the definitive test will be at least 50% of the next higher treatment, unless information concerning the concentration-effect curve indicates that a different dilution factor would be more appropriate.

Test solutions will be inoculated with 10,000 cells/mL. Three "biological" replicates per experimental group will be prepared for evaluating cell densities. One or more additional "analytical" replicates will be included in the test, as needed, to provide test solution for concentration verification on Day 3 of the exposure. An abiotic replicate at the highest concentration will be included in the test and will be sampled on Day 3 and Day 4. In order to control bias, the position of the flasks will be determined by indiscriminate draw daily during the exposure period. No other potential sources of bias are expected to affect the results of the study.

The response of the algae will be measured in terms of cell density, biomass expressed as area under the growth curve, and growth rate. An EC50 (i.e., the theoretical test concentration that produces a 50% reduction in the measured parameter) value for cell density will be calculated for each 24 hour interval. EC10, EC50 and EC90 values for cell density, biomass ($E_bC$) and growth rate ($E_rC$) will be

calculated, if possible, at the 72 and 96 hour intervals. The no observed adverse effect concentration (NOAEC), which is the highest test concentration that induces no adverse inhibitory effect on growth, will be determined relative to each parameter at 72 and 96 hours based upon evaluation of the statistical results and the dose-response pattern. At the end of the 96-hour exposure, algistatic effects will be differentiated from algicidal effects in those treatments which are maximally inhibited.

## MATERIALS AND METHODS

### Test Substance

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice Standards (GLP). The Sponsor is responsible for providing Wildlife International Ltd. written verification that the test substance has been characterized according to GLPs prior to its use in the study. If written verification of GLP test substance characterization is not provided to Wildlife International Ltd., it will be noted in the compliance statement of the final report. The attached form **IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR** (Appendix I) is to be used to provide information necessary for GLP compliance.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

### Preparation of Test Solutions

The test substance will be administered to *Selenastrum* in an aqueous medium. This route of administration was selected because it represents the most likely route of exposure to algae, which exists in aquatic environments.

Test solutions will be prepared by diluting one or more stock solutions with algal culture medium to achieve the target concentrations. Stocks will be prepared in algal culture medium. All weights, volumes and dilutions used to prepare the test solutions will be documented in the raw data.

### Test Organism

The freshwater green alga, *Selenastrum capricornutum*, will be used in this test. This species is representative of an important group of algae and was selected for use in the test based upon past use and ease of handling in the laboratory. Stock cultures, obtained from the Culture Collection of Algae at the University of Texas at Austin or another supplier, will be maintained in culture medium at Wildlife International Ltd. for a minimum of two weeks prior to use in a toxicity test. Algae used in toxicity tests

will be in exponential growth phase, which is defined as the period of growth when algal cells are dividing at a constant rate.

Just prior to beginning the test, an inoculum of the stock culture will be prepared so that each milliliter of inoculum contains enough cells to provide an initial cell density of approximately 10,000 cells/mL in each replicate.

Culture Medium

Culture medium prepared according to Wildlife International Ltd. Standard Operating Procedures will be used as dilution water. The concentrations of the components in the medium are presented in Table 1 (3). Stock nutrient solutions will be prepared by adding reagent-grade chemicals to Wildlife International Ltd. well water purified by reverse-osmosis. Appropriate volumes of the stock nutrient solutions will then be diluted with purified well water to prepare the medium. The medium will be filter sterilized (0.2 $\mu$m or autoclaved) prior to use. The final pH of the medium should be 7.5 ± 0.1. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and results of the analyses will be summarized in the final report.

Specifications for acceptable levels of contaminants have not been established for culture medium. However, there are no levels of contaminants reasonably expected to be present in the medium that are considered to interfere with the purpose or conduct of the study.

Test Apparatus

Test chambers will be sterile, 250-mL polycarbonate Erlenmeyer flasks plugged with foam stoppers. Each test chamber will hold 100 mL of test solution. Test chambers will be indiscriminately positioned on a mechanical shaker table in an environmental chamber and will be shaken continuously at approximately 100 rpm. Test chambers will be labeled with the project number, test concentration, and replicate.

Environmental Conditions

Test flasks will be held at 24 ± 2°C under continuous fluorescent lighting (e.g., cool white light tubes) at an intensity of approximately 4300 ± 10% lux. Light intensity will be measured at five locations on the shaker table daily during the test. Temperature will be measured twice daily in the environmental chamber using a liquid-in-glass thermometer.

The pH of each treatment and the control will be measured at test initiation and termination using a Fisher Accumet Model 915 pH meter or equivalent. Samples will be collected from the batch of test solution prepared at test initiation for each treatment and control group for the measurement of pH. At test termination, pH will be measured in pooled test solution samples collected from the three replicates of each treatment and control group.

Biological Measurements

Cell densities will be monitored during the test by conducting cell counts using a hemacytometer and a microscope. One sample will be collected from each replicate of the treatment and control groups at approximately 24 hour intervals during the exposure period. The samples will be evaluated immediately. Each sample will be diluted using an electrolyte solution (Isoton®), as needed, to maintain counting accuracy. The cell density of the sample will be calculated based on the mean number of cells per grid.

