PFOS:
A 96-HOUR TOXICITY TEST WITH THE MARINE DIATOM (*Skeletonema costatum*)

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-113A

3M LAB REQUEST NO. U2723

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

AUTHORS:

Debbie Desjardins
Cary A. Sutherland
Raymond L. Van Hoven, Ph.D.
Henry O. Krueger, Ph.D.

STUDY INITIATION DATE: January 28, 2000

STUDY COMPLETION DATE: March 27, 2001

Submitted to

3M Corporation
Environmental Laboratory
935 Bush Avenue
St. Paul, Minnesota 55106

Wildlife International, Ltd.

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

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A 96-Hour Toxicity Test with the Marine Diatom (*Skeletonema costatum*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-113A

STUDY COMPLETION: March 27, 2001

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, (ENV/MC/CHEM(98)17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984 with the following exceptions:

The test and reference substances were not characterized in accordance with full GLP compliance prior to its use in the study; 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 has been recharacterized in accordance with GLP (September 7, 2000).

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

STUDY DIRECTOR:

Cary A. Sutherland
Laboratory Supervisor

3/27/01
DATE

SPONSOR:

Sponsor

5/15/01
DATE

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, (ENV/MC/CHEM(98)17); 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-113			
Test Substance Preparation	March 2, 2000	March 2, 2000	March 2, 2000
Matrix Fortifications	March 3, 2000	March 6, 2000	March 14, 2000
Definitive Trial 454A-113A			
Analytical Data and Draft Report	July 11 & 12, 2000	July 12, 2000	July 14, 2000
Biological Data and Draft Report	July 17 & 18, 2000	July 18, 2000	August 28, 2000
Analytical Data and Second Draft Report	March 12, 2001	March 12, 2001	March 13, 2001
Biological Data and Second Draft Report	March 26, 2001	March 26, 2001	March 27, 2001
Final Report	March 27, 2001	March 27, 2001	March 27, 2001

Marshall T. Hynson
Quality Assurance Program Supervisor

3/27/2001
DATE

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFOS: A 96-Hour Toxicity Test with the Marine Diatom (*Skeletonema costatum*)

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STUDY DIRECTOR:

Cary A. Sutherland
Laboratory Supervisor

3/27/01
DATE

MANAGEMENT:

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

3/27/01
DATE

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SUMMARY

SPONSOR:	3M Corporation
SPONSOR'S REPRESENTATIVE:	Rochelle R. Robideau
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International, Ltd. Easton, MD 21601

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER:	454A-113A
TEST SUBSTANCE:	PFOS (Perfluorooctanesulfonate, Potassium Salt) IUPAC Name: 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
STUDY:	PFOS: A 96-Hour Toxicity Test with the Marine Diatom (*Skeletonema costatum*)
NOMINAL TEST CONCENTRATIONS:	Negative Control and 3.46 mg a.i./L
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control and 3.20 mg a.i./L
TEST DATES:	Experimental Start (OECD) – March 2, 2000 Experimental Start (EPA) – May 19, 2000 Exposure Termination – May 23, 2000 Experimental Termination – May 23, 2000
LENGTH OF EXPOSURE:	96 Hours

TEST ORGANISM:	Marine Diatom (*Skeletonema costatum*)
SOURCE OF TEST ORGANISMS:	Wildlife International, Ltd. Easton, Maryland 21601

SUMMARY (Continued)

CELL DENSITY:	
72-HOUR EC50:	> 3.20 mg a.i./L
95% CONFIDENCE LIMITS:	Not calculable
96-HOUR EC10, EC50 and EC90:	> 3.20 mg a.i./L
95% CONFIDENCE LIMITS:	Not calculable
72-HOUR NOAEC:	3.20 mg a.i./L
96-HOUR NOAEC:	3.20 mg a.i./L

AREA UNDER THE GROWTH CURVE:	
72-HOUR EC50:	> 3.20 mg a.i./L
95% CONFIDENCE LIMITS:	Not calculable
96-HOUR EC10, EC50 and EC90 :	> 3.20 mg a.i./L
95% CONFIDENCE LIMITS:	Not calculable
72-HOUR NOAEC:	3.20 mg a.i./L
96-HOUR NOAEC:	3.20 mg a.i./L

GROWTH RATE:	
72-HOUR EC50:	> 3.20 mg a.i./L
95% CONFIDENCE LIMITS:	Not calculable
96-HOUR EC10, EC50 and EC90:	> 3.20 mg a.i./L
95% CONFIDENCE LIMITS:	Not calculable
72-HOUR NOAEC:	3.20 mg a.i./L
96-HOUR NOAEC:	3.20 mg a.i./L

All values are mean measured test concentrations.

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for 3M Corporation at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. An initial trial of the test was conducted from March 3, 2000 to March 7, 2000. However, the test was repeated due to low analytical recoveries in the first trial. The in-life phase of the definitive test was conducted from May 19, 2000 to May 23, 2000. Raw data generated by Wildlife International, Ltd and a copy of the final report are filed under Project Number 454A-113A in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of the study was to evaluate the toxicity of PFOS (Perfluorooctanesulfonate, Potassium Salt) to the growth of the marine diatom, *Skeletonema costatum*, during a 96-hour exposure period.

EXPERIMENTAL DESIGN

The marine diatom, *Skeletonema costatum*, was exposed to a single test concentration and a negative (culture medium) control under static conditions for 96 hours. Six replicate test chambers were maintained in the treatment and control groups. One additional replicate for each treatment and control group was maintained for analytical sampling at 72 hours. In addition, two "abiotic" replicates (test solution without algae) were maintained in the treatment group to determine the stability of the test substance under the conditions of administration. The nominal test concentration was selected in consultation with the Sponsor and was based upon the results of range finding tests and the approximate solubility of PFOS in saltwater algal medium. The nominal test concentration was 3.46 mg active ingredient (a.i.)/L. The mean measured test concentration was determined from samples of test medium collected from the treatment and control groups at test initiation, at approximately 72 hours, and at test termination.

At test initiation an inoculum of the algal cells was added to each test chamber to achieve an initial nominal concentration of approximately 77,000 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. EC10, EC50 and EC90 values could not be calculated using statistical methods because observed effects were below these levels. Because there were no statistically significant effects at the test concentration (3.20 mg a.i./L) EC10, EC50 and EC90 values can be taken as an estimate of the no-observed-adverse-effect-concentration (NOAEC). The no-observed-adverse-effect-concentration (NOAEC) was determined based upon statistical evaluation of the 72-hour and 96-hour results.

MATERIALS AND METHODS

The study was conducted based on the procedures outlined in the protocol, "PFOS: A 96-Hour Toxicity Test with the Marine Diatom (*Skeletonema costatum*)". 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).

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 September 7, 2000 indicated a purity of 86.9% and expiration date of August 31, 2001. The test substance was stored at ambient room temperature.

Preparation of Test Concentrations

The nominal test concentration was 3.46 mg a.i./L, based on a test substance purity of 86.9%. A single test solution was prepared in saltwater algal medium. The test solution was sonicated for approximately 30 minutes and was stirred with a magnetic stir plate for approximately 43 hours to aid in the solubilization of the test substance. The test solution appeared clear and colorless.

Test Organism

The marine diatom, *Skeletonema costatum*, was selected as the test species for this study. The species is representative of an important group of 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 the Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory at Oban Argyll, Scotland, 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).

Culture Medium

The algal cells were cultured and tested in saltwater algal medium (2). 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 appropriate volumes of the stock nutrient solutions to artificial saltwater (Appendix I). The pH of the medium was 8.1, and the medium was sterilized by filtration (0.22 µm) prior to use. Analyses were performed at least once annually to determine the concentrations of selected organic and inorganic constituents in the well water used by Wildlife International, Ltd. The results of the most recent GLP analyses performed to measure the concentrations of selected contaminants in the well water are presented in Appendix II.

Test Apparatus

Test chambers were sterile, 250-mL glass Erlenmeyer flasks plugged with foam stoppers, and contained 100 mL of test or control medium. The test chambers were labeled with the project number, concentration and replicate, and were indiscriminately positioned daily 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 approximately 100 rpm.

Environmental Conditions

Test flasks were held in an environmental chamber at a temperature of 20 ± 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 a photoperiod of 14 hours of cool-white fluorescent lighting at a target light intensity of 4300 ± 430 lux, and 10 hours of darkness. Light intensity was measured at the four corners and the middle of the shaker table at test initiation using a SPER Scientific Model 840006 light meter.

