

AR226-0099

PFOS:
A SEMI-STATIC LIFE-CYCLE TOXICITY TEST
WITH THE CLADOCERAN (*Daphnia magna*)

FINAL REPORT

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-109
3M LAB REQUEST NO. U2723

U.S. Environmental Protection Agency
Series 850 – Ecological Effects
OPPTS Number 850.1300

OECD Guideline 211

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STUDY INITIATION DATE: July 2, 1999

STUDY COMPLETION DATE: February 28, 2000

AMENDED REPORT DATE: April 26, 2000

Submitted to

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Easton, Maryland 21601
(410) 822-8600

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-109

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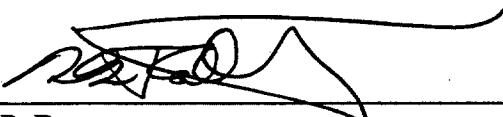
AMENDED REPORT: April 26, 2000

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 (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 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:

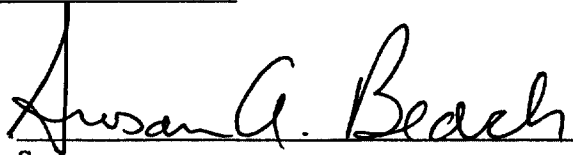


Kurt R. Drott
Senior Biologist

4/26/00

DATE

SPONSOR APPROVAL:



Sponsor

4/27/00

DATE

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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:

ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO:	
		STUDY DIRECTOR:	MANAGEMENT:
Test Substance Preparation	July 9, 1999	July 9, 1999	February 23, 2000
Test Initiation	July 12, 1999	July 12, 1999	July 13, 1999
Observations	July 27, 1999	July 27, 1999	July 28, 1999
Draft Report and Data	October 12 and 13, 1999	October 13, 1999	October 13, 1999
Analytical Data and Draft Report	October 12 and 13, 1999	October 14, 1999	October 14, 1999
Final Report	February 28, 2000	February 28, 2000	February 28, 2000
Amended Report	April 25 and 26, 2000	April 26, 2000	April 26, 2000

James H. Coleman
James H. Coleman
Quality Assurance Representative

4-26-00
DATE

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REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFOS: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-109

STUDY DIRECTOR:

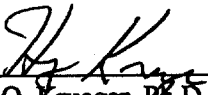


Kurt R. Drottar
Senior Biologist

4/26/00

DATE

MANAGEMENT:



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

4/26/00

DATE

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SUMMARY

SPONSOR:	3M Corporation
SPONSOR'S REPRESENTATIVE:	Susan A. Beach
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	454A-109
TEST SUBSTANCE:	PFOS (Perfluorooctane Sulfonic Acid Potassium Salt)
STUDY:	PFOS: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (<i>Daphnia magna</i>)
NOMINAL TEST CONCENTRATIONS	Negative Control, 1.4, 2.9, 5.7, 11, 23 and 46 mg a.i./L
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control, 1.5, 2.9, 5.6, 12, 24 and 48 mg a.i./L
TEST DATES:	Experimental Start – July 12, 1999 Biological Termination – August 2, 1999 Experimental Termination – August 4, 1999
LENGTH OF TEST:	21 Days

TEST ORGANISM:	Neonate Cladocerans (<i>Daphnia magna</i>)
SOURCE OF TEST ORGANISMS:	Wildlife International Ltd. cultures Easton, Maryland 21601
AGE OF TEST ORGANISMS:	Juveniles < 24 hours old

NOEC:	12 mg a.i./L
LOEC:	24 mg a.i./L
MATC:	17 mg a.i./L

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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 July 12, 1999 to August 2, 1999. Raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 454A-109 in archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of this study was to determine the effects of Perfluorooctane Sulfonic Acid Potassium Salt (PFOS) on the survival, growth and reproduction of the cladoceran (*Daphnia magna*) during a 21-day exposure period under semi-static test conditions.

EXPERIMENTAL DESIGN

Daphnids were exposed to a geometric series of six test concentrations and a negative (dilution water) control. Ten replicate test chambers were maintained in each treatment and control group with one daphnid in each test chamber. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the results of an exploratory range finding toxicity test. Nominal test concentrations were 1.4, 2.9, 5.7, 11, 23 and 46 mg a.i./L. Mean measured concentrations were determined from new and corresponding old solutions during each week of the test. Daphnids were transferred to new solutions every Monday, Wednesday and Friday during the test.

First-generation daphnids were observed daily for survival, the onset of reproduction, and clinical signs of toxicity. With the onset of reproduction, the number of second-generation neonates were counted (number of live neonates, number of dead or immobile neonates and number of aborted eggs) every Monday, Wednesday and Friday during the test. If available, twenty second-generation neonates from each treatment and the control were exposed under static test conditions for 48 hours. At test termination, the length and dry weight of each first-generation daphnid was determined. The no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC) were determined by examination of the mortality, growth and reproduction data. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and LOEC.

MATERIALS AND METHODS

The study was conducted based on the procedures outlined in the protocol, "PFOS: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)". The protocol was based on procedures outlined in the U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.1300 (1); OECD Guideline 211: *Daphnia magna* Reproduction Test (2); *Standard Evaluation Procedure, Daphnia magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test* (3); and ASTM Standard E 1193-87, *Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna* (4).

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

A primary stock solution was prepared by adding the test substance to UV sterilized dilution water at a concentration of 46 mg a.i./L. The test solution was stirred with an electric stainless steel top-down mixer until all the test substance was dissolved. The primary stock solution was proportionally diluted with UV sterilized dilution water to prepare the other five test concentrations. New solutions were prepared for each renewal day. After mixing, all test solutions appeared clear and colorless. All reported test concentrations were adjusted for the purity of the active ingredient in the test substance (90.49%).

Test Organism

The cladoceran, *Daphnia magna*, was selected as the test species for this study. Daphnids are representative of an important group of aquatic invertebrates and were selected for use in the test based upon past history of use and ease of culturing in the laboratory. Daphnid neonates used in the test were less than 24-hours old and were obtained from cultures maintained by Wildlife International Ltd., Easton, Maryland.

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Adult daphnids were cultured at approximately the same temperature as used during the test in Wildlife International, Ltd. well water. Daphnids in the cultures were held for at least 27 days prior to collection of the juveniles for testing. The adults showed no signs of disease or stress during the holding period. During the 14-day holding period preceding the test, water temperatures ranged from 20.0 to 20.3°C. The pH of the water ranged from 8.3 to 8.5 and dissolved oxygen ranged from 8.2 to 8.8 mg/L. Instrumentation and methods used for water measurements are described in the *Environmental Conditions* section of this report.

Neonate daphnids (<24 hours old) were obtained for testing from six individual adult daphnids. At test initiation, the juvenile daphnids were collected from the cultures and placed in glass beakers one to two at a time until each beaker contained 10 daphnids. The beakers were then impartially assigned to treatment groups. One daphnid was then transferred to each test chamber using a wide-bore pipet underneath the air/water interface. Daphnids in the cultures and those used in the test were fed a mixture of yeast, Cerophyll®, and trout chow (YCT, 1800 mg TSS/L), as well as a suspension of the freshwater green alga, *Selenastrum capricornutum* (3.5×10^7 cells/mL). During the test, each test chamber was fed 0.3 mL of YCT and 0.60 mL of *Selenastrum* once daily.

Test Apparatus

Test chambers were 250-mL plastic (Nalgene®) beakers containing approximately 200 mL of test solution. The depth of test solution in a representative test chamber was approximately 5 cm. Test chambers were randomly positioned by treatment group in an environmental chamber set to maintain a temperature of $20 \pm 1^\circ\text{C}$. The water bath was enclosed in a plexiglass hood to minimize the potential for cross-contamination. The test chambers were labelled with the project number, test concentration and replicate.

Dilution Water

The water used for culturing and testing was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International Ltd. site. The well water is characterized as moderately-hard water. The specific conductance, hardness, alkalinity, and pH of the well water during the four-week period immediately preceding the test are presented in Appendix I.

The well water was passed through a sand filter to remove particles greater than approximately 25 μm , and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to use, the water again was filtered to

0.45 μm and passed through a UV sterilizer to remove microorganisms and fine particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International Ltd. are presented in Appendix II.

Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity was measured at test initiation and at weekly intervals thereafter with a SPER Scientific Ltd. light meter. Light intensity ranged from 329 to 383 lux at the surface of the water.

Temperature was measured in alternating replicates of each treatment and the control group at test initiation, in old and new test solutions on renewal days, and at test termination using a liquid-in-glass thermometer. Temperature was also measured continuously in one negative control replicate using a Fulscope ER/C Recorder. The target test temperature during the test was $20 \pm 1^\circ\text{C}$. Dissolved oxygen was measured daily in alternating replicates during the first seven days of the test, in old and new solutions on renewal days, and at test termination. Measurements of pH were made in alternating replicates at test initiation, in old and new solutions on renewal days and at test termination. Hardness, alkalinity and specific conductance were measured in the negative control and the highest test concentration with surviving daphnids at test initiation and at approximately weekly intervals thereafter.

Measurements of pH were made using a Fisher Accumet Model 915 pH meter, and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Specific conductance was measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter. Hardness and alkalinity measurements were made by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* (5).

Biological Observations and Measurements

The first generation daphnids were observed daily during the test for survival, the onset of reproduction, and clinical signs of toxicity. The criteria for death included white opaque coloration, lack of movement of appendages, absence of heartbeat and lack of response to gentle prodding. Immobilization was defined as lack of movement except for minor

spontaneous random movement of the appendages. The presence of eggs in the brood pouch, aborted eggs, males or ephippia were also recorded.

With the onset of reproduction, the number of second-generation neonates were counted (number of live neonates, number of dead or immobile neonates and number of aborted eggs) and recorded every Monday, Wednesday and Friday during the test. At each observation period, the live first-generation daphnids were retained and the second-generation daphnids were discarded. On Day 14, the second-generation neonates were collected and used for the second-generation acute exposure. At test termination, the length (measured from the apex of the head to the base of the spine) and dry weight were determined for each surviving first-generation daphnid. The individual daphnids were removed from the test chambers and placed into tared aluminum drying pans. The length of each daphnid was determined using a caliper. The pans were then placed in a drying oven at approximately 60°C for approximately 43 hours. The pans were removed from the drying oven and placed in a desiccator to cool. When cool, the pans were weighed using a Mettler AE-240 analytical balance.

Statistical Analyses

Statistical analyses were performed on survival of first-generation daphnids, the number of live young and the length and dry weight of the surviving first-generation daphnids. Survival data were analyzed using Fisher's exact test. Reproduction and growth (length and dry weight) data were evaluated for normality and homogeneity of variance using the Shapiro-Wilk's test and Bartlett's test, respectively. Dunnett's test was then used to identify treatment groups that were statistically significant in comparison to the negative control ($p \leq 0.05$).

All statistical tests were performed on a personal computer using TOXSTAT Version 3.5 (6) statistical software.

Analytical Chemistry

Water samples were collected from new and old solutions each week during the test. Duplicate samples of new solutions were collected from the freshly prepared solutions. Duplicate samples of the old solutions were collected from a composite of the remaining replicate test chambers. Samples were collected in plastic (Nalgene®) bottles and were analyzed as soon as possible without storage. Analytical procedures used in the analysis of the samples are provided in Appendix III.

RESULTS AND DISCUSSION

Verification of Test Concentrations

Results of analyses to measure concentrations of PFOS in water samples collected during the test are presented in Table 1 and the analytical chemistry report (Appendix III). Nominal concentrations used in the study were 1.4, 2.9, 5.7, 11, 23 and 46 mg a.i./L. Measured values of new samples ranged from 94 to 121% of nominal values, whereas measured values from the old solutions ranged from 90 to 108% of nominal values. Consequently, PFOS was stable throughout the renewal periods. When measured concentrations of samples collected during each week of the test were averaged, the mean measured concentrations were 1.5, 2.9, 5.6, 12, 24 and 48 mg a.i./L, which represented 107, 100, 98, 109, 104 and 104% of nominal, respectively. The results of the study were based on mean measured concentrations.