Samples of test solutions will be collected from each replicate per treatment and control group at the end of the test. Each replicate sample will be examined microscopically for atypical cell morphology (e.g., changes in shape, size or color). Growth of cells in the flasks also will be assessed for aggregations or flocculations of cells and adherence of the cells to the test chamber.

At the end of the test, algistatic effects, which result in the inhibition of cell growth, will be differentiated from algicidal effects, which result in the death of cells. Aliquots (0.5 mL) of test solutions will be taken from each replicate test chamber where growth is maximally inhibited. Maximally inhibited treatments are those in which it cannot be visually determined if live algal cells are present (e.g. absence of color to the test solution). These aliquots will be combined and diluted with algal medium to a concentration of the test substance that theoretically should not affect growth. A negative control will be prepared by diluting a 0.5-mL aliquot from one negative control replicate to 100 mL with algal medium. Growth of these subcultures will be monitored for up to 9 days to determine whether inhibition of growth observed during the test is reversible. Samples will be collected for cell counts at recovery phase initiation, termination and at approximately 3-day intervals between initiation and termination. The recovery phase may be terminated once algal growth is sufficient to indicate that the algal cells have fully recovered from effects due to the test substance. This will be determined through visual examination of the recovery test solutions.

Sampling for Analytical Measurements

Samples of the exposure solutions will be collected on Days 0, 3 and 4 and analyzed for test substance concentrations. Day 0 samples will be taken from each batch of test solution prior to their distribution among the replicate test chambers. Day 3 samples will be collected from the extra replicate test chamber(s) included in the test to provide sample for that analysis and from the abiotic replicates. If more than one replicate is included for the Day 3 sample analysis, then the solutions from each replicate will be composited to provide the sample for that treatment. Day 4 samples will be composited solutions from the three replicates remaining at the end of the test.

Samples collected on Days 3 and 4 may be centrifuged or filtered to remove algal cells prior to analysis. Samples will be analyzed immediately or placed in an appropriate storage container (e.g., polypropylene or polyethylene bottle) and stored until analyzed. The sample scheme is summarized below:

PROPOSED NUMBERS OF VERIFICATION SAMPLES

| Experimental Group | Day 0 | Day 3 | Day 4 |
|---|---|---|---|
| Control | 1 | 1 | 1 |
| Solvent Control (if needed) | 1 | 1 | 1 |
| Level 1-Low Concentration | 1 | 1 | 1 |
| Level 2 | 1 | 1 | 1 |
| Level 3 | 1 | 1 | 1 |
| Level 4 | 1 | 1 | 1 |
| Level 5-High Concentration | 1 | 1 | 1 |
| Totals | 7 | 7 | 7 |

The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical chemistry phase of the study.

Analytical Chemistry

Chemical analysis of the samples will be performed by Wildlife International Ltd. The analytical method used will be based upon methodology provided by the Sponsor and identified in Appendix II. Modifications made to the analytical method will be documented in the raw data and described in the final report.

Statistical Analyses

Biomass expressed as area under the growth curve will be calculated for the treatment and control groups using the following formula:

$$A = ((N_1-N_o)/2)(t_1)+((N_1+N_2-2N_o)/2)(t_2-t_1)+...+((N_{n-1}+N_n-2N_o)/2)(t_n-t_{n-1})$$

Where:

$A$ = Area under the growth curve
$N_o$ = Measured number of cells/mL at $t_o$
$N_1$ =Measured number of cells/mL at $t_1$
$N_2$ =Measured number of cells/mL at $t_2$
$N_n$ =Measured number of cells/mL at $t_n$
$t_1$ = Time of first measurement after beginning of test (hours)
$t_2$ = Time of second measurement after beginning of test (hours)
$t_n$ = Time of nth measurement after beginning of test (hours)

Growth rates ($\mu$) will be calculated for the treatment and control groups using the following formula:

$$\mu = \frac{\ln N_n - \ln N_0}{t_n - t_0}$$

$\mu$ = Average specific growth rate
$N_o$ = Measured number of cells/mL at $t_o$
$N_n$ = Measured number of cells/mL at $t_n$
$t_0$ = Time of beginning of test (hours)
$t_n$ = Time of nth measurement after beginning of test (hours)

Percent inhibition will be calculated for each treatment group based upon mean cell density, biomass and growth rate using the following formula:

$$\text{Percent Inhibition} = \frac{\text{Mean Control Response} - \text{Mean Treatment Response}}{\text{Mean Control Response}} \times 100$$

EC values will be determined, when possible, using linear interpolation or other suitable techniques with treatment response (i.e. cell densities, biomass and growth rate) and exposure concentration data for each 24-hour interval of the test.