The pH of the medium prepared for the treatment and control groups 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 the treatment and control groups. 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 six "biological" replicates per treatment and control group at 24-hour intervals during the 96-hour exposure, and were held for a maximum of two days under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. 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 1.0×10^3 cells/mL.

Samples of test solution were collected from each replicate per treatment and control group at the end of the test. These samples were pooled within their respective treatment, and subsamples were removed and examined microscopically for atypical cell morphology (e.g., changes in cell shape or color). Growth of cells in the replicate test chambers also was assessed for aggregations (clumping) of cells and adherence of the cells to the test chamber.

Statistical Analyses

Cell densities, areas under the growth curve, growth rates and percent inhibition were calculated using "The SAS System for Windows", Release 6.12 (3). Area under the growth curve was calculated for each replicate of the control and treatment groups using the following formula:

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

where: A = Area
N_o = Nominal number of cells/mL at t_o
N_1 = 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 n^{th} measurement after beginning of test (hours)

Growth rates were calculated for each replicate of the control and treatment groups using the following formula:

$$\mu = \frac{\ln N_n - \ln N_o}{t_n - t_o}$$

where: μ = Average specific growth rate
N_o = Nominal number of cells/mL at t_o
N_n = Measured number of cells/mL at t_n
t_o = Time of beginning of the test (hours)
t_n = Time of n^{th} measurement after beginning of test (hours)

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

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

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 for cell

densities, area under the growth curve and growth rates could not be calculated using statistical methods. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were evaluated for normality using the Shapiro-Wilk's test and for equality of variance using an F-test. Since the data was normally distributed with equal variances, the treatment group was compared to the control group using analysis of variance (ANOVA) and a 2-sample t-test. Results of the statistical analyses were used to determine the NOAEC values at 72 and 96 hours.

Analytical Chemistry

Samples of test medium were collected from the negative control and the 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 the treatment and control groups. Samples collected at 72 hours were collected from the additional "analytical" replicates. Samples collected at test termination were a composite of the remaining biotic replicates for each treatment and control group. The 3.46 mg a.i./L abiotic replicates were sampled at 72 and 96 hours to determine the stability of the test substance under the conditions of administration. The samples were placed in glass scintillation vials and were analyzed immediately without storage. The 72 and 96-hour samples were centrifuged approximately 10 minutes at approximately 2000 rpm prior to analysis. 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. The nominal concentration used in this study was 3.46 mg a.i./L. The sample collected at the beginning of the test had a measured concentration that was 94.2% of nominal. Samples collected at 72 hours and at test termination had recoveries of 92.2 and 91.2% of nominal, respectively. The abiotic replicates from the 3.46 mg a.i./L treatment had recoveries of 97.1 and 86.8% of nominal at 72 and 96 hours, respectively, which were comparable to the biotic replicate recoveries. When the values obtained at test initiation, at 72 hours and at test termination were averaged, the mean measured test concentration

was 3.20 mg a.i./L, representing 92.5% of the nominal concentration. The mean measured test concentration was used in the determination of EC values.

Observations and Measurements

Measurements of temperature, light intensity and pH are presented in Tables 2, 3 and 4, respectively. The temperatures ranged from 20.2 to 21.4°C and were within the range established for the test (20 ± 2°C). The light intensity ranged from 3880 to 4710 lux and was within the desired range for the test (approximately 3870 to 4730 lux). Measurements of pH were 8.0 on Day 0 and 8.4 on Day 4.

The effect of PFOS upon *Skeletonema costatum* was determined by evaluating differences in cell densities, areas under the growth curve and growth rates. Mean values for each parameter were used to calculate growth inhibition for each 24-hour period. Mean cell densities, areas under the growth curve and growth rates, and the corresponding percent inhibition, are presented in Tables 5, 6 and 7, respectively. Cell density, area under the growth curve and growth rate for each individual replicate are presented in Appendices IV, V and VI, respectively, while cell densities are illustrated graphically in Figures 1 and 2. EC 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). At 72 and 96 hours, growth in the 3.20 mg a.i./L treatment group was comparable to growth in the control group. After 72 hours of exposure, percent inhibition in the 3.20 mg a.i./L treatment group in relation to cell density, area under the growth curve and growth rate was –16, –4.8 and -4.6%, respectively. After 96 hours, percent inhibition for the three parameters was –4.8, –7.3 and –1.3%, respectively. There were no significant differences ($p>0.05$) in cell density, area under the growth curve or growth rate between the 3.20 mg a.i./L treatment group and the control group at 72 and 96 hours. Consequently, the NOAEC at 72 and 96 hours was 3.20 mg a.i./L.

Visual and Microscopic Observations

After 96 hours of exposure, there were no noticeable changes in cell morphology, nor were there signs of aggregation (clumping) or adherence of the algae to the test flasks in the 3.20 mg a.i./L treatment

group. After 96 hours of exposure, adherence of cells to the test chamber was observed in the negative control group. However, this was not observed in the PFOS treatment group.

Reversibility of Growth Inhibition

After 96 hours of exposure, there was no significant inhibition of growth in the 3.20 mg a.i./L treatment group, the single concentration tested. Therefore, a recovery phase was not conducted.

CONCLUSIONS

A single concentration of PFOS was tested at the approximate solubility of the test substance in saltwater algal medium. The 72 and 96-hour EC50, based on cell density, area under the growth curve and growth rate, was > 3.20 mg a.i./L, the single concentration tested. Since there was no significant inhibition of growth in the treatment group during the test, the 72 and 96-hour NOAEC was 3.20 mg a.i./L.

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 **ASTM Standard Guide 1218-90E.** 1990. *Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae*. American Society for Testing and Materials. Philadelphia, Pennsylvania.

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

4 **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:	Marine Diatom, *Skeletonema costatum*
Dilution Water:	Saltwater 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	< LOQ	--
	72[3]	< LOQ		
	96[4]	< LOQ		
3.46	0	3.26	3.20	92.5
	72	3.19		
	96	3.15		
3.46 (abiotic)	72[5]	3.36	3.18	91.9
	96[5]	3.00		

[1] Limit of Quantitation (LOQ) was 0.480 mg a.i./L.
[2] 0-hour samples were collected from individual batches of test solution prepared for the treatment and control groups for test initiation.
[3] 72-hour samples were collected from the additional analytical replicate.
[4] 96-hour samples were composites of test solution collected from each of the six replicates per treatment and control group.
[5] 72 and 96-hour samples were collected from the additional abiotic replicates.

Table 2

Temperature Measurements

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Marine Diatom, *Skeletonema costatum*
Dilution Water:	Saltwater Algal Medium

Time (Day)	Temperature [1] (°C)	
	Measurement 1	Measurement 2
0	21.2	21.1
1	20.4	21.4
2	21.3	20.8
3	20.2	21.3
4	20.3	21.2

[1] Temperature Measurement 2 was taken at least 4 hours after Measurement 1, with the exception of test termination (Day 4).

Table 3

Light Intensity Measurements

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Marine Diatom, *Skeletonema costatum*
Dilution Water:	Saltwater Algal Medium

Test Day	Light Intensity Measurements [1] (lux)				
	No. 1	No. 2	No. 3	No. 4	No.5
0	3970	3880	4710	4450	4130

[1] Light intensity was measured at five locations surrounding the test flasks on the shaker table.

Table 4

pH Measurements

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Marine Diatom, *Skeletonema costatum*
Dilution Water:	Saltwater Algal Medium

Mean Measured Concentration (mg a.i./L)	pH Measurements	
	Day 0[1]	Day 4[2]
Negative Control	8.0	8.4
3.20	8.0	8.4

[1] Day 0 samples were collected from the batches of test solution prepared for the treatment and control groups at test initiation.
[2] Day 4 samples were collected from the pooled replicates per treatment and control group.

Table 5

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

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Marine Diatom, *Skeletonema costatum*
Dilution Water: Saltwater Algal Medium

Mean Measured Concentration (mg a.i./L)	24 Hours		48 Hours		72 Hours		96 Hours	
	Mean Cell Density[1] (cells/mL)	Percent Inhibition[1]	Mean Cell Density[1] (cells/mL)	Percent Inhibition[1]	Mean Cell Density[1,2] (cells/mL)	Percent Inhibition[1]	Mean Cell Density[1,2] (cells/mL)	Percent Inhibition[1]
Negative Control	297,333	--	1,064,167	--	1,848,333	--	2,481,667	--
3.20	275,167	7.5	1,041,667	2.1	2,140,000	-16	2,601,667	-4.8

[1] Values calculated using SAS 6.12. Manual calculations may differ slightly.
[2] The treatment group response was not significantly different from the negative control using an F-test ($p < 0.05$).