Physical and Chemical Measurements of Water

Measurements of temperature, dissolved oxygen and pH are presented in Tables 2, 3 and 4, respectively. Water temperature measurements were within the range of $20 \pm 1^\circ\text{C}$ established for the test. Dissolved oxygen concentrations remained ≥ 8.3 mg/L (91% of saturation) throughout the test. Measurements of pH ranged from 8.1 to 8.5.

Measurements of specific conductance, hardness and alkalinity in the negative control and the highest treatment group are presented in Table 5. No apparent differences were observed for any of these parameters throughout the exposure period.

Survival and Biological Observations

A summary of the results of the observations of mortality and sublethal signs of toxicity for first-generation daphnids are shown in Table 6. Daily observations are provided in Appendix V. After 21 days of exposure, survival in the negative control group was 100%. Survival in the 1.5, 2.9, 5.6, 12, 24 and 48 mg a.i./L treatment groups was 90, 90, 90, 90, 0 and 0%, respectively. At test termination, all surviving daphnids exposed to PFOS appeared normal. Fisher's exact test showed that survival in the 24 and 48 mg a.i./L treatment groups was statistically different when compared to the negative control ($p \leq 0.05$).

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Reproduction

A summary of the mean number of live young produced by the first-generation daphnids is presented in Table 7. A complete listing of the number of neonates produced is provided in Appendix V. Daphnids in the control and PFOS treatment groups ≤ 12 mg a.i./L started producing neonates on Day 9. There were no observations of aborted eggs in any treatment group. Reproduction averaged 122 young per adult in the negative control. Reproduction in the 1.5, 2.9, 5.6 and 12 mg a.i./L treatment groups averaged 142, 136, 132 and 119 young per adult, respectively. The Bonferroni t-test showed that reproduction was not significantly reduced in any treatment group ≤ 12 mg a.i./L ($p > 0.05$). The 24 and 48 mg a.i./L treatment groups were not included in the statistical analysis of reproduction due to a statistically significant effect on survival.

Growth

A summary of the mean lengths and mean dry weights of the surviving first-generation daphnids are presented in Table 8. Individual measurements are provided in Appendix VI. The mean length and dry weight of first-generation daphnids in the negative control was 4.65 mm and 0.695 mg, respectively. The Bonferroni t-test showed that mean length and dry weight in the PFOS treatment groups ≤ 12 mg a.i./L were not significantly reduced in comparison to the negative control ($p > 0.05$).

Second-Generation Acute Exposure

The results of the second-generation acute exposure are presented in Table 9. After 48-hours of exposure, survival in the negative control was 95%. Survival in the 1.5, 2.9, 5.6, 12 and 24 mg a.i./L treatment groups was 100, 100, 100, 90 and 0%, respectively. Fisher's exact test showed that survival in the 24 mg a.i./L treatment group was significantly different from the negative control ($p \leq 0.05$).

CONCLUSIONS

There were no adverse effects on survival, reproduction or growth of *Daphnia magna* exposed to PFOS at concentrations ≤ 12 mg a.i./L for 21 days. *Daphnia magna* exposed to 24 and 48 mg a.i./L had significantly reduced survival. Consequently, the LOEC for this study, based on survival was 24 mg a.i./L. The NOEC was 12 mg a.i./L and the MATC was calculated to be 17 mg a.i./L. The results of the second-generation acute exposure also indicated a NOEC of 12 mg a.i./L.

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REFERENCES

- 1 U.S. Environmental Protection Agency. 1996. Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1300: *Daphnia Chronic Toxicity Test*.
- 2 OECD Guideline for Testing of Chemicals. 1997. Guideline 211: *Daphnia magna Reproduction Test*.
- 3 U.S. Environmental Protection Agency. 1986. *Standard Evaluation Procedure, Daphnia magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test*. Hazard Evaluation Division. Office of Pesticide Programs, EPA 540/9-86-141.
- 4 American Society for Testing and Materials. 1991. *Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna*, ASTM E 1193-87. Philadelphia, PA.
- 5 APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater*. 16th Edition, American Public Health Association. American water Works Association. Water Pollution Control Federation, New York.
- 6 West, Inc. and D.D. Gulley. 1996. TOXSTAT, Version 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

Table 1

Summary of Analytical Chemistry Data

Sponsor:	3M Corporation							
Test Substance:	PFOS							
Test Organism:	Cladoceran, <i>Daphnia magna</i>							
Dilution Water:	Well Water							
Nominal Test Concentration (mg a.i./L)	Day 0 (New)	Day 2 (Old)	Day 11 (New)	Day 14 (Old)	Day 18 (New)	Day 21 (Old)	Mean Measured Concentration (mg a.i./L)	Percent of Nominal
Negative Control	<LOQ ¹ <LOQ	<LOQ <LOQ	<LOQ <LOQ	<LOQ <LOQ	<LOQ <LOQ	<LOQ <LOQ	<LOQ	—
1.4	1.78 1.72	1.58 1.56	1.38 1.47	1.36 1.32	1.38 1.43	1.50 1.45	1.5	107
2.9	3.20 3.05	3.01 3.07	2.75 2.77	2.85 2.71	2.79 2.81	2.81 2.82	2.9	100
5.7	5.97 5.87	5.65 5.72	5.63 5.59	5.36 5.39	5.58 5.75	5.24 5.37	5.6	98
11	11.5 11.5	11.6 11.8	11.3 11.3	11.2 11.6	11.8 11.6	11.5 11.3	12	109
23	24.2 23.1	24.0 24.6	22.8 22.5	23.6 23.1	24.8 25.0	— ² — ²	24	104
46	47.3 48.0	49.1 49.4	— ² — ²	— —	— —	— —	48	104

¹The limit of quantitation (LOQ) was 0.458 mg a.i./L.²Measurements discontinued due to 100% mortality.

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Table 2

Temperature of Water in the Test Chambers¹

Sponsor:	3M Corporation										
Test Substance:	PFOS										
Test Organism:	Cladoceran, <i>Daphnia magna</i>										
Dilution Water:	Well Water										
	Temperature (°C)										
Mean Measured Test Concentration (mg a.i./L)	Day 0	Day 2		Day 4		Day 7		Day 9		Day 11	
	New	Old	New	Old	New	Old	New	Old	New	Old	New
Negative Control	19.4	19.6	19.5	19.5	19.6	19.8	19.8	20.0	20.1	20.1	19.8
1.5	19.4	19.6	19.5	19.5	19.6	19.8	19.8	20.0	20.1	20.0	19.8
2.9	19.4	19.6	19.5	19.5	19.6	19.8	19.8	20.0	20.1	20.0	19.8
5.6	19.4	19.6	19.5	19.5	19.6	19.8	19.8	20.1	20.1	20.0	19.8
12	19.4	19.5	19.5	19.5	19.6	19.8	19.8	20.1	20.1	20.0	19.8
24	19.4	19.5	19.5	19.5	19.7	19.8	19.8	20.1	20.1	20.1	19.9
48	19.4	19.5	19.5	-- ²	--	--	--	--	--	--	--

¹Temperature measured continuously during the test ranged from 19.0°C to 20.0°C.

²No measurement taken due to 100% mortality.

¹Temperature measured continuously during the test ranged from 19.0°C to 20.0°C.²No measurement taken due to 100% mortality.

Table 2 (Continued)

Temperature of Water in the Test Chambers¹

Sponsor:	3M Corporation						
Test Substance:	PFOS						
Test Organism:	Cladoceran, <i>Daphnia magna</i>						
Dilution Water:	Well Water						
Mean Measured Test Concentration (mg a.i./L)	Temperature (°C)						
	Day 14		Day 16		Day 18		Day 21
	Old	New	Old	New	Old	New	Old
Negative Control	19.9	19.8	19.9	19.8	20.1	20.1	20.1
1.5	19.8	19.8	19.9	19.8	20.1	20.1	20.0
2.9	19.8	19.8	19.9	19.9	20.0	20.1	20.0
5.6	19.9	19.9	19.9	19.9	20.1	20.1	20.0
12	19.9	19.9	19.9	19.9	20.1	20.1	20.0
24	19.9	19.9	20.0	19.9	20.1	-- ²	--
48	--	--	--	--	--	--	--

¹ Temperature measured continuously during the test ranged from 19.0°C to 20.0°C.

² Measurements discontinued due to 100% mortality.

Table 3

Dissolved Oxygen Content of Water in the Test Chambers¹

Sponsor:	3M Corporation										
Test Substance:	PFOS										
Test Organism:	Cladoceran, <i>Daphnia magna</i>										
Dilution Water:	Well Water										
Mean Measured Test Concentration (mg a.i./L)	Dissolved Oxygen Concentration (mg/L)										
	Day 0	Day 1	Day 2		Day 3	Day 4		Day 5	Day 6	Day 7	
	New	Old	Old	New	Old	Old	New	Old	Old	Old	New
Negative Control	8.3	8.3	8.6	8.9	8.7	8.6	8.6	8.7	8.8	8.7	8.8
1.5	8.3	8.3	8.6	8.9	8.8	8.6	8.8	8.8	8.8	8.7	8.8
2.9	8.3	8.3	8.6	8.9	8.8	8.6	8.8	8.8	8.9	8.7	8.8
5.6	8.3	8.3	8.6	8.9	8.8	8.6	8.8	8.8	8.9	8.7	8.8
12	8.3	8.3	8.6	9.0	8.8	8.6	8.8	8.8	8.8	8.7	8.8
24	8.4	8.3	8.6	8.9	8.8	8.6	8.8	8.8	8.9	8.7	8.8
48	8.4	8.4	8.6	8.9	- ²	-	-	-	-	-	-

¹A dissolved oxygen concentration of 5.4 mg/L represents 60% saturation at 20°C in freshwater²Measurements discontinued due to 100% mortality

Table 3 (Continued)

Dissolved Oxygen Content of Water in the Test Chambers¹

Sponsor:	3M Corporation										
Test Substance:	PFOS										
Test Organism:	Cladoceran, <i>Daphnia magna</i>										
Dilution Water:	Well Water										
Mean Measured Test Concentration (mg a.i./L)	Dissolved Oxygen Concentration (mg/L)										
	Day 9		Day 11		Day 14		Day 16		Day 18		Day 21
	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Negative Control	8.7	8.8	8.4	8.7	8.4	8.8	8.8	8.8	8.4	8.7	8.6
1.5	8.7	8.9	8.4	8.8	8.4	8.8	8.8	8.8	8.4	8.7	8.5
2.9	8.7	8.9	8.4	8.8	8.4	8.8	8.8	8.8	8.5	8.8	8.5
5.6	8.7	8.8	8.5	8.8	8.4	8.8	8.8	8.8	8.4	8.7	8.6
12	8.7	8.8	8.4	8.8	8.4	8.8	8.8	8.8	8.4	8.7	8.5
24	8.7	8.9	8.4	8.8	8.5	8.9	8.9	8.9	8.4	-- ²	--
48	--	--	--	--	--	--	--	--	--	--	--

¹A dissolved oxygen concentration of 5.4 mg/L represents 60% saturation at 20°C in freshwater.²No measurement taken due to 100% mortality.

Table 4
pH of Water in the Test Chambers

Sponsor:	3M Corporation										
Test Substance:	PFOS										
Test Organism:	Cladoceran, <i>Daphnia magna</i>										
Dilution Water:	Well Water										
	pH										
Mean Measured Test Concentration (mg a.i./L)	Day 0	Day 2		Day 4		Day 7		Day 9		Day 11	
	New	Old	New	Old	New	Old	New	Old	New	Old	New
Negative Control	8.3	8.3	8.1	8.4	8.2	8.4	8.4	8.3	8.2	8.3	8.1
1.5	8.4	8.4	8.2	8.4	8.2	8.5	8.3	8.4	8.2	8.3	8.2
2.9	8.4	8.4	8.2	8.4	8.2	8.5	8.3	8.4	8.3	8.2	8.2
5.6	8.4	8.4	8.2	8.5	8.2	8.5	8.4	8.4	8.3	8.3	8.2
12	8.4	8.4	8.3	8.5	8.3	8.5	8.4	8.4	8.3	8.3	8.2
24	8.4	8.4	8.3	8.5	8.4	8.5	8.5	8.5	8.4	8.3	8.3
48	8.5	8.4	8.4	-- ¹	--	--	--	--	--	--	--
¹ Measurements discontinued due to 100% mortality.											