A no observed adverse effect concentration (NOAEC) will be selected based on an evaluation of the dose-response pattern and the results of the statistical analysis using individual replicate values of the cell density, biomass and growth rate. Statistically significant differences between the control and the treatment groups will be identified using analysis of variance (ANOVA) and a test to compare treatment mean responses to the control response (e.g., Dunnett's test or Bonferroni's t-test). Data will be assessed for normality and homogeneity of variances prior to performing the ANOVA. Transformations will be used to correct any condition of non-normality or unequal error variances, if possible. If a solvent control is used in addition to the negative control, their responses will be compared using Student's t-test. Data from the two control groups will be pooled for treatment level analyses if no statistical differences exist. Otherwise, all comparisons will be made to the solvent control group. Statistical comparisons for the ANOVA and the comparison of mean responses will be carried out using TOXSTAT® V3.5 software (4) or equivalent.

## RECORDS TO BE MAINTAINED

Records to be maintained for data generated at Wildlife International Ltd. will include, but not be limited to:

1. Copy of signed protocol.
2. Identification and characterization of the test substance, if provided by Sponsor.
3. Dates of initiation and termination of the test.
4. Source of algae.
5. Culture conditions.

6. Growth measurements.
7. Calculation and preparation of test concentrations.
8. Observations.
9. The methods used to analyze test substance concentrations and the results of analytical measurements.
10. Statistical calculations.
11. Test conditions and physical/chemical measurements.
12. Copy of final report.

## FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International Ltd. The report will include, but not be limited to the following, when applicable:

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures, as stated in the approved protocol, including all changes to the protocol.
5. The test substance identification including name, chemical abstract number or code number, strength, purity, composition, and other information provided by the Sponsor.
6. Stability and solubility of the test substance under the conditions of administration, if provided by the Sponsor.
7. A description of the methods used to conduct the test.
8. A description of the test organisms, including the source, scientific name, age or life stage, light intensity and photoperiod.
9. A description of the preparation of the test solutions.
10. The methods used to allocate organisms to test chambers and begin the test, the number of organisms and chambers per treatment, and the duration of the test.
11. A description of circumstances that may have affected the quality or integrity of the data.

12. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
13. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data and analytical chemistry data, and a statement of the conclusions drawn from the analyses. A graph plotting the concentration response curve.
14. Statistical methods used to evaluate the data.
15. The signed and dated reports of each of the individual scientists or other professionals involved in the study.
16. The location where raw data and final report are to be stored.
17. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.

18. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed by the Study Director.

## CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

## GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (OCDE/GD (92) 32, Environment Monograph No. 45); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). Each study conducted by Wildlife International Ltd. is routinely examined by the Wildlife International Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of

compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report.

## REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400: Algal Toxicity, Tiers I and II.

2 **OECD Guideline for Testing of Chemicals.** 1984. *201: Alga, Growth Inhibition Test.*

3 **ASTM Standard Guide 1218-90E.** 1990. *Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae.*

4 **West, Inc. and D. D. Gulley.** 1996. *TOXSTAT Release 3.5.* Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

TABLE 1

FRESHWATER ALGAL MEDIUM CONSTITUENTS

| Component | Nominal Concentration |
|---|---|
| $MgCl_2 \cdot 6H_2O$ | 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 |
| $FeCl_3 \cdot 6H_2O$ | 0.1598 mg/L |
| $CoCl_2 \cdot 6H_2O$ | 1.428 µg/L |
| $Na_2MoO_4 \cdot 2H_2O$ | 7.26 µg/L |
| $CuCl_2 \cdot 2H_2O$ | 0.012 µg/L |
| $Na_2EDTA \cdot 2H_2O$ | 0.300 mg/L |
| $NaNO_3$ | 25.50 mg/L |
| $MgSO_4 \cdot 7H_2O$ | 14.70 mg/L |
| $K_2HPO_4$ | 1.044 mg/L |
| $NaHCO_3$ | 15.00 mg/L |

The pH of the medium will be adjusted, as necessary, to 7.5 ± 0.1 using 0.1 N NaOH or 10% HCl.

APPENDIX I
IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): PFOS (Perfluorooctane Sulfonic Acid Potassium Salt

Reference Standard (if applicable): Analytical Standard: N/A

Internal Standard: 1,1,2,2H,H,H,H Perfluorooctane Sulfonic Acid

Test Substance Sample Code or Batch Number: Lot 217

Test Substance Purity (% Active Ingredient): 98.9 Expiration Date: 2008

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? Yes ____ No X

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International Ltd.

Ambient room temperature

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? Yes ____ No X

Other pertinent stability information: ____

IV. Test Concentrations:

X Adjust test concentration to 100% a.i. based upon the purity (%) given above.

____ Do not adjust test concentration to 100% a.i. Test the material AS IS.

V. Toxicity Information:

Mammalian: Rat LD50 251 mg/kg Mouse LD50 N/A

Aquatic:

| Invertebrate Toxicity (EC/LC50) | Fish Toxicity (LC50) |
|---|---|
| Daphnia magna: 27 mg/L | Rainbow Trout: 11 mg/L |
| Daphnia magna: 50 mg/L | Fathead Minnow: 38 mg/L |

Other Toxicity Information (including findings of chronic and subchronic tests):

Please see MSDS

APPENDIX II

Analytical Method to be Provided by Sponsor