Table 6

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

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Marine Diatom, *Skeletonema costatum*
Dilution Water: Saltwater Algal Medium

Mean Measured Concentration (mg a.i./L)	0 - 24 Hours		0 - 48 Hours		0 - 72 Hours		0 - 96 Hours	
	Mean Area[1]	Percent Inhibition[1]	Mean Area[1]	Percent Inhibition[1]	Mean Area[1,2]	Percent Inhibition[1]	Mean Area[1,2]	Percent Inhibition[1]
Negative Control	2,644,000	--	17,134,000	--	50,236,000	--	100,348,000	--
3.20	2,378,000	10	16,332,000	4.7	52,664,000	-4.8	107,716,000	-7.3

[1] Values calculated using SAS 6.12. Manual calculations may differ slightly.
[2] The treatment group response was not significantly different from the negative control using an F-test ($p < 0.05$).

Table 7

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

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Marine Diatom, *Skeletonema costatum*
Dilution Water: Saltwater Algal Medium

Mean Measured Concentration (mg a.i./L)	0 - 24 Hours		0 - 48 Hours		0 - 72 Hours		0 - 96 Hours	
	Mean Growth Rate[1] (cells/mL/hr)	Percent Inhibition[1]	Mean Growth Rate[1] (cells/mL/hr)	Percent Inhibition[1]	Mean Growth Rate[1,2] (cells/mL/hr)	Percent Inhibition[1]	Mean Growth Rate[1,2] (cells/mL/hr)	Percent Inhibition[1]
Negative Control	0.0561	--	0.0546	--	0.0441	--	0.0362	--
3.20	0.0530	5.5	0.0542	0.84	0.0461	-4.6	0.0366	-1.3

[1] Values calculated using SAS 6.12. Manual calculations may differ slightly.
[2] The treatment group response was not significantly different from the negative control using an F-test ($p < 0.05$).

Table 8

EC Values Based on Cell Density Over the 96-Hour Exposure Period

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Marine Diatom, *Skeletonema costatum*
Dilution Water:	Saltwater 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	--	> 3.20	--[1]	Not Determined	--
48 Hours	Not Determined	--	> 3.20	--[1]	Not Determined	--
72 Hours	> 3.20	--[1]	> 3.20	--[1]	> 3.20	--[1]
96 Hours	> 3.20	--[1]	> 3.20	--[1]	> 3.20	--[1]

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

Table 9

EC Values Based on Area Under the Growth Curve Over the 96-Hour Exposure Period

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Marine Diatom, *Skeletonema costatum*
Dilution Water:	Saltwater 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	--	> 3.20	--[1]	Not Determined	--
48 Hours	Not Determined	--	> 3.20	--[1]	Not Determined	--
72 Hours	> 3.20	--[1]	> 3.20	--[1]	> 3.20	--[1]
96 Hours	> 3.20	--[1]	> 3.20	--[1]	> 3.20	--[1]

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

Table 10

EC Values Based on Growth Rate Over the 96-Hour Exposure Period

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Marine Diatom, *Skeletonema costatum*
Dilution Water:	Saltwater 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	--	> 3.20	--[1]	Not Determined	--
48 Hours	Not Determined	--	> 3.20	--[1]	Not Determined	--
72 Hours	> 3.20	--[1]	> 3.20	--[1]	> 3.20	--[1]
96 Hours	> 3.20	--[1]	> 3.20	--[1]	> 3.20	--[1]

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

Figure 1. Negative control algal growth, expressed as cell density, during the 96-hour exposure period.

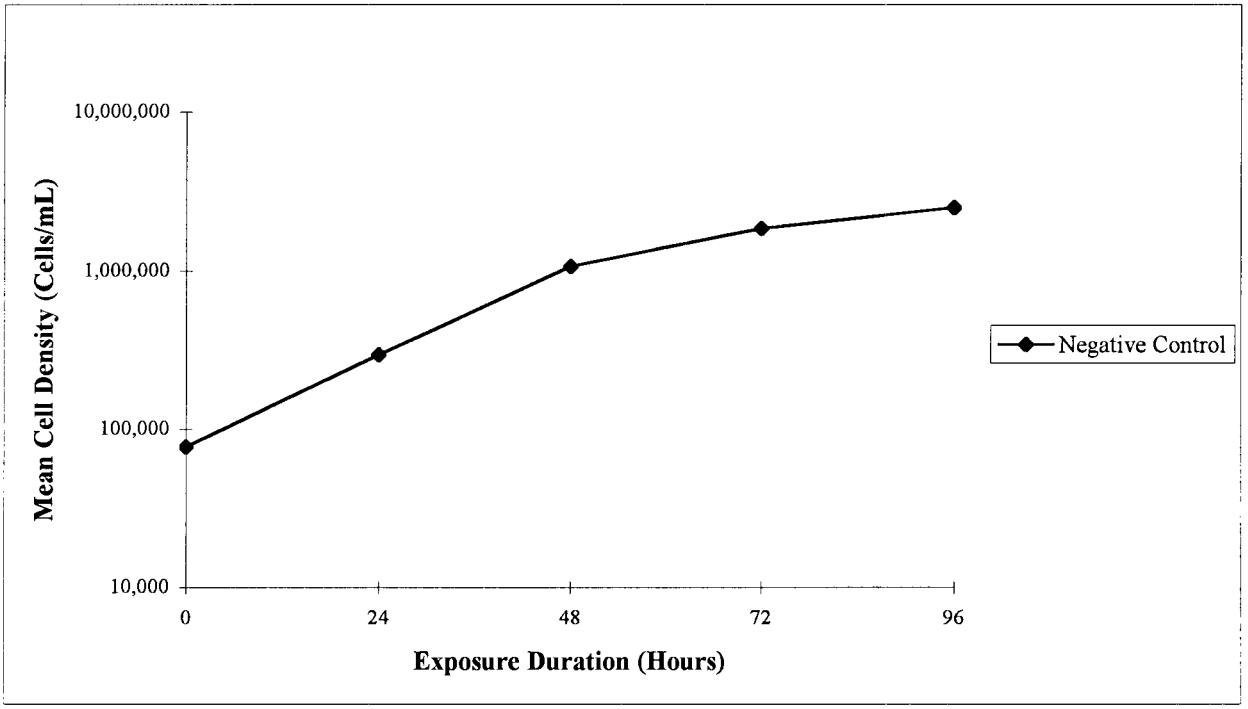
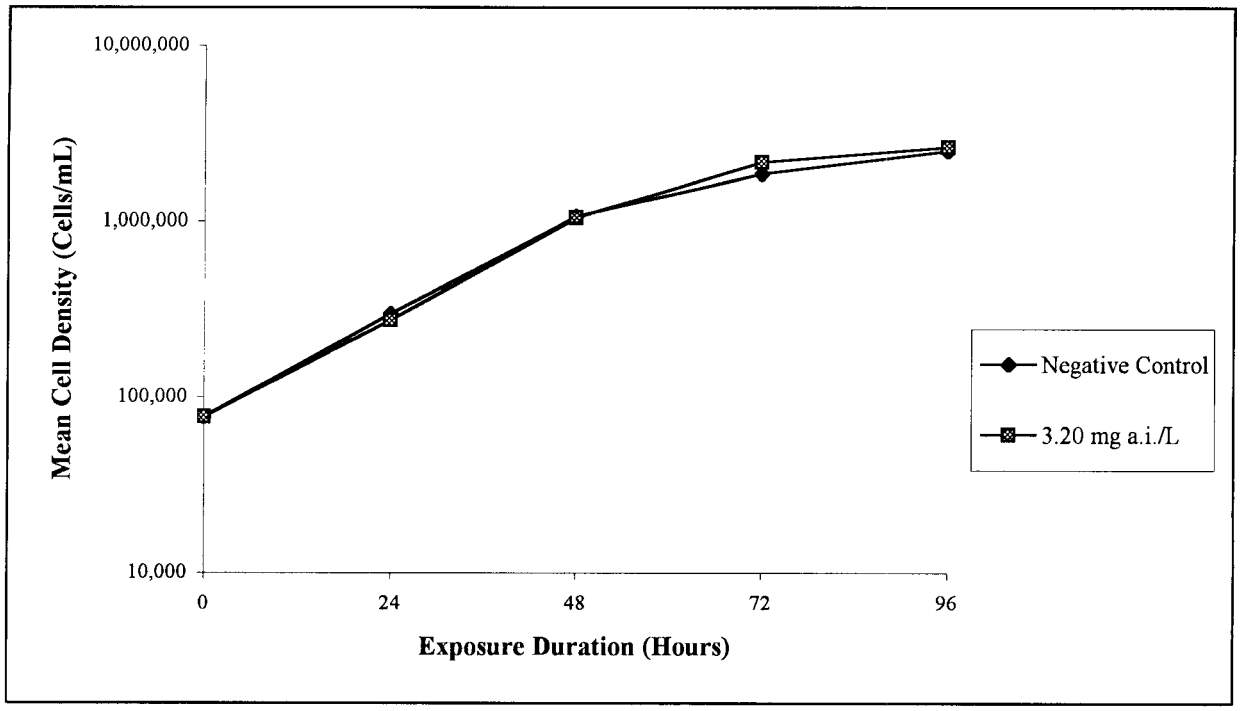


Figure 2. Treatment response, expressed as cell density, over the 96-hour exposure period.