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Table 4 (Continued)
pH of Water in the Test Chambers

Sponsor:	3M Corporation						
Test Substance:	PFOS						
Test Organism:	Cladoceran, <i>Daphnia magna</i>						
Dilution Water:	Well Water						
Mean Measured Test Concentration (mg a.i./L)	pH						
	Day 14		Day 16		Day 18		Day 21
	Old	New	Old	New	Old	New	Old
Negative Control	8.3	8.2	8.3	8.1	8.3	8.2	8.3
1.5	8.3	8.2	8.3	8.1	8.3	8.2	8.3
2.9	8.3	8.2	8.3	8.1	8.3	8.2	8.3
5.6	8.3	8.3	8.3	8.1	8.3	8.2	8.3
12	8.4	8.3	8.3	8.2	8.3	8.3	8.3
24	8.4	8.3	8.3	8.3	8.4	-- ¹	--
48	--	--	--	--	--	--	--

¹Measurements discontinued due to 100% mortality.

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Table 5

Specific Conductance, Hardness and Alkalinity in the Negative Control
and Highest Treatment Group Test Chambers

Sponsor:	3M Corporation			
Test Substance:	PFOS			
Test Organism:	Cladoceran, <i>Daphnia magna</i>			
Dilution Water:	Well Water			

Negative Control				
Parameter	Time			
	Day 0	Day 7	Day 14	Day 21
Specific Conductance (μ mhos/cm)	310	320	310	315
Hardness (mg/L as CaCO ₃)	136	128	132	128
Alkalinity (mg/L as CaCO ₃)	178	176	172	170

Parameter	Time			
	Day 0 ¹	Day 7 ²	Day 14 ²	Day 21 ³
Specific Conductance (μ mhos/cm)	310	320	310	320
Hardness (mg/L as CaCO ₃)	136	128	132	128
Alkalinity (mg/L as CaCO ₃)	178	176	174	174
48 mg a.i./L ¹ / 24 mg a.i./L ² / 12 mg a.i./L ³				

Table 6

Summary of Cumulative Percent Mortality and Treatment-Related Effects¹ for First-Generation Daphnids

Sponsor:	3M Corporation								
Test Substance:	PFOS								
Test Organism:	Cladoceran, <i>Daphnia magna</i>								
Dilution Water:	Well Water								
Mean Measured Test Concentration (mg a.i./L)	Cumulative Observations to Day 7			Cumulative Observations to Day 14			Cumulative Observations to Day 21		
	% Mortality	% Immobile	Effects	% Mortality	% Immobile	Effects	% Mortality	% Immobile	Effects
Negative Control	0	0	10 AN	0	0	10 AN	0	0	10 AN
1.5	0	0	10 AN	0	0	10 AN	10	0	9 AN
2.9	0	0	10 AN	0	0	10 AN	10	0	9 AN
5.6	0	0	10 AN	0	0	10 AN	10	0	9 AN
12	0	0	10 AN	10	0	9 AN	10	0	9 AN
24	70	0	3 AN	90	0	1 AN	100*	0	--
48	100	0	--	100	0	--	100*	0	--

¹ Observed Effects: AN = Appears Normal.
 * Indicates a significant difference from the negative control on Day 21 using Fisher's exact test ($p \leq 0.05$).

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Table 7

Summary of *Daphnia magna* Reproduction During the Life-Cycle Toxicity Test

Sponsor:	3M Corporation				
Test Substance:	PFOS				
Test Organism:	Cladoceran, <i>Daphnia magna</i>				
Dilution Water:	Well Water				
Mean Measured Test Concentration (mg a.i./L)	Number of Surviving Individually-Exposed Daphnids	Mean Live Young/ Surviving Adult Daphnid ($\pm$ SD)	First Day of Reproduction	Total Number of Dead/Immobile Neonates	Total Number of Aborted Eggs
Negative Control	10	122 ($\pm$ 19.2)	9	0	0
1.5	9	142 ($\pm$ 24.7)	9	0	0
2.9	9	136 ($\pm$ 17.9)	9	0	0
5.6	9	132 ($\pm$ 19.5)	9	0	0
12	9	119 ($\pm$ 26.5)	9	1	0
24 ¹	0	--	11	10	0
48 ¹	0	--	None	--	--
¹ This treatment was excluded from the statistical analyses of reproduction due to a statistically significant survival effect.					

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Table 8

Summary of Length and Dry Weight of Surviving
Individually-Exposed First-Generation Daphnids

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Cladoceran, <i>Daphnia magna</i>
Dilution Water:	Well Water

Mean Measured Test Concentration (mg a.i./L)	Number of Individually-Exposed Surviving Daphnids	Total Length (mm) Mean ($\pm$ SD)	Dry Weight (mg) Mean ($\pm$ SD)
Negative Control	10	4.65 ($\pm$ 0.111)	0.695 ($\pm$ 0.100)
1.5	9	4.66 ($\pm$ 0.118)	0.669 ($\pm$ 0.0623)
2.9	9	4.62 ($\pm$ 0.100)	0.724 ($\pm$ 0.110)
5.6	9	4.61 ($\pm$ 0.124)	0.727 ($\pm$ 0.0665)
12	9	4.59 ($\pm$ 0.102)	0.723 ($\pm$ 0.0661)
24	0	--	--
48	0	--	--

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Table 9

Cumulative Percent Mortality/Immobility and Treatment-Related Effects¹ for Second-Generation Daphnids

Sponsor		3M Corporation											
Test Substance:		PFOS											
Test Organism:		Cladoceran, <i>Daphnia magna</i>											
Dilution Water:		Well Water											
Mean Measured Concentration (mg a.i./L)	Replicate	Daphnia/ Replicate	5 Hours			24 Hours			Percent Immobile and Dead	48 Hours			Percent Immobile and Dead
			Number Dead	Number Immobile	Effects	Number Dead	Number Immobile	Effects		Number Dead	Number Immobile	Effects	
Negative Control	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	5
	B	10	0	0	10 AN	0	0	10 AN		1	0	9 AN	
1.5	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	0
	B	10	0	0	10 AN	0	0	10 AN		0	0	10 AN	
2.9	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	0
	B	10	0	0	10 AN	0	0	10 AN		0	0	10 AN	
5.6	A	10	0	0	10 AN	0	0	10 AN	0	0	0	10 AN	0
	B	10	0	0	10 AN	0	0	10 AN		0	0	10 AN	
12	A	10	0	0	10 AN	0	0	10 AN	0	1	0	9 AN	10
	B	10	0	0	10 AN	0	0	10 AN		1	0	9 AN	
24	A	4	0	0	4 AN	1	1	1AN,1C	50	4	0	—	100*
	B	4	0	0	4 AN	2	0	1AN,1C		4	0	—	

¹ Observed Effects: AN = Appears Normal, C = Lethargic

* Indicates a significant difference from the negative control at 48 hours using Fisher's exact test ($p \leq 0.05$).

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APPENDIX I

Specific Conductance, Hardness, Alkalinity and pH of Well Water Measured
During the 4-Week Period Immediately Preceding the Test

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Cladoceran, <i>Daphnia magna</i>
Dilution Water:	Well Water

	Mean	Range
Specific Conductance (μ mhos/cm)	329 (N = 4)	315 - 340
Hardness (mg/L as CaCO ₃)	124 (N = 4)	120 - 128
Alkalinity (mg/L as CaCO ₃)	169 (N = 4)	164 - 172
pH	8.2 (N = 4)	8.0 - 8.3

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APPENDIX II
Analyses of Pesticides, Organics, Metals and Other Inorganics
in Wildlife International Ltd. Well Water¹

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 ³	<	0.20	µg/L
Molybdenum ³	<	2.0	µg/L
Nickel ³	<	2.0	µg/L
Potassium 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.

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APPENDIX III

THE ANALYSIS OF PFOS IN FRESHWATER

IN SUPPORT OF

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454A-109

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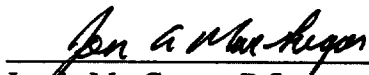
REPORT APPROVAL

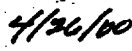
SPONSOR: 3M Corporation

TITLE: PFOS: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454A-109

PRINCIPAL INVESTIGATOR:


Jon A. MacGregor, B.S.
Scientist


DATE

MANAGEMENT:


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


DATE

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Introduction

Freshwater samples were collected from a semi-static life-cycle toxicity study designed to determine the effects of PFOS (Perfluorooctane Sulfonic Acid Potassium Salt) to the cladoceran (*Daphnia magna*). This study was conducted by Wildlife International Ltd. and identified as Project No.: 454A-109. 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 between July 12, 1999 and August 2, 1999 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 was stored under ambient conditions.

Analytical Method

The method used for the analysis of the freshwater 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 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 100LC Mass Spectrometer equipped with a Perkin-

Elmer TurboIonSpray ion source. HPLC separations were achieved using a Keystone Betasil C₁₈ analytical column (50 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 0.00915 to 0.0915 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 0.458 mg a.i./L calculated as the product of the lowest calibration standard analyzed (0.00915 mg a.i./L) and the dilution factor of the matrix blank samples (50).

Matrix Blank and Fortification Samples

Six 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.

Freshwater was fortified at 0.915, 9.15 and 54.9 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 104%. A representative ion chromatogram of a matrix fortification is presented in Figure 6.

Example Calculations

Sample number 454A-109-3, nominal concentration of 1.4 mg a.i./L in freshwater.

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: 0.500 mL

Final Volume: 25.0 mL

Dilution Factor: 50.0

PFOS Peak Area: 3091181

Internal Standard Peak Area: 3169689

Peak Area Ratio: 0.97523

Calibration curve equation.

Slope: 2.42974

Intercept: 0.11482

Curve is weighted (1/x)

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

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

Calculated concentration: 1.78 mg a.i./L

Note: manual calculations may differ.

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

Calculated recovery: 121%

Note: manual calculation may differ.

RESULTS

Sample Analysis

Freshwater samples were collected from the semi-static life-cycle toxicity study with the cladoceran (*Daphnia magna*) at test initiation, July 12, 1999 (Day 0), on July 14, 1999 (Day 2 old solutions), on July 23, 1999 (Day 11 – new solutions), on July 26, 1999 (Day 14 – old solutions), on July 30, 1999 (Day 18 – new solutions) and at test termination on August 2, 1999 (Day 21 old solutions). The measured concentrations of PFOS in the samples collected at initiation of exposure of the test organisms (Hour 0) ranged from 96.5 to 121% of the nominal concentrations (Table 4). Samples collected at Day 2, Day 11, Day 14, and Day 18 had measured concentration ranges of 97.8 to 108%, 94.3 to 101%, 89.8 to 103% and 94.5 to 109% of nominal values, respectively. Samples collected at test termination (Day 21) had a measured concentration range of 91.0 to 102% of nominal values. A representative ion chromatogram of a test sample is shown in Figure 7.

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Table 1

Typical HPLC/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 (50 mm x 2 mm I.D., 3 µm particle size)
OVEN TEMPERATURE:	30°C
STOP TIME:	5.00 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 4.3 minutes
INTERNAL STANDARD RETENTION TIME:	Approximately 3.0 minutes
PFOS MONITORED MASS:	498.6 amu
INTERNAL STANDARD MONITORED MASS:	426.7 amu

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Table 2

Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured Concentration of PFOS ¹ (mg a.i./L)
Number (454A-109-)	Type	
MAB-1	Matrix Blank	< LOQ
MAB-2	Matrix Blank	< LOQ
MAB-3	Matrix Blank	< LOQ
MAB-4	Matrix Blank	< LOQ
MAB-5	Matrix Blank	< LOQ
MAB-6	Matrix Blank	< LOQ

¹ The limit of quantitation (LOQ) was 0.458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.00915 mg a.i./L) and the dilution factor of the matrix blank samples (50).

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Table 3

Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (454A-109-)	Concentrations of PFOS (mg a.i./L)		Percent Recovered
	Fortified	Measured	
MAS-1	0.915	0.988	108
MAS-4	0.915	1.02	111
MAS-7	0.915	0.952 ¹	104
MAS-10	0.915	0.942	103
MAS-13	0.915	0.997	109
MAS-16	0.915	0.979	107
MAS-2	9.15	9.33	102
MAS-5	9.15	9.33	102
MAS-8	9.15	9.33	102
MAS-11	9.15	9.15	100
MAS-14	9.15	9.15	100
MAS-17	9.15	9.61	105
MAS-3	54.9	59.9	109
MAS-6	54.9	56.7	103
MAS-9	54.9	55.3	101
MAS-12	54.9	55.9	102
MAS-15	54.9	57.3	104
MAS-18	54.9	55.7	102

Mean = 104

Standard Deviation = 3.32

CV = 3.19%

N = 18

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 Reported value represents mean of duplicate re-analyses (0.970 and 0.933 mg a.i./L).

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Table 4

Measured Concentrations of PFOS in Freshwater Samples from a
Cladoceran Semi-Static Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-109-)	Sampling Time (Day)	PFOS Measured Concentration ¹ (mg a.i./L)	Percent of Nominal
0.0	1	0	< LOQ	--
	2	0	< LOQ	--
	15	2	< LOQ	--
	16	2	< LOQ	--
	29	11	< LOQ	--
	30	11	< LOQ	--
	41	14	< LOQ	--
	42	14	< LOQ	--
	53	18	< LOQ	--
	54	18	< LOQ	--
	65	21	< LOQ	--
	66	21	< LOQ	--
1.4	3	0	1.78	121
	4	0	1.72	117
	17	2	1.58	108
	18	2	1.56	107
	31	11	1.38	94.3
	32	11	1.47	101
	43	14	1.36	93.1
	44	14	1.32	89.8
	55	18	1.38	94.2
	56	18	1.43	97.4
	67	21	1.50	102
	68	21	1.45	98.5

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 0.458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.00915 mg a.i./L) and the dilution factor of the matrix blank samples (50).

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Table 4 (continued)

Measured Concentrations of PFOS in Freshwater Samples from a
Cladoceran Semi-Static Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-109-)	Sampling Time (Day)	PFOS Measured Concentration ¹ (mg a.i./L)	Percent of Nominal
2.9	5	0	3.20	113
	6	0	3.05	107
	19	2	3.01	106
	20	2	3.07	108
	33	11	2.75	96.9
	34	11	2.77	97.6
	45	14	2.85	101
	46	14	2.71	95.5
	57	18	2.79	98.3
	58	18	2.81	99.1
	69	21	2.81	99.0
	70	21	2.82	99.2
5.7	7	0	5.97	104
	8	0	5.87	102
	21	2	5.65	98.1
	22	2	5.72	99.1
	35	11	5.63	97.6
	36	11	5.59	96.9
	47	14	5.36	93.0
	48	14	5.39	93.5
	59	18	5.58	96.8
	60	18	5.75	99.6
	71	21	5.24	91.0
	72	21	5.37	93.2

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 0.458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.00915 mg a.i./L) and the dilution factor of the matrix blank samples (50).

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Table 4 (continued)

Measured Concentrations of PFOS in Freshwater Samples from a
Cladoceran Semi-Static Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-109-)	Sampling Time (Day)	PFOS Measured Concentration ¹ (mg a.i./L)	Percent of Nominal
11	9	0	11.5	96.5
	10	0	11.5	97.0
	23	2	11.6	97.8
	24	2	11.8	99.3
	37	11	11.3	95.0
	38	11	11.3	94.5
	49	14	11.2	94.0
	50	14	11.6	97.5
	61	18	11.8	99.4
	62	18	11.6	97.4
	73	21	11.5	97.1
	74	21	11.3	94.5
23	11	0	24.2	106
	12	0	23.1	101
	25	2	24.0	105
	26	2	24.6	108
	39	11	22.8	99.6
	40	11	22.5	98.3
	51	14	23.6	103
	52	14	23.1	101
	63	18	24.8	108
	64	18	25.0	109
46	13	0	47.3	103
	14	0	48.0	105
	27	2	49.1	107
	28	2	49.4	108

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 0.458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.00915 mg a.i./L) and the dilution factor of the matrix blank samples (50).

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

Prepare matrix fortification samples by spiking the requisite volume of PFOS stock solutions directly into filtered freshwater 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 LCMS analysis.

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

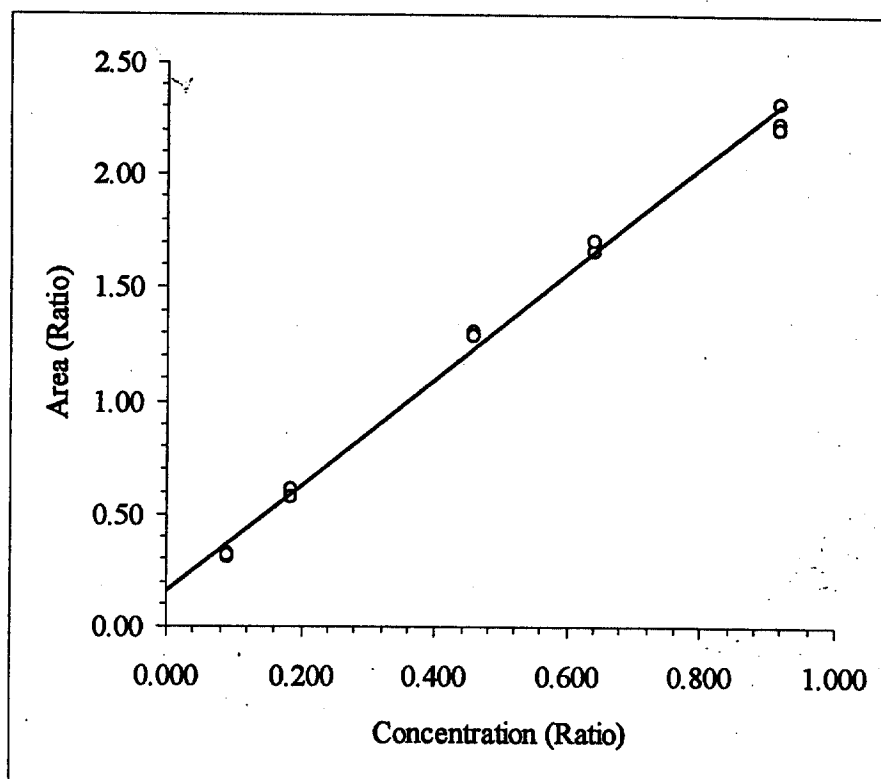


Figure 2. A typical calibration curve for PFOS. Slope = 2.42974; Intercept = 0.11482; $r = 0.9974$. Curve is weighted ($1/x$).

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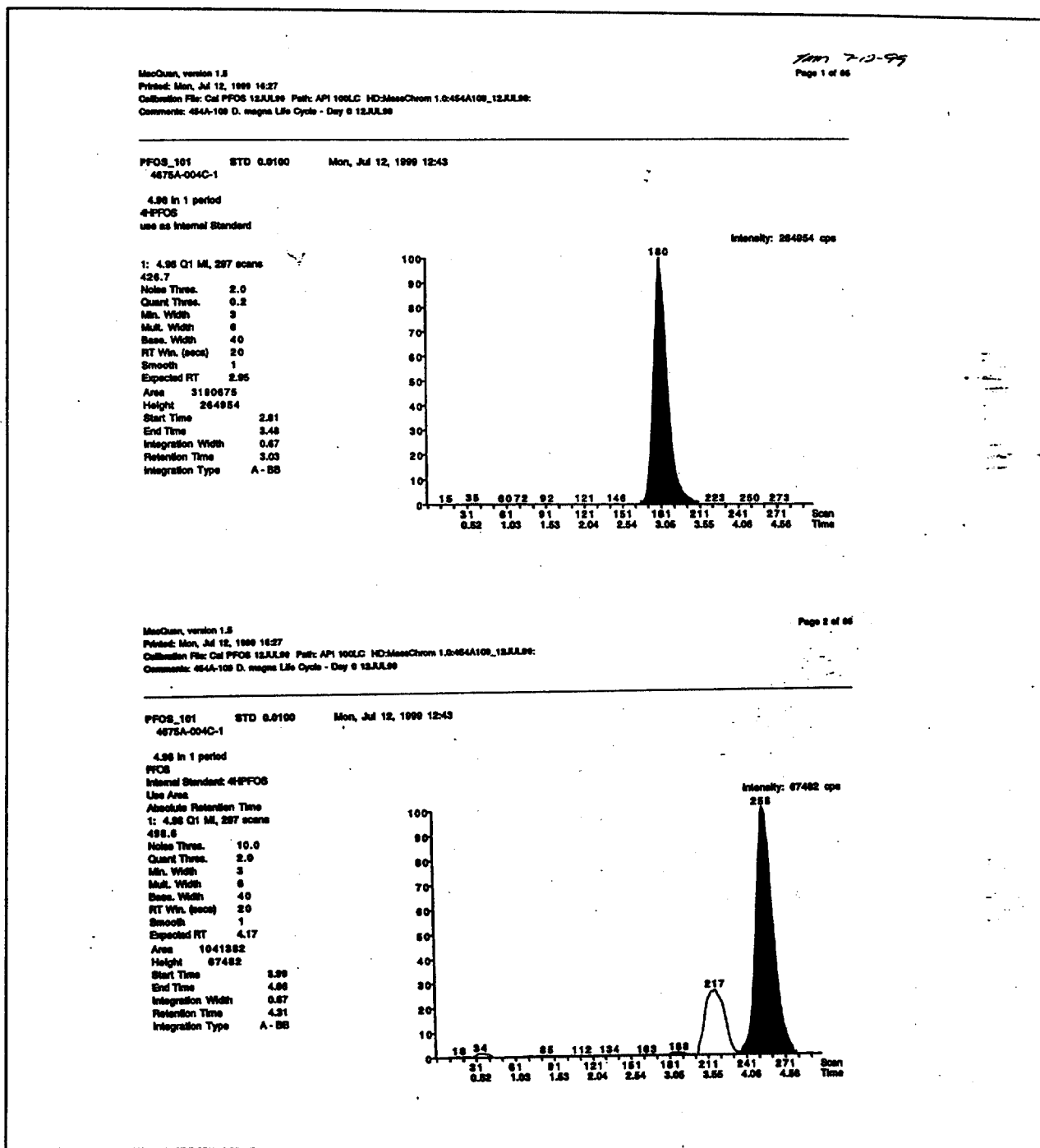


Figure 3. A representative ion chromatogram of a low-level (0.00915 mg a.i./L) PFOS standard.