APPENDIX I

Saltwater Algal Medium Constituents[1]

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Marine Diatom, *Skeletonema costatum*
Dilution Water:	Saltwater Algal Medium

Component[1]	Nominal Concentration[2]
$FeCl_3 \cdot 6H_2O$	0.72 mg/L
$MnCl_2 \cdot 4H_2O$	2.16 mg/L
$ZnSO_4 \cdot 7H_2O$	0.675 mg/L
$CuSO_4 \cdot 5H_2O$	2.36 µg/L
$CoCl_2 \cdot 6H_2O$	6.06 µg/L
H_3BO_3	17.1 mg/L
$Na_2EDTA \cdot 2H_2O$	15.0 mg/L
K_3PO_4	3.0 mg/L
$NaNO_3$	50.0 mg/L
$Na_2SiO_3 \cdot 9H_2O$	20.0 mg/L
Thiamine Hydrochloride	0.25 mg/L
Biotin	0.05 µg/L
B_{12}	0.5 µg/L

[1] The components were added to instant saltwater at a salinity of approximately 30 parts per thousand.

[2] The pH of the medium was 8.1.

APPENDIX II

Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Well Water[1]

Component	Measured Concentration	Component	Measured Concentration
Pesticides and Organics			
Aclonifen	<0.03 µg/L	Dimethomorf	<0.05 µg/L
Alachlor	<0.01 µg/L	Disulfoton	<0.02 µg/L
Ametryn	<0.01 µg/L	DMST	<0.05 µg/L
Atrazin	<0.01 µg/L	Dodemorf	<0.01 µg/L
Azinphos-ethyl	<0.04 µg/L	Endosulfan-α	<0.01 µg/L
Azinphos-methyl	<0.08 µg/L	Endosulfan-β	<0.01 µg/L
Azoxystrobin	<0.25 µg/L	Endosulfan-sulfaat	<0.02 µg/L
Bifenthrin	<0.05 µg/L	Epoxiconazole	<0.05 µg/L
Bioallethrin	<0.05 µg/L	Eptam	<0.02 µg/L
Bitertanol	<0.05 µg/L	Esfenvaleraat	<0.02 µg/L
Bromacil	<0.05 µg/L	Ethion	<0.05 µg/L
Bromophos	<0.02 µg/L	Ethofumesaat	<0.02 µg/L
Bromophos-ethyl	<0.02 µg/L	Ethoprophos	<0.01 µg/L
Broompropylaat	<0.02 µg/L	Etridiazole	<0.02 µg/L
Bupirimaat	<0.05 µg/L	Etrimphos	<0.05 µg/L
Carbaryl	<0.05 µg/L	Fenarimol	<0.05 µg/L
Carbofuran	<0.03 µg/L	Fenchlorphos	<0.01 µg/L
Carboxin	<0.02 µg/L	Fenitrothion	<0.03 µg/L
Chlorfenvinphos	<0.02 µg/L	Fenoxycarb	<0.03 µg/L
Chloridazon	<0.05 µg/L	Fenpiclonil	<0.05 µg/L
Chlorpropham	<0.02 µg/L	Fenpropathrin	<0.25 µg/L
Chlorpyriphos	<0.01 µg/L	Fenpropimorf	<0.01 µg/L
Chlorpyriphos-methyl	<0.01 µg/L	Fenthion	<0.01 µg/L
Chlorthalonil	<0.04 µg/L	Fenvaleraat	<0.02 µg/L
Coumaphos	<0.02 µg/L	Fluazifop-butyl	<0.02 µg/L
Cyanazin	<0.05 µg/L	Fluoroglycofen-ethyl	<0.02 µg/L
Cyfluthrin	<0.05 µg/L	Fluroxypyr-meptyl	<0.05 µg/L
Cypermethrin	<0.25 µg/L	Flutolanil	<0.02 µg/L
Cyproconazole	<0.05 µg/L	Fonophos	<0.01 µg/L
Deltamethrin	<0.02 µg/L	Furalaxyl	<0.02 µg/L
Demeton	<0.02 µg/L	Heptenophos	<0.02 µg/L
Demeton-o	<0.02 µg/L	Imazalil	<0.01 µg/L
Desethylatrazin	<0.01 µg/L	Iprodion	<0.05 µg/L
Desisopropylatrazin	<0.02 µg/L	Kresoxim-methyl	<0.02 µg/L
Desmetryn	<0.01 µg/L	Lenacil	<0.05 µg/L
Diazinon	<0.01 µg/L	Lindane	<0.02 µg/L
Dichlobenil	<0.01 µg/L	Malathion	<0.02 µg/L
Dichloran	<0.03 µg/L	Metalaxyl	<0.05 µg/L
Dichlorbenzamide	<0.02 µg/L	Metamitron	<0.05 µg/L
Dichlorfenthion	<0.01 µg/L	Metazachlor	<0.02 µg/L
Dichlorfluanid	<0.03 µg/L	Methidathion	<0.02 µg/L

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on October 14 and 15, 1999.

Continued

APPENDIX II

Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Well Water[1]
Page 2

Pesticides And Organics (Page 2)			
Component	Measured Concentration	Component	Measured Concentration
Dichlorvos	<0.01 µg/L	Methoxychlor	<0.01 µg/L
Dicofol	<0.25 µg/L	Metolachlor	<0.01 µg/L
Diethyltoluamide	<0.02 µg/L	Metribuzin	<0.02 µg/L
Difenoconazole	<0.03 µg/L	Mevinphos	<0.01 µg/L
Dimethoate	<0.02 µg/L	Nitrothal-Isopropyl	<0.05 µg/L
Paclobutazole	<0.05 µg/L	Pyrifenox-1	<0.01 µg/L
Parathion	<0.01 µg/L	Pyrifenox-2	<0.01 µg/L
Parathion-methyl	<0.01 µg/L	Pyrimethanil	<0.01 µg/L
Penconazole	<0.05 µg/L	Quizalofop-ethyl	<0.02 µg/L
Pendimethalin	<0.03 µg/L	Simazin	<0.01 µg/L
Permethrin-cis	<0.01 µg/L	Sulfotep	<0.02 µg/L
Permethrin-trans	<0.01 µg/L	Tebuconazole	<0.05 µg/L
Phosalon	<0.05 µg/L	Tebufenpyrad	<0.05 µg/L
Phosmet	<0.02 µg/L	Terbutryn	<0.01 µg/L
Phosphamidon-cis	<0.05 µg/L	Terbutylazin	<0.01 µg/L
Pirimicarb	<0.01 µg/L	Tetrachlorvinphos	<0.01 µg/L
Pirimiphos-ethyl	<0.01 µg/L	Tetrahydroftaalimide	<0.05 µg/L
Pirimiphos-methyl	<0.01 µg/L	Tetramethrin	<0.01 µg/L
Prochloraz	<0.02 µg/L	Thiabendazole	<0.05 µg/L
Procymidon	<0.01 µg/L	Thiometon	<0.04 µg/L
Prometryn	<0.01 µg/L	Tolclophos-methyl	<0.01 µg/L
Propachlor	<0.01 µg/L	Tolylfluanid	<0.04 µg/L
Propazin	<0.01 µg/L	Triadimefon	<0.05 µg/L
Propham	<0.02 µg/L	Triadimenol	<0.05 µg/L
Propiconazool	<0.05 µg/L	Triallaat	<0.02 µg/L
Propoxur	<0.03 µg/L	Triazophos	<0.02 µg/L
Propyzamide	<0.02 µg/L	Trifluralin	<0.02 µg/L
Prosulfocarb	<0.02 µg/L	Vamidothion	<0.01 µg/L
Pyrazophos	<0.03 µg/L	Vinchlozolin	<0.01 µg/L
Metals			
Magnesium	11.0 mg/L	Nickel	<1.1 µg/L
Sodium	18.0 mg/L	Copper	<0.7 µg/L
Calcium	29 mg/L	Zinc	<0.25 µg/L
Iron	<0.015 mg/L	Molybdenum	<0.3 µg/L
Potassium	1.1 mg/L	Silver	<0.2 µg/L
Aluminum	<0.02 mg/L	Cadmium	<0.1 µg/L
Manganese	<0.1 µg/L	Arsenic	<0.5 µg/L
Beryllium	<0.2 µg/L	Mercury	<0.025 µg/L
Chromium	<0.5 µg/L	Selenium	<0.5 µg/L
Cobalt	<0.2 µg/L		

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on October 14 and 15, 1999.