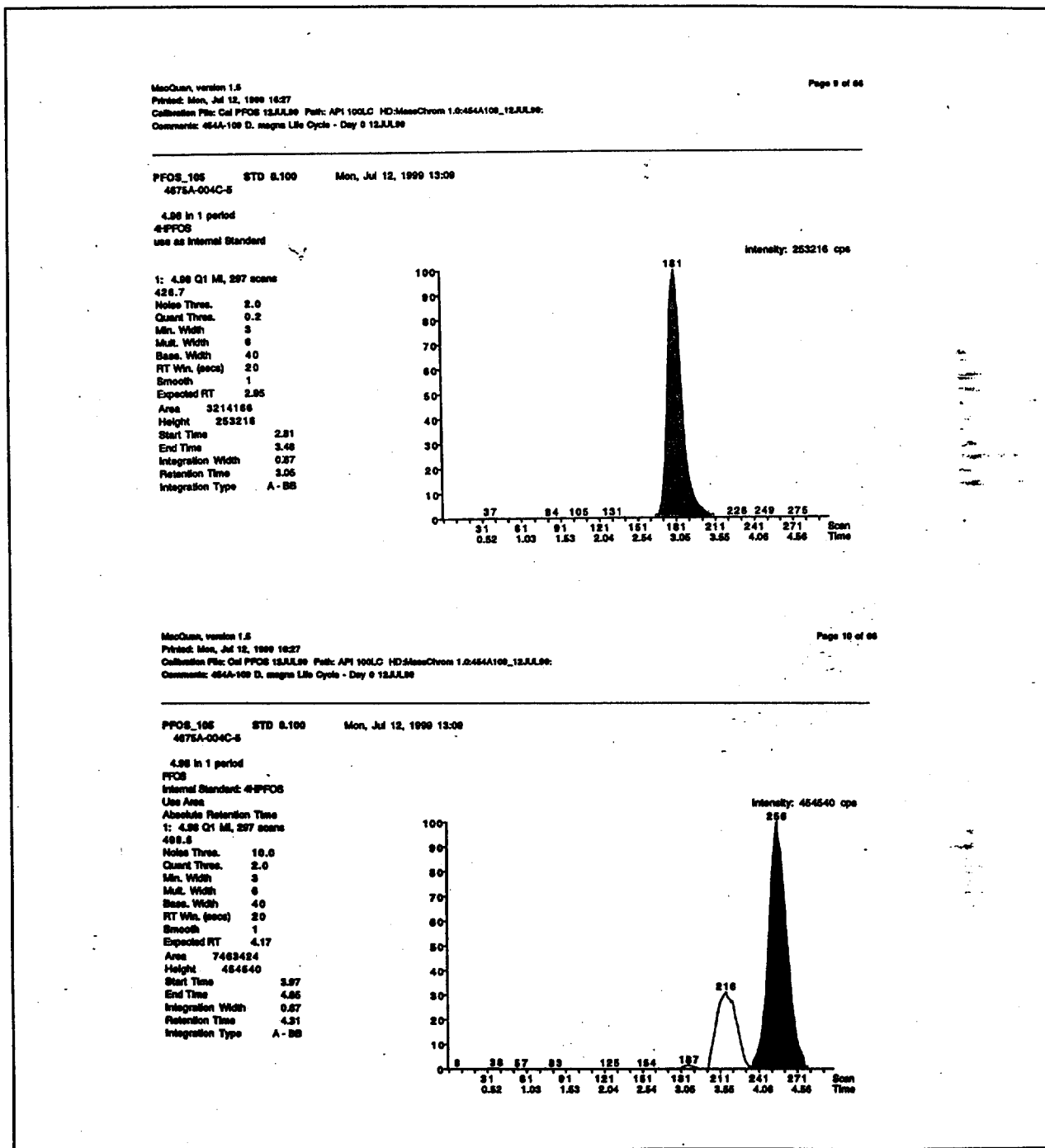
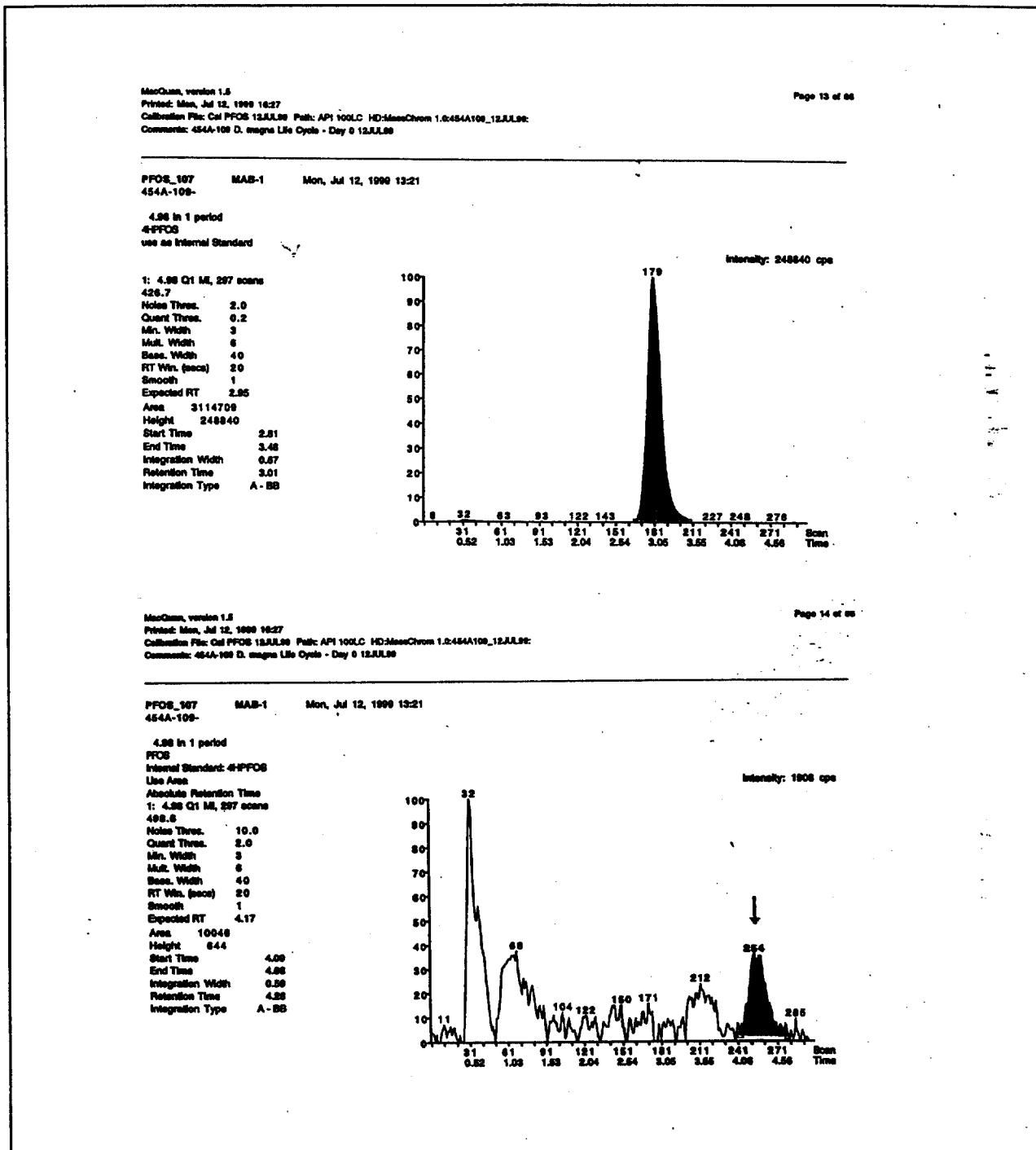


Figure 4. A representative ion chromatogram of a high-level (0.0915 mg a.i./L) PFOS standard.



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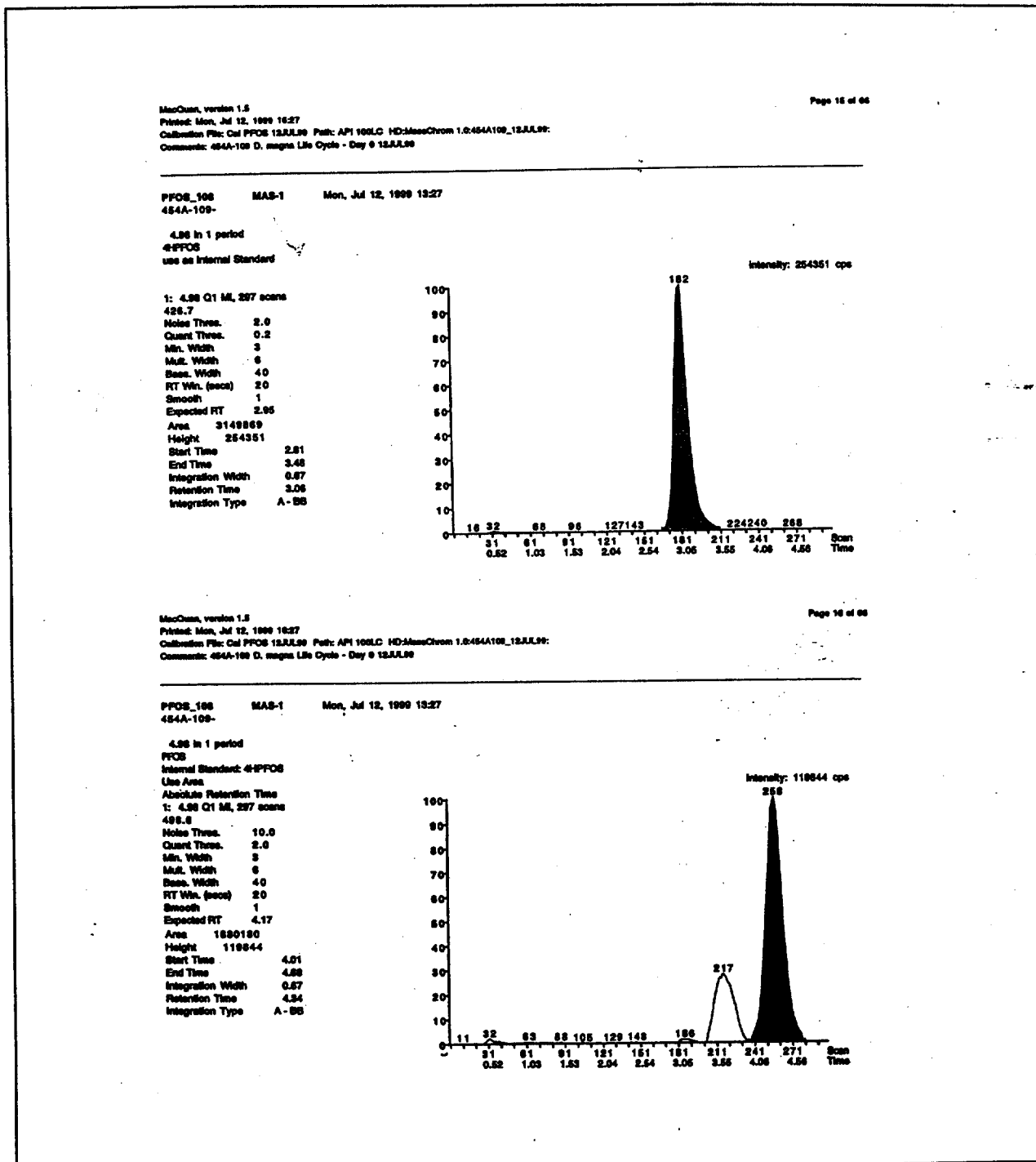


Figure 6. A representative ion chromatogram of a matrix fortification sample (454A-109-MAS-1).

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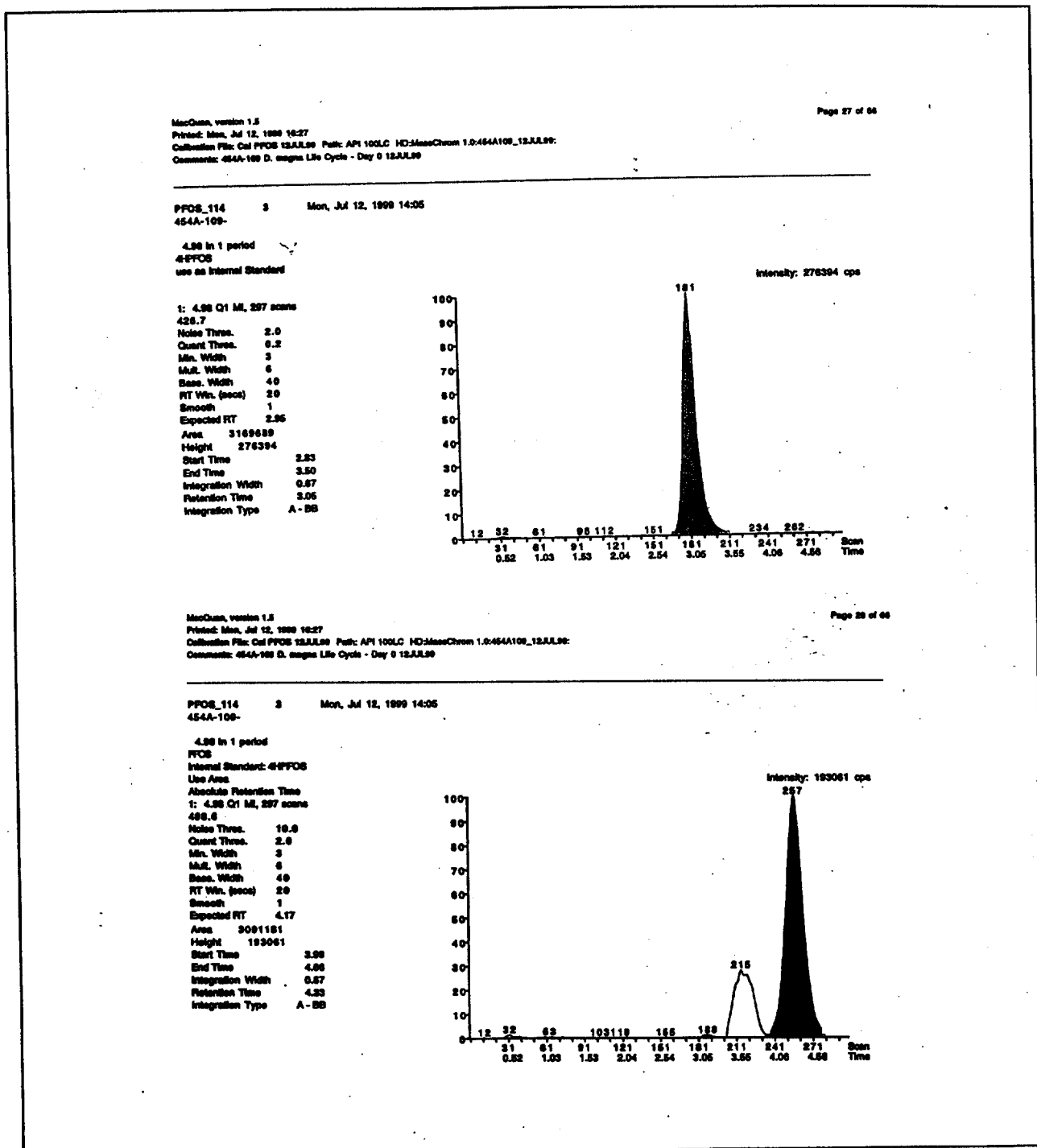


Figure 7. A representative ion chromatogram of a test sample (454A-109-3).

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APPENDIX IV

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 1			Day 2			Day 3		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
1.5	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
2.9	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

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APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor: 3M Corporation											
Test Substance: PFOS											
Test Organism: Cladoceran, <i>Daphnia magna</i>											
Dilution Water: Well Water											
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 1			Day 2			Day 3		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
5.6	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
12	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
24	A	1	0	0	1 AN	0	0	1 AN	0	1	-
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	1	-
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	1	-
	F	1	0	0	1 AN	0	0	1 AN	0	1	-
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 C	0	1	-
	J	1	0	0	1 AN	0	0	1 AN	0	1	-

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 1			Day 2			Day 3		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
48	A	1	0	0	1 AN	0	1	--	0	1	--
	B	1	0	0	1 AN	0	1	--	0	1	--
	C	1	0	0	1 AN	0	1	--	0	1	--
	D	1	0	0	1 AN	0	1	--	0	1	--
	E	1	0	0	1 AN	0	1	--	0	1	--
	F	1	0	0	1 AN	0	1	--	0	1	--
	G	1	0	0	1 AN	0	1	--	0	1	--
	H	1	0	0	1 AN	0	1	--	0	1	--
	I	1	0	0	1 AN	0	1	--	0	1	--
	J	1	0	0	1 AN	0	1	--	0	1	--

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

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APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor: 3M Corporation											
Test Substance: PFOS											
Test Organism: Cladoceran, <i>Daphnia magna</i>											
Dilution Water: Well Water											
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 4			Day 5			Day 6		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
1.5	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
2.9	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 4			Day 5			Day 6		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
5.6	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
12	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
24	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	1	--	0	1	--	0	1	--
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	1	--	0	1	--	0	1	--
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	1	--	0	1	--	0	1	--
	J	1	0	1	--	0	1	--	0	1	--

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

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APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 4			Day 5			Day 6		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
48	A	1	0	1	-	0	1	-	0	1	-
	B	1	0	1	-	0	1	-	0	1	-
	C	1	0	1	-	0	1	-	0	1	-
	D	1	0	1	-	0	1	-	0	1	-
	E	1	0	1	-	0	1	-	0	1	-
	F	1	0	1	-	0	1	-	0	1	-
	G	1	0	1	-	0	1	-	0	1	-
	H	1	0	1	-	0	1	-	0	1	-
	I	1	0	1	-	0	1	-	0	1	-
	J	1	0	1	-	0	1	-	0	1	-
¹ Observed Effects: AN = Appeared Normal; C = Lethargic.											
² All organisms on Day 0 appeared normal.											

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APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 7			Day 8			Day 9		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
1.5	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
2.9	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L.)	Replicate	Initial Number Exposed ²	Day 7			Day 8			Day 9		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
5.6	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
12	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
24	A	1	0	1	—	0	1	—	0	1	—
	B	1	0	1	—	0	1	—	0	1	—
	C	1	0	1	—	0	1	—	0	1	—
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	1	—	0	1	—	0	1	—
	F	1	0	1	—	0	1	—	0	1	—
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	1	0	—
	I	1	0	1	—	0	1	—	0	1	—
	J	1	0	1	—	0	1	—	0	1	—

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 7			Day 8			Day 9		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
48	A	1	0	1	-	0	1	-	0	1	-
	B	1	0	1	-	0	1	-	0	1	-
	C	1	0	1	-	0	1	-	0	1	-
	D	1	0	1	-	0	1	-	0	1	-
	E	1	0	1	-	0	1	-	0	1	-
	F	1	0	1	-	0	1	-	0	1	-
	G	1	0	1	-	0	1	-	0	1	-
	H	1	0	1	-	0	1	-	0	1	-
	I	1	0	1	-	0	1	-	0	1	-
	J	1	0	1	-	0	1	-	0	1	-

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

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APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 10			Day 11			Day 12		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
1.5	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
2.9	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor: 3M Corporation											
Test Substance: PFOS											
Test Organism: Cladoceran, <i>Daphnia magna</i>											
Dilution Water: Well Water											
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 10			Day 11			Day 12		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
5.6	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
12	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 C
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
24	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	1	--	0	1	--	0	1	--
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	1	--	0	1	--	0	1	--
	G	1	0	0	1 AN	0	0	1 AN	0	1	--
	H	1	0	1	--	0	1	--	0	1	--
	I	1	0	1	--	0	1	--	0	1	--
	J	1	0	1	--	0	1	--	0	1	--

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

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APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor: 3M Corporation											
Test Substance: PFOS											
Test Organism: Cladoceran, <i>Daphnia magna</i>											
Dilution Water: Well Water											
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 10			Day 11			Day 12		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
48	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	1	--	0	1	--	0	1	--
	D	1	0	1	--	0	1	--	0	1	--
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	1	--	0	1	--	0	1	--
	G	1	0	1	--	0	1	--	0	1	--
	H	1	0	1	--	0	1	--	0	1	--
	I	1	0	1	--	0	1	--	0	1	--
	J	1	0	1	--	0	1	--	0	1	--
¹ Observed Effects: AN = Appeared Normal; C = Lethargic.											
² All organisms on Day 0 appeared normal.											

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APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor: 3M Corporation											
Test Substance: PFOS											
Test Organism: Cladoceran, <i>Daphnia magna</i>											
Dilution Water: Well Water											
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 13			Day 14			Day 15		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
1.5	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
2.9	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation										
Test Substance:		PFOS										
Test Organism:		Cladoceran, <i>Daphnia magna</i>										
Dilution Water:		Well Water										
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 13			Day 14			Day 15			
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	
5.6	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
12	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	H	1	0	1	—	0	1	—	0	1	—	
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
24	A	1	0	1	—	0	1	—	0	1	—	
	B	1	0	1	—	0	1	—	0	1	—	
	C	1	0	1	—	0	1	—	0	1	—	
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN	
	E	1	0	1	—	0	1	—	0	1	—	
	F	1	0	1	—	0	1	—	0	1	—	
	G	1	0	1	—	0	1	—	0	1	—	
	H	1	0	1	—	0	1	—	0	1	—	
	I	1	0	1	—	0	1	—	0	1	—	
	J	1	0	1	—	0	1	—	0	1	—	

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 13			Day 14			Day 15		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
48	A	1	0	1	--	0	1	--	0	1	--
	B	1	0	1	--	0	1	--	0	1	--
	C	1	0	1	--	0	1	--	0	1	--
	D	1	0	1	--	0	1	--	0	1	--
	E	1	0	1	--	0	1	--	0	1	--
	F	1	0	1	--	0	1	--	0	1	--
	G	1	0	1	--	0	1	--	0	1	--
	H	1	0	1	--	0	1	--	0	1	--
	I	1	0	1	--	0	1	--	0	1	--
	J	1	0	1	--	0	1	--	0	1	--

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

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APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor: 3M Corporation											
Test Substance: PFOS											
Test Organism: Cladoceran, <i>Daphnia magna</i>											
Dilution Water: Well Water											
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 16			Day 17			Day 18		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
1.5	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
2.9	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 16			Day 17			Day 18		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
5.6	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	1	-
12	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	1	-	0	1	-	0	1	-
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
24	A	1	0	1	-	0	1	-	0	1	-
	B	1	0	1	-	0	1	-	0	1	-
	C	1	0	1	-	0	1	-	0	1	-
	D	1	0	0	1 AN	0	1	-	0	1	-
	E	1	0	1	-	0	1	-	0	1	-
	F	1	0	1	-	0	1	-	0	1	-
	G	1	0	1	-	0	1	-	0	1	-
	H	1	0	1	-	0	1	-	0	1	-
	I	1	0	1	-	0	1	-	0	1	-
	J	1	0	1	-	0	1	-	0	1	-

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

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APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 16			Day 17			Day 18		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
48	A	1	0	1	-	0	1	-	0	1	-
	B	1	0	1	-	0	1	-	0	1	-
	C	1	0	1	-	0	1	-	0	1	-
	D	1	0	1	-	0	1	-	0	1	-
	E	1	0	1	-	0	1	-	0	1	-
	F	1	0	1	-	0	1	-	0	1	-
	G	1	0	1	-	0	1	-	0	1	-
	H	1	0	1	-	0	1	-	0	1	-
	I	1	0	1	-	0	1	-	0	1	-
	J	1	0	1	-	0	1	-	0	1	-

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor: 3M Corporation											
Test Substance: PFOS											
Test Organism: Cladoceran, <i>Daphnia magna</i>											
Dilution Water: Well Water											
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 19			Day 20			Day 21		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
1.5	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	1	-
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
2.9	A	1	0	0	1 AN	0	0	1 AN	0	1	-
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor: 3M Corporation											
Test Substance: PFOS											
Test Organism: Cladoceran, <i>Daphnia magna</i>											
Dilution Water: Well Water											
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 19			Day 20			Day 21		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
5.6	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	1	-	0	1	-	0	1	-
12	A	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	B	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	C	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	D	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	E	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	F	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	G	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	H	1	0	1	-	0	1	-	0	1	-
	I	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
	J	1	0	0	1 AN	0	0	1 AN	0	0	1 AN
24	A	1	0	1	-	0	1	-	0	1	-
	B	1	0	1	-	0	1	-	0	1	-
	C	1	0	1	-	0	1	-	0	1	-
	D	1	0	1	-	0	1	-	0	1	-
	E	1	0	1	-	0	1	-	0	1	-
	F	1	0	1	-	0	1	-	0	1	-
	G	1	0	1	-	0	1	-	0	1	-
	H	1	0	1	-	0	1	-	0	1	-
	I	1	0	1	-	0	1	-	0	1	-
	J	1	0	1	-	0	1	-	0	1	-

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

APPENDIX IV (Continued)

Cumulative Percent Mortality and Treatment-Related Effects¹ - First-Generation Daphnids

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Initial Number Exposed ²	Day 19			Day 20			Day 21		
			Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
48	A	1	0	1	—	0	1	—	0	1	—
	B	1	0	1	—	0	1	—	0	1	—
	C	1	0	1	—	0	1	—	0	1	—
	D	1	0	1	—	0	1	—	0	1	—
	E	1	0	1	—	0	1	—	0	1	—
	F	1	0	1	—	0	1	—	0	1	—
	G	1	0	1	—	0	1	—	0	1	—
	H	1	0	1	—	0	1	—	0	1	—
	I	1	0	1	—	0	1	—	0	1	—
	J	1	0	1	—	0	1	—	0	1	—

¹ Observed Effects: AN = Appeared Normal; C = Lethargic.² All organisms on Day 0 appeared normal.