APPENDIX III

THE ANALYSIS OF PFOS IN SALTWATER ALGAL MEDIUM

IN SUPPORT OF

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454A-113A

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFOS: A 96-Hour Toxicity Test with the Marine Diatom (*Skeletonema costatum*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454A-113A

PRINCIPAL INVESTIGATOR:

Raymond L. Van Hoven, Ph.D.
Scientist

3/27/01
DATE

MANAGEMENT:

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

3/27/01
DATE

Introduction

Saltwater algal medium samples were collected from a 96-hour toxicity test designed to determine the effects of PFOS (Perfluorooctanesulfonate, Potassium Salt) to the marine diatom (*Skeletonema costatum*). This study was conducted by Wildlife International, Ltd. and identified as Project No.: 454A-113A. 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 May 19, May 22, and 23, 2000 and were analyzed on each sample receipt day.

Test Substance and Internal Standard

The test substance used for this study was Wildlife International, Ltd. identification number 4675. The test substance was used to prepare calibration 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 (hereafter referred to as 4H PFOS) was stored under ambient conditions.

Analytical Method

The method used for the analysis of the saltwater algal medium samples was developed at Wildlife International, Ltd. and entitled "Analytical Method for the Determination of PFOS in Freshwater, Saltwater, and Algal Medium". 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 centrifuged, as necessary, and 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 the 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 3000 Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. HPLC separations were achieved using a Keystone Betasil C_{18} analytical column (50 mm × 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 0.00480 to 0.0480 mg a.i./L, were analyzed with the samples. The same and most prominent peak response for PFOS was utilized to monitor PFOS in all calibration, quality control, and study samples. No attempt was made to quantify PFOS on the basis of individual isomeric components. 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 0.480 mg a.i./L calculated as the product of the lowest calibration standard analyzed (0.00480 mg a.i./L) and the dilution factor of the matrix blank samples (100).

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 ion chromatogram of a matrix blank is presented in Figure 5.

Saltwater algal medium was fortified at 2.40 and 4.80 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 88.5%. A representative ion chromatogram of a matrix fortification is presented in Figure 6.

Example Calculations

Sample number 454A-113A-4, nominal concentration of 3.46 mg a.i./L in saltwater algal medium.

Peak Area Ratio = Analyte Peak Area/Internal Standard Peak Area

Concentration Ratio = Concentration of Analyte/Concentration of Internal Standard

Internal Standard Concentration: 0.100 mg/L

Initial Volume: 0.100 mL

Final Volume: 10.0 mL

Dilution Factor: 100

PFOS Peak Area: 1242173

Internal Standard Peak Area: 2202273

Peak Area Ratio: 0.5640

Calibration curve equation.

Slope: 1.6984

Intercept: 0.0227

Curve is weighted (1/x)

$$\text{PFOS (mg a.i./L) at Instrument} = \frac{\text{Peak area ratio - (Y-intercept)}}{\text{Slope}} \times \text{Internal Standard Concentration}$$

$$= \frac{0.5640 - 0.0227}{1.6984} \times 0.100$$

$$= 0.0319$$

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

$$= 0.0319 \times 100$$

$$= 3.19$$

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

$$= \frac{3.19}{3.46} \times 100$$

$$= 92.2\%$$

RESULTS

Sample Analysis

Saltwater algal medium samples were collected from the 96-hour toxicity test with the marine diatom (*Skeletonema costatum*) at test initiation, May 19, 2000 (Day 0), on May 22, 2000 (Day 3) and at test termination,

May 23, 2000 (Day 4). The measured concentration of PFOS in the sample collected at initiation of exposure of the test organisms (Hour 0) was 94.2% of the nominal concentration. Samples collected at Day 3 had a measured concentration range of 92.2 to 97.1% of nominal values. Samples collected at test termination (Day 4) had a measured concentration range of 86.8 to 91.2% of nominal values (Table 4). Samples from the abiotic 3.46 mg a.i./L treatment group were comparable to samples from the 3.46 mg a.i./L treatment group with the marine diatom present (Table 4). A representative ion chromatogram of a test sample is shown in Figure 7.

Table 1

Typical HPLC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 3000 Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in selective ion monitoring mode (SIM).
ANALYTICAL COLUMN:	Keystone Betasil C_{18} column (50 mm × 2 mm I.D., 3-μm particle size)
OVEN TEMPERATURE:	30°C
STOP TIME:	5.00 minutes
FLOW RATE:	220 μL/minute
MOBILE PHASE:	70.0% Methanol : 30.0% NANOpure® Water containing 0.1% Formic Acid
INJECTION VOLUME:	5.0 μL
PFOS RETENTION TIME:	Approximately 3.5 minutes
INTERNAL STANDARD RETENTION TIME:	Approximately 2.3 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-113A-)	Type	
MAB-1	Matrix Blank	< LOQ
MAB-2	Matrix Blank	< LOQ
MAB-3	Matrix Blank	< LOQ

[1] The limit of quantitation (LOQ) was 0.480 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.00480 mg a.i./L) and the dilution factor of the matrix blank samples (100).

Table 3

Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (454A-113A-)	Concentrations of PFOS (mg a.i./L)		Percent Recovered[2]
	Fortified[1]	Measured[1]	
MAS-1	2.40	2.02	84.3
MAS-3	2.40	2.21	92.1
MAS-5	2.40	2.05	85.4
MAS-2	4.80	4.34	90.4
MAS-4	4.80	4.52	94.2
MAS-6	4.80	4.05	84.3
		Mean	= 88.5
		Standard Deviation	= 4.33
		CV	= 4.90%
		N	= 6

[1] Concentrations were corrected for change in test substance purity (90.49% to 86.9%) per Certificate of Analysis dated September 7, 2000.

[2] Results were generated using MacQuan version 1.6 software. Manual calculations may differ slightly since fortified and measured concentrations were corrected for change in test substance purity and rounded for reporting purposes.

Table 4

Measured Concentrations of PFOS in Saltwater Algal Medium Samples
from a Marine Diatom 96-hour Toxicity Test

Nominal Test Concentration[1] (mg a.i./L)	Sample Number (454A-113A-)	Sampling Time (Day)	PFOS Measured Concentration[1,2] (mg a.i./L)	Percent of Nominal[3]
0.0	1	0	< LOQ	--
(Negative Control)	3	3	< LOQ	--
	6	4	< LOQ	--
3.46	2	0	3.26	94.2
	4	3	3.19	92.2
	7	4	3.15	91.2
3.46	5	3	3.36	97.1
(Abiotic)	8	4	3.00	86.8

[1] Concentrations were corrected for change in test substance purity (90.49% to 86.9%) per Certificate of Analysis dated September 7, 2000.

[2] The limit of quantitation (LOQ) was 0.480 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.00480 mg a.i./L) and the dilution factor of the matrix blank samples (100).

[3] Results were generated using MacQuan version 1.6 software. Manual calculations may differ slightly since nominal and measured concentrations were corrected for change in test substance purity and rounded for reporting purposes.

METHOD OUTLINE FOR THE ANALYSIS OF PFOS IN SALTWATER ALGAL MEDIUM

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

↓

Centrifuge all samples, as necessary, for approximately ten minutes at approximately 2000 rpm.

↓

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 LCMS analysis.