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APPENDIX V

Neonate Production

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Day									Total
		2	5	7	9	11	14	16	18	21	
Negative Control	A	0	0	0	0	27	40	0	25	26	118
	B	0	0	0	9	41	0	45	4	14	113
	C	0	0	0	0	14	43	0	27	10	94
	D	0	0	0	3	29	48	0	38	25	143
	E	0	0	0	8	40	41	0	35	27	151
	F	0	0	0	0	24	37	0	34	31	126
	G	0	0	0	14	0	38	39	18	20	129
	H	0	0	0	0	21	29	0	21	29	100
	I	0	0	0	9	32	45	0	31	23	140
	J	0	0	0	0	20	33	34	0	18	105
$\bar{x} (\pm SD) = 122 (\pm 19.2)$											
1.5	A	0	0	0	0	21	43	0	36	31	131
	B	0	0	0	2	0	39	39	0	26	106
	C	0	0	0	6	37	47	0	38	35	163
	D	0	0	0	0	23	44	0	0	32	99 ¹
	E	0	0	0	0	20	33	35	0	31	119
	F	0	0	0	5	38	51	0	36	30	160
	G	0	0	0	0	25	48	31	0	22	126
	H	0	0	0	16	46	44	1	41	34	182
	I	0	0	0	10	37	27	12	40	33	159
	J	0	0	0	3	28	41	0	37	27	136
$\bar{x} (\pm SD) = 142 (\pm 24.7)$											
2.9	A	0	0	0	0	13	37	35	7	17	109 ¹
	B	0	0	0	7	38	41	4	41	36	167
	C	0	0	0	11	37	38	0	42	34	162
	D	0	0	0	0	25	45	33	0	20	123
	E	0	0	0	0	24	43	34	3	23	127
	F	0	0	0	19	0	47	36	0	14	116
	G	0	0	0	0	19	44	35	0	24	122
	H	0	0	0	0	17	40	43	0	35	135
	I	0	0	0	15	41	0	38	33	15	142
	J	0	0	0	0	26	46	29	0	28	129
$\bar{x} (\pm SD) = 136 (\pm 17.9)$											

¹ This value excluded from calculation of the mean due to adult death.

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APPENDIX V (Continued)
Neonate Production

Sponsor:		3M Corporation									
Test Substance:		PFOS									
Test Organism:		Cladoceran, <i>Daphnia magna</i>									
Dilution Water:		Well Water									
Mean Measured Test Concentration (mg a.i./L)	Replicate	Day									Total
		2	5	7	9	11	14	16	18	21	
5.6	A	0	0	0	0	21	38	31	0	35	125
	B	0	0	0	5	34	43	0	31	33	146
	C	0	0	0	0	22	37	19	0	26	104
	D	0	0	0	0	27	41	29	0	20	117
	E	0	0	0	0	18	43	38	0	29	128
	F	0	0	0	11	36	52	0	37	34	170
	G	0	0	0	0	17	38	4	34	32	125
	H	0	0	0	5	29	46	0	38	30	148
	I	0	0	0	0	14	39	40	0	34	127
	J	0	0	0	0	19	35	19	-	-	73 ¹
											$\bar{x} (\pm SD) = 132 (\pm 19.5)$
12	A	0	0	0	0	19	39	0	0	28	86
	B	0	0	0	0	16	40	34	17	20	127
	C	0	0	0	0	24	36	35	0	34	129
	D	0	0	0	18	0	38	34	19	0	109
	E	0	0	0	0	26	36	37	0	34	133
	F	0	0	0	10	37	0	40	37	15	139
	G	0	0	0	10	34	41	0	39	41	165
	H	0	0	0	0	20	-	-	-	-	20 ¹
	I	0	0	0	0	11	30	32	0	30	103
	J	0	0	0	0	13	27	29	0	14	83
											$\bar{x} (\pm SD) = 119 (\pm 26.5)$
24	A	0	-	-	-	-	-	-	-	-	0
	B	0	-	-	-	-	-	-	-	-	0
	C	0	-	-	-	-	-	-	-	-	0
	D	0	0	0	0	0	8	24	-	-	32 ¹
	E	0	-	-	-	-	-	-	-	-	0
	F	0	-	-	-	-	-	-	-	-	0
	G	0	0	0	0	9	-	-	-	-	9 ¹
	H	0	0	0	0	-	-	-	-	-	0
	I	0	-	-	-	-	-	-	-	-	0
	J	0	-	-	-	-	-	-	-	-	0
											$\bar{x} = 0$
48	A	-	-	-	-	-	-	-	-	-	0
	B	-	-	-	-	-	-	-	-	-	0
	C	-	-	-	-	-	-	-	-	-	0
	D	-	-	-	-	-	-	-	-	-	0
	E	-	-	-	-	-	-	-	-	-	0
	F	-	-	-	-	-	-	-	-	-	0
	G	-	-	-	-	-	-	-	-	-	0
	H	-	-	-	-	-	-	-	-	-	0
	I	-	-	-	-	-	-	-	-	-	0
	J	-	-	-	-	-	-	-	-	-	0
											$\bar{x} = 0$

¹ This value excluded from calculation of the mean due to adult death.

¹ This value excluded from calculation of the mean due to adult death.

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APPENDIX VI

Length and Dry Weight of Surviving First-Generation Daphnids

Sponsor:	3M Corporation			
Test Substance:	PFOS			
Test Organism:	Cladoceran, <i>Daphnia magna</i>			
Dilution Water:	Well Water			
Mean Measured Test Concentration (mg a.i./L)	Individual Length (mm)	Mean Length (mm $\pm$ SD)	Individual Dry Weights (mg)	Mean Dry Weights (mg $\pm$ SD)
Negative Control	4.70	4.65 ($\pm$ 0.111)	0.73	0.695($\pm$ 0.100)
	4.65		0.68	
	4.55		0.87	
	4.65		0.65	
	4.55		0.60	
	4.80		0.69	
	4.55		0.75	
	4.50		0.61	
	4.75		0.55	
	4.80		0.82	
1.5	4.45	4.66 ($\pm$ 0.118)	0.68	0.669 ($\pm$ 0.0623)
	4.60		0.70	
	4.70		0.63	
	--		--	
	4.80		0.81	
	4.55		0.61	
	4.75		0.67	
	4.80		0.63	
	4.60		0.68	
	4.65		0.61	
2.9	--	4.62 ($\pm$ 0.100)	--	0.724 ($\pm$ 0.110)
	4.75		0.63	
	4.50		0.68	
	4.65		0.70	
	4.70		0.94	
	4.55		0.72	
	4.45		0.80	
	4.70		0.82	
	4.60		0.60	
	4.65		0.63	

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APPENDIX VI (Continued)

Length and Dry Weight of Surviving First-Generation Daphnids

Sponsor:	3M Corporation			
Test Substance:	PFOS			
Test Organism:	Cladoceran, <i>Daphnia magna</i>			
Dilution Water:	Well Water			
Mean Measured Test Concentration (mg a.i./L)	Individual Length (mm)	Mean Length (mm $\pm$ SD)	Individual Dry Weights (mg)	Mean Dry Weights (mg $\pm$ SD)
5.6	4.45	4.61 ($\pm$ 0.124)	0.71	0.727 ($\pm$ 0.0665)
	4.70		0.69	
	4.50		0.83	
	4.70		0.75	
	4.65		0.82	
	4.80		0.68	
	4.45		0.62	
	4.55		0.73	
	4.65		0.71	
	--		--	
12	4.70	4.59 ($\pm$ 0.102)	0.76	0.723 ($\pm$ 0.0661)
	4.60		0.64	
	4.75		0.76	
	4.65		0.65	
	4.45		0.82	
	4.60		0.65	
	4.55		0.70	
	--		--	
	4.45		0.74	
	4.55		0.79	

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APPENDIX VII

Changes to Protocol

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

1. The proposed experimental start and termination dates were amended to the protocol.
2. During the second generation exposure, neonates were observed at 5, 24 and 48 hours.

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APPENDIX VIII

Personnel Involved in the Study

The following key Wildlife International Ltd. 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. Jon A. MacGregor, Scientist
4. Mark A. Mank, Laboratory Supervisor
5. Kurt R. Drottar, Senior Biologist

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APPENDIX IX

Report Amendment

1. Original Report: Pages 1-4, 6 and 31
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 9
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, and NOEC, LOEC and MATC 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.

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APPENDIX IX

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Report Amendment

AMENDMENT SIGNATURES:



Kurt R. Drott
Study Director

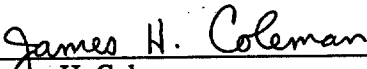
4/26/00
DATE



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

4/26/00
DATE

REVIEWED BY:



James H. Coleman
Quality Assurance

4-26-00
DATE

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AMENDED

PROTOCOL

PFOS: A SEMI-STATIC LIFE-CYCLE TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*)

U.S. Environmental Protection Agency
Series 850 – Ecological Effects Test Guidelines
OPPTS Number 850.1300

OECD Guideline 211

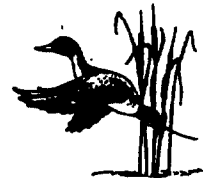
3M Lab Request No. U2723

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

June 10, 1999

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WILDLIFE INTERNATIONAL, LTD

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PFOS: A SEMI-STATIC LIFE-CYCLE TOXICITY TEST
WITH THE CLADOCERAN (*Daphnia magna*)

SPONSOR:

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

SPONSOR'S REPRESENTATIVE:

Ms. Susan A. Beach

TESTING FACILITY:

Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601

STUDY DIRECTOR:

Kurt Drottar
Senior Aquatic Biologist

LABORATORY MANAGEMENT:

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

FOR LABORATORY USE ONLY

Proposed Dates:	
Experimental Start Date: _____	Experimental Termination Date: _____
Project No.: _____	
Test Concentrations: <u>Negative Control, 1.6, 3.1, 6.3, 13, 25 and 50 mg a.i./L</u>	
Test Substance No.: <u>4675</u> Reference Substance No. (if applicable): <u>4526</u>	

PROTOCOL APPROVAL

STUDY DIRECTOR

DATE

LABORATORY MANAGEMENT

DATE

Susan A. Beach
SPONSOR'S REPRESENTATIVE

6/30/99
DATE

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INTRODUCTION

Wildlife International, Ltd. will conduct a semi-static life-cycle toxicity test with the cladoceran, *Daphnia magna*, for the Sponsor at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures outlined in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.1300 (1); OECD Guideline 211: *Daphnia magna* Reproduction Test (2); *Standard Evaluation Procedure, Daphnia Magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test* (3); and ASTM E 1193-87 *Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna* (4). 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 effects of the test substance on the survival, growth, and reproduction of the cladoceran, *Daphnia magna*, under semi-static test conditions, for a period of 21 days.

EXPERIMENTAL DESIGN

Daphnids will be exposed to a geometric series of six test concentrations, and a negative (dilution water) control for 21 days. Individual neonate daphnids will be placed in ten replicate test chambers impartially assigned to each test concentration and control. Individually exposed daphnids will be observed for survival, reproduction and growth.

Nominal test concentrations will be selected in consultation with the Sponsor, and will be based upon information such as the results of exploratory range-finding toxicity data, known toxicity data, physical/chemical properties of the test substance or other relevant information. Each test substance concentration will be 50% of the next higher one. Water samples will be collected at specified intervals for analysis of the test substance. Results of the analyses will be used to calculate mean measured test concentrations. New test solutions will be prepared three times per week (e.g., Monday, Wednesday, Friday), and the test organisms will be transferred from the old to the new solutions.