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

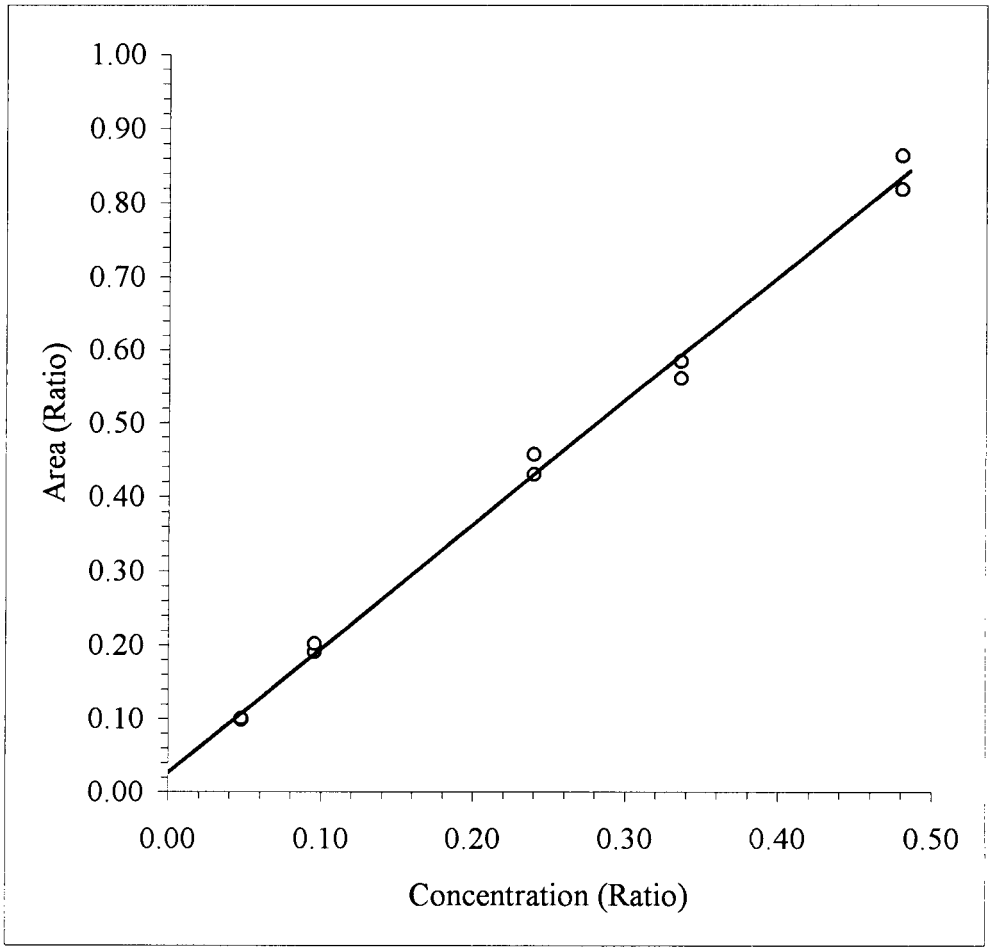


Figure 2. A typical calibration curve for PFOS. Slope = 1.6984; Intercept = 0.0227; r = 0.9981. Curve is weighted (1/x).

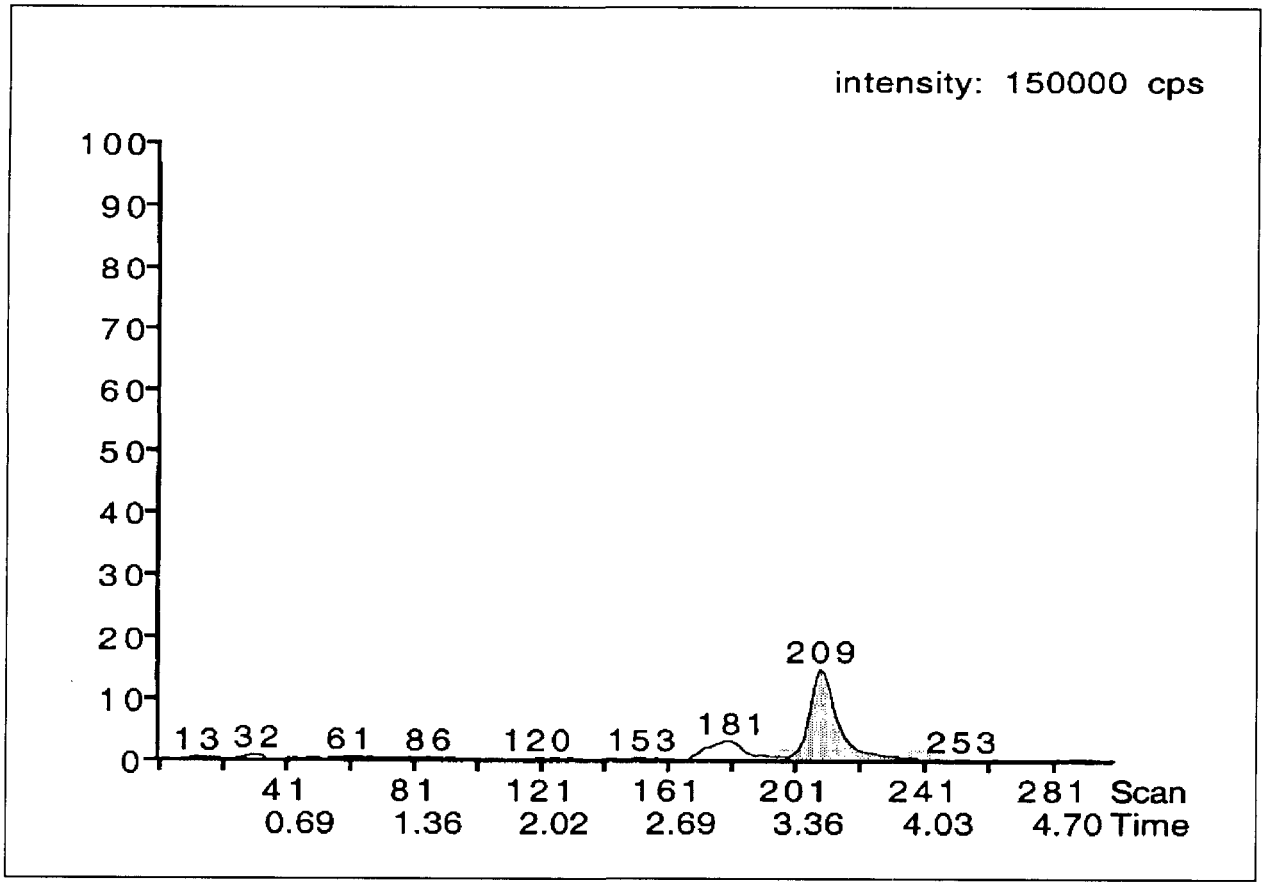


Figure 3. A representative ion chromatogram of a low-level (0.00480 mg a.i./L) PFOS standard.

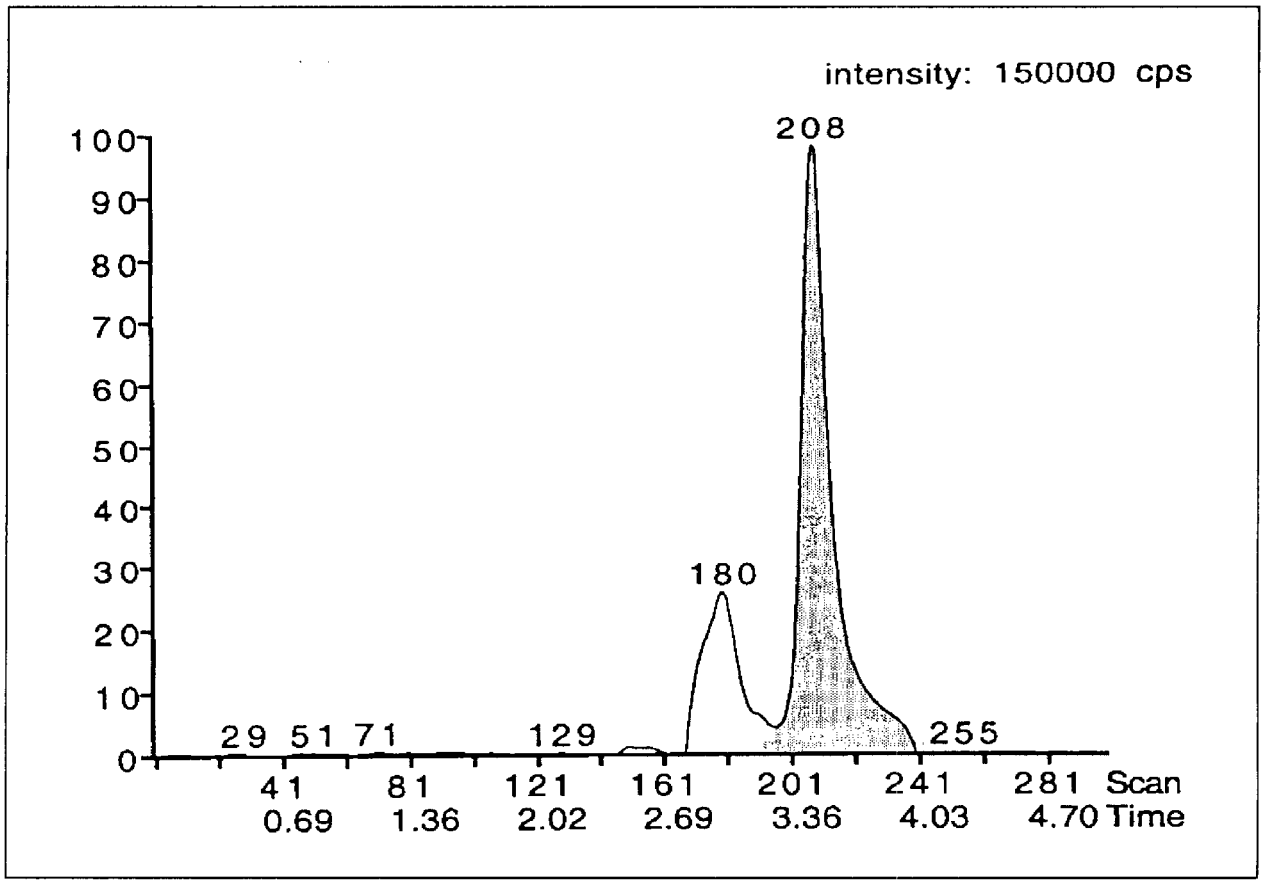


Figure 4. A representative ion chromatogram of a high-level (0.0480 mg a.i./L) PFOS standard.

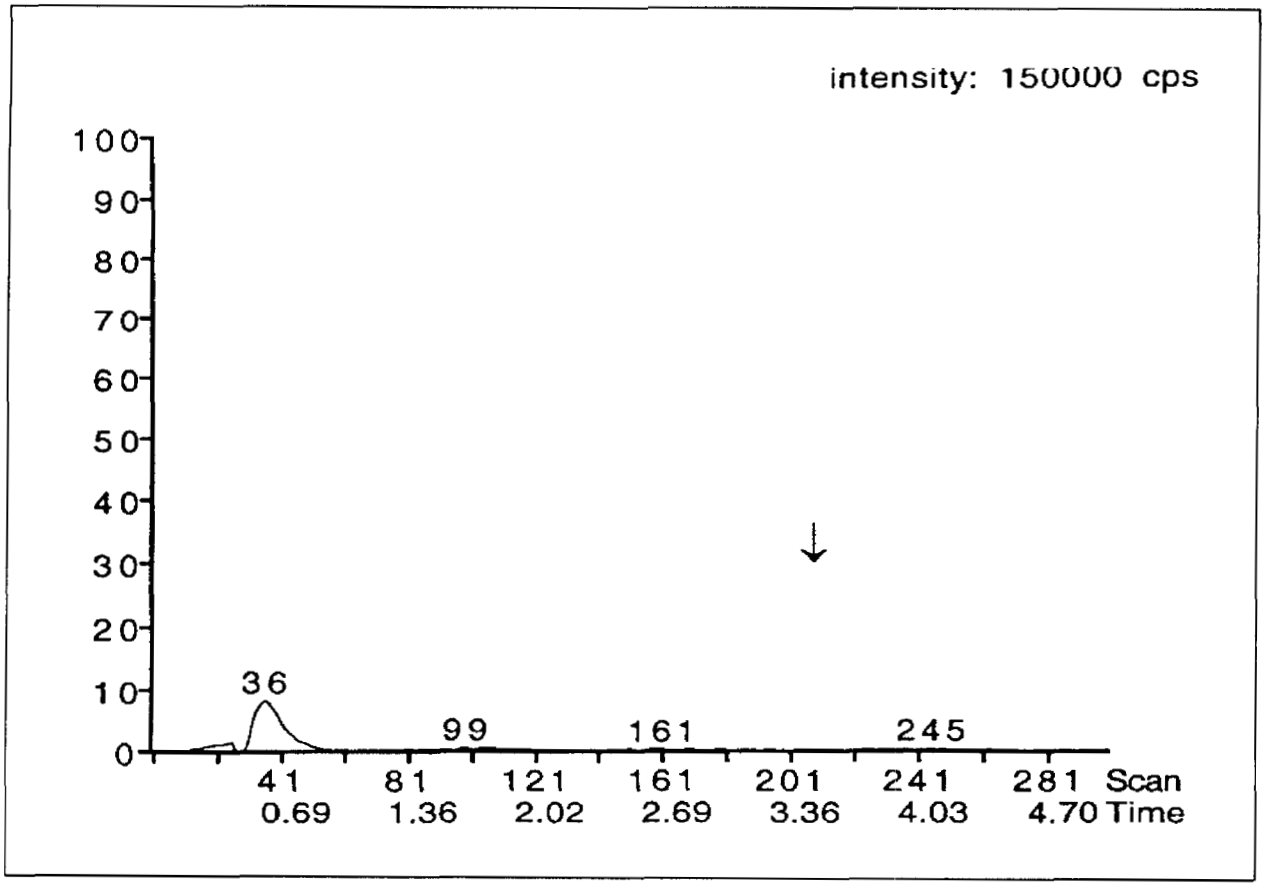


Figure 5. A representative ion chromatogram of a matrix blank sample (454A-113A-MAB-2). The arrow indicates the retention time of PFOS.

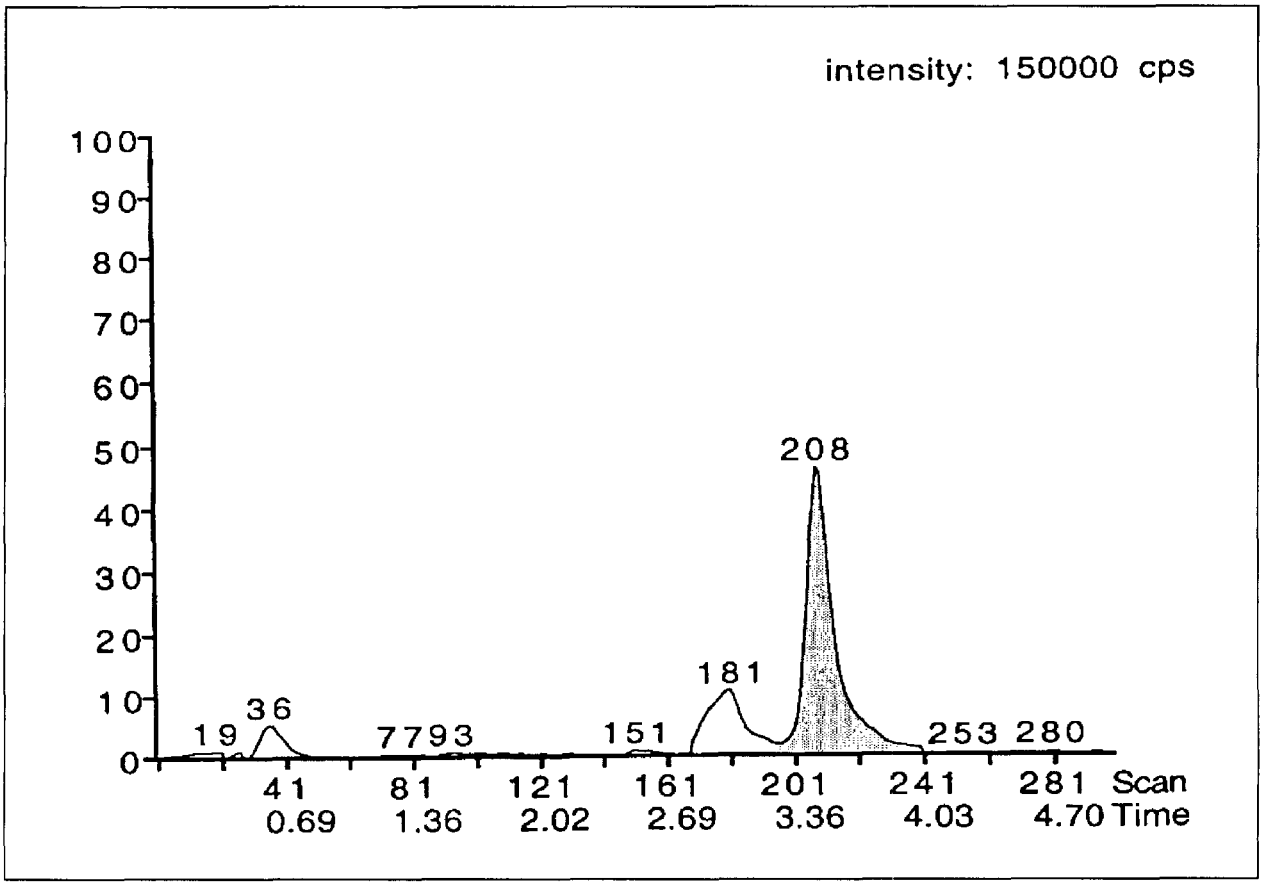


Figure 6. A representative ion chromatogram of a matrix fortification sample (454A-113A-MAS-4).

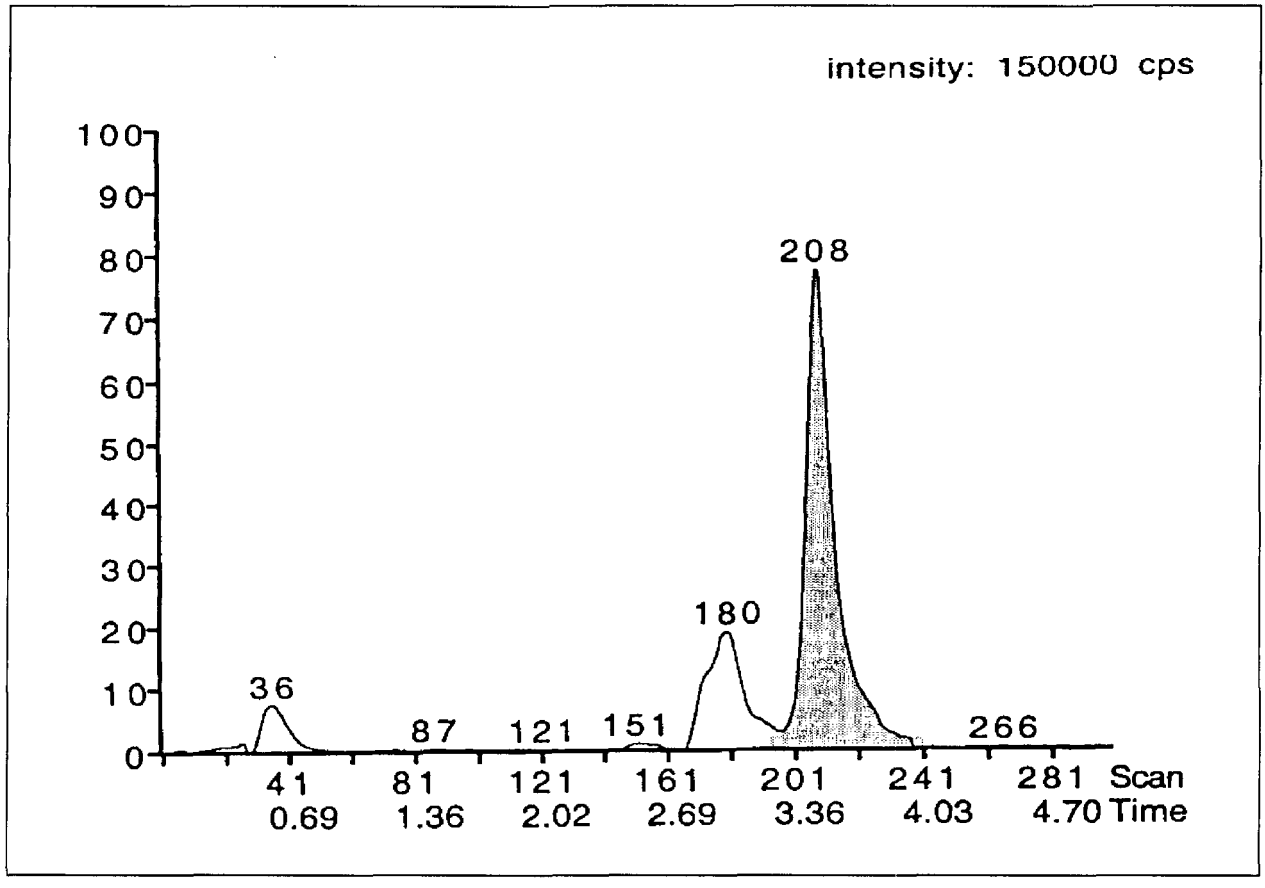


Figure 7. A representative ion chromatogram of a test sample (454A-113A-4).

APPENDIX IV

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

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Marine Diatom, *Skeletonema costatum*
Dilution Water: Saltwater 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	296,000	1,085,000	1,880,000	2,610,000
	B	316,000	1,130,000	1,860,000	2,370,000
	C	356,000	970,000	1,740,000	2,500,000
	D	267,000	925,000	2,100,000	2,410,000
	E	259,000	1,215,000	1,880,000	2,530,000
	F	290,000	1,060,000	1,630,000	2,470,000
3.20	A	290,000	1,140,000	2,160,000	2,220,000
	B	263,000	1,135,000	2,360,000	2,670,000
	C	250,000	1,110,000	2,140,000	2,450,000
	D	288,000	875,000	1,940,000	2,740,000
	E	255,000	945,000	1,900,000	2,690,000
	F	305,000	1,045,000	2,340,000	2,840,000

[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 77,000 cells/mL at test initiation (0 hours).

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: Marine Diatom, *Skeletonema costatum*
Dilution Water: Saltwater 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	2,628,000	17,352,000	51,084,000	103,116,000
	B	2,868,000	18,372,000	52,404,000	101,316,000
	C	3,348,000	17,412,000	48,084,000	97,116,000
	D	2,280,000	14,736,000	49,188,000	101,460,000
	E	2,184,000	18,024,000	53,316,000	104,388,000
	F	2,556,000	16,908,000	47,340,000	94,692,000
3.20	A	2,556,000	17,868,000	55,620,000	106,332,000
	B	2,232,000	17,160,000	57,252,000	115,764,000
	C	2,076,000	16,548,000	53,700,000	106,932,000
	D	2,532,000	14,640,000	46,572,000	100,884,000
	E	2,136,000	14,688,000	46,980,000	100,212,000
	F	2,736,000	17,088,000	55,860,000	116,172,000

APPENDIX VI

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

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Marine Diatom, *Skeletonema costatum*
Dilution Water: Saltwater 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	0.0561	0.0551	0.0444	0.0367
	B	0.0588	0.0560	0.0442	0.0357
	C	0.0638	0.0528	0.0433	0.0363
	D	0.0518	0.0518	0.0459	0.0359
	E	0.0505	0.0575	0.0444	0.0364
	F	0.0553	0.0546	0.0424	0.0361
3.20	A	0.0553	0.0561	0.0463	0.0350
	B	0.0512	0.0561	0.0475	0.0369
	C	0.0491	0.0556	0.0462	0.0360
	D	0.0550	0.0506	0.0448	0.0372
	E	0.0499	0.0522	0.0445	0.0370
	F	0.0574	0.0543	0.0474	0.0376

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 and the test concentrations for the first trial, and to add the test substance identification number.

2. The protocol was amended to change the experimental design to a limit test with six biological replicates in each of the treatment and control groups, and to correct a typographical error in the initial cell density.

3. The protocol was amended to change the experimental start and termination dates and the test concentrations for the repeat test, and to change the test apparatus from plastic to glass.

4. Two abiotic replicates, rather than one, were prepared at the test concentration, with one replicate sampled on each of Days 3 and 4.

5. The test chambers were inoculated with a calculated volume of the algal stock culture, rather than with 1 mL of an inoculum prepared from the stock culture.

5. The nominal concentration was calculated based on a test substance purity of 90.49%.

5. The nominal concentration was calculated based on a test substance purity of 86.9%.

5. The protocol was amended to change the test substance name from Perfluorooctane Sulfonic Acid, Potassium Salt to Perfluorooctanesulfonate, Potassium Salt.

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. Van Hoven, Ph.D., Scientist
4. Cary A. Sutherland, Laboratory Supervisor
5. Debbie Desjardins, Biologist