To control bias, neonate daphnids will be impartially assigned to exposure chambers at test initiation. No other potential sources of bias are expected to affect the results of the study. Observations of the survival of the first-generation daphnids will be made daily. The presence of eggs in the brood patch, males

or winter eggs also will be recorded daily. With the onset of reproduction, the number of young present in each test chamber will be counted every Monday, Wednesday and Friday during the test and at test termination. An acute exposure will also be performed for any treatment groups which produce young. Twenty neonates from each treatment group and the control will be exposed under static test conditions for 48 hours. The results of the test will be used to calculate the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The NOEC is defined as the highest concentration that produces no statistically-significant effect on survival, reproduction, or growth. The LOEC is defined as the lowest concentration that produces a statistically significant effect on survival, reproduction, or growth. The maximum acceptable toxicant concentration (MATC) will be calculated as the geometric mean of the NOEC and LOEC.

MATERIALS AND METHODS

Test Substance

Information on the characterization of test, control or reference substances is required by OECD Principles of Good Laboratory Practice. The Sponsor is responsible for providing Wildlife International, Ltd. written verification that the test substance has been characterized according to GLPs. 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 Concentrations

The test substance will be mixed directly into dilution water. The solutions will be mixed until the test material is dissolved. Once dissolved, the solution will be distributed into test chambers or diluted with dilution water to achieve the desired test concentrations.

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Test Organism

The cladoceran, *Daphnia magna*, has been selected as the test species for this study. Daphnids are representative of an important group of aquatic invertebrates, and have been selected for use in the test based upon past use history and ease of culturing in the laboratory. Daphnid neonates to be used in the test will be less than 24 hours old and will be obtained from cultures maintained at Wildlife International, Ltd., Easton, Maryland. The identity of the species will be verified using appropriate taxonomic keys such as Pennak (5).

Daphnids will be cultured in water from the same source and at approximately the same temperature as will be used during the test. Daphnids in the cultures producing neonates for the test will be held for at least 10 days prior to collection of the neonates for testing. Neonates from daphnids that show signs of disease or stress will not be used as test organisms. Daphnids in holding that produce ephippia also will not be used to supply neonates for testing.

Daphnids in the cultures will be fed once daily. The diet will be a mixture of yeast, Cerophyll®, and trout chow (YCT), supplemented with a suspension of the freshwater green alga *Selenastrum capricornutum*. Specifications for acceptable levels of contaminants in daphnid diets have not been established. However, there are no known levels of contaminants reasonably expected to be present in the diet that are considered to interfere with the purpose or conduct of the test.

Neonates will be obtained for testing from at least three individual adults. Prior to test initiation, the neonates will be collected from cultures and transferred to glass beakers. The daphnids will be released into the test compartments below the water surface using a wide-bore pipette. Daphnids will be fed daily during the test using the same diet as used in the cultures. Daphnids will be fed 0.3 mL of YCT and 0.6 mL of *Selenastrum* once daily. The frequency of feeding will be documented in the raw data and the final report.

Dilution Water

Water used for the culturing and testing of daphnids will be obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The water will be passed through a sand filter and pumped into a 37,800-L storage tank where the water will be aerated with spray nozzles. Prior to use the water will be filtered to 0.45 µm and passed through an ultraviolet sterilizer in order to remove

microorganisms and fine particles. Water used for culturing and testing is characterized as moderately hard. Typical values for hardness, alkalinity, pH and specific conductance are approximately:

Hardness, mg/L as CaCO ₃	145
Alkalinity, mg/L as CaCO ₃	190
pH	8.1
Specific Conductance, μ mhos/cm	330

Hardness, alkalinity, pH and specific conductance will be measured weekly to monitor the consistency of the well water. Means and ranges of the measured parameters for the four-week period preceding the test will be provided in the final report. 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 most recent GLP compliant analyses will be summarized in the final report.

Test Apparatus

Test chambers will consist of 250-mL Nalgene® beakers containing 200 mL of test solution. Test chambers will be randomly positioned by treatment group in a temperature-controlled water bath or an environmental chamber. Test chambers will be labeled with the project number, test concentration and replicate.

Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing will be provided by fluorescent tubes that emit wavelengths similar to natural sunlight (e.g., Colortone® 50). A photoperiod of 16 hours of light and 8 hours of dark will be controlled with an automatic timer. A 30-minute transition of low light intensity will be provided when lights go on and off to avoid sudden changes in light intensity. Light intensity will be measured just prior to test initiation and at approximately weekly intervals thereafter with a SPER Scientific Ltd. light meter or equivalent.

The target test temperature will be $20 \pm 1^\circ \text{C}$. Temperature will be measured in one alternating replicate of each treatment and the control group at test initiation, in the old and new solutions on renewal days, and at test termination using a liquid-in-glass thermometer. Temperature also will be measured with a continuous recorder in one negative control replicate. Recorder measurements will be verified with a liquid-in-glass thermometer prior to test initiation and at least weekly thereafter.

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Dissolved oxygen will be measured in one alternating replicate of each treatment and the control group daily during the first week of the test, in the old and new solutions on renewal days, and at test termination using a Yellow Springs Instruments Model 51B dissolved oxygen meter, or equivalent. In the event that dissolved oxygen levels fall below 60% saturation, dissolved oxygen will be measured in every test chamber and appropriate actions will be taken after consultation with the Sponsor. Measurements of pH will be made in one alternating replicate of each treatment and the control group at test initiation, in the old and new solutions on renewal days, and at test termination using a Fisher Accumet Model 915 pH meter, or equivalent. If a treatment level reaches 100% mortality, dissolved oxygen, pH and temperature measurements will be taken at that time, then discontinued.

Hardness, alkalinity, and specific conductance will be measured in the negative (dilution water) control and the highest test concentration at test initiation and at approximately weekly intervals thereafter. Hardness and alkalinity measurements will be made by titration using procedures based on methods in *Standard Methods for the Examination of Water and Wastewater* (6). Specific conductance will be measured using a Yellow Springs Instruments Model 33 Salinity-Conductivity-Temperature meter, or equivalent.

Biological Observations

The first generation daphnids will be observed daily during the test for survival, the onset of reproduction, and clinical signs of toxicity. The criteria for death include absence of heartbeat, white opaque coloration, lack of movement of appendages and lack of response to gentle prodding. Immobilization is defined as a lack of movement except for minor spontaneous random movement of the appendages. Examples of clinical signs of toxicity include inability to maintain position in the water column, uncoordinated swimming and cessation of feeding. The presence of eggs in the brood pouch, aborted eggs, males, or ephippia also will be recorded. After the onset of reproduction, twenty neonates from each treatment group and the control will be exposed for 48-hours under static test conditions. The neonates will be observed at 24 and 48 hours for mortality/immobility and clinical signs of toxicity.

With the onset of reproduction, the number of second-generation daphnids will be counted and recorded approximately three times per week. Second generation neonates will be observed for clinical signs of toxicity. Second-generation daphnids not used for the acute exposure will be discarded. The

length and dry weight of the surviving first-generation daphnids will be measured at the end of the test. The test will not be considered acceptable if control survival is <80% or if the number of young produced by each surviving daphnid living the full 21 days is less than an average of 60.

Sampling for Analytical Measurements

Water samples will be collected from the new and old solutions during each week of the test to determine concentrations of the test substance. Duplicate samples for the new solutions will be collected from the freshly prepared solutions, whereas samples from the old solutions will be obtained from a composite of all replicate test chambers for each control and treatment level. In the event that 100% mortality occurs in any treatment, then sampling of that treatment will terminate following the next sampling interval. Samples will be collected at mid-depth and analyzed immediately. The sample scheme is summarized below:

PROPOSED NUMBERS OF SAMPLES						
Experimental Group	Week 1		Week 2		Week 3	
	New ^a	Old ^b	New ^a	Old ^b	New ^a	Old ^b
Control	2	2	2	2	2	2
Level 1-Low Concentration	2	2	2	2	2	2
Level 2	2	2	2	2	2	2
Level 3	2	2	2	2	2	2
Level 4	2	2	2	2	2	2
Level 5	2	2	2	2	2	2
Level 6-High Concentration	2	2	2	2	2	2
TOTALS	14	14	14	14	14	14

^a New solutions (duplicate samples collected from intermediate chambers)
^b Old solutions (duplicate samples from a composite of all replicate test chambers from each control and test level)

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.

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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.

Data Analyses

Statistical analyses will be performed on the survival of first-generation daphnids, the number of live young produced by each individually-exposed daphnid, and the length and dry weight of the individually-exposed first-generation daphnids.

To evaluate whether or not survival is affected by the treatments, 2 X 2 contingency tables and the chi-square test will be used on survival data (i.e., numbers of alive and dead daphnids). Analyses of reproduction and growth (weight and length) data will be evaluated for normality and homogeneity of variances. If the data are deemed normal with homogeneous variances, hypothesis testing using analysis of variance (ANOVA) and multiple means tests (e.g., Dunnett's, Bonferroni, Scheffe) will be used. If the data fail the tests for normality or homogeneity, then data transformations will be tried in an attempt to correct the condition. When the data transformations fail to correct for non-normality or heterogeneity of variances, nonparametric procedures will be used to identify statistically significant differences among the experimental groups.

The statistical analyses of survival, growth and reproduction data will aid but may not be exclusively used in the determination of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC). The maximum acceptable toxicant concentration (MATC) will be calculated as the geometric mean of the NOEC and LOEC.

RECORDS TO BE MAINTAINED

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

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.

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3. Dates of initiation and termination of the study.
4. Length and dry weight measurements.
5. Reproduction parameters.
6. Test organism culture records.
7. Results of rangefinding tests, when applicable.
8. Stock solution calculation and preparation.
9. Daily observations.
10. Water chemistry calculations (e.g., hardness and alkalinity).
11. If applicable, the methods used to analyze test substance concentrations and the results of analytical measurements.
12. Statistical calculations.
13. Test conditions and physical/chemical measurements.
14. Calculation and preparation of test concentrations.
15. 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 the following, when applicable:

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed. It is the responsibility of the Sponsor to provide the final date that data are recorded for chemistry, pathology and/or supporting evaluations that may be generated at other laboratories.
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 changes in the original 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 or contracted to Wildlife International, Ltd.
7. A description of the methods used to conduct the test.

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8. A description of the test organisms, including the source, scientific name, age, life stage, feed types, light intensity and photoperiod.
9. A description of the preparation of the test solutions, 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.
10. A description of circumstances that may have affected the quality or integrity of the data.
11. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
12. 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.
13. Statistical methods used to evaluate the data.
14. The signed and dated reports of each of the individual scientists or other professionals involved in the study.
15. The location where raw data and final report are to be stored.
16. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and findings reported to the Study Director and Management.
17. 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 and dated 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. 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 filed with the raw data. All changes to the protocol will be indicated in the final report.

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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 (ENV/MC/CHEM (98) 17); 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.

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REFERENCES

- 1 U.S. Environmental Protection Agency. 1996. Series 850- Ecological Effects Test Guidelines (draft), OPPTS Number 850.1300: *Daphnid Chronic Toxicity Test*.
- 2 OECD Guideline for Testing of Chemicals. 1997. Guideline 211: *Daphnia magna* Reproduction Test.
- 3 U.S. Environmental Protection Agency. 1982. *Standard Evaluation Procedure, Daphnia magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test*. EPA 540/9-86-141. Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C.
- 4 American Society for Testing and Materials Standard E1193-96. 1997. *Standard Guide for Conducting Life-Cycle Toxicity Tests with Daphnia magna*. American Society for Testing and Materials. Philadelphia, PA.
- 5 Pennak, R.W. 1978. *Freshwater Invertebrates of the United States*. 2nd Ed. 365 p.
- 6 APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater*. 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

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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 x No

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International Ltd.

Ambient

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

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

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APPENDIX II

Analytical Method Provided by Sponsor

Samples will be analyzed based upon procedures provided by the Sponsor in the following analytical methods:

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

A copy of the above method will be maintained in the raw data. The actual methodology used to analyze the test samples will be documented in the raw data and summarized in the final report.

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WILDLIFE INTERNATIONAL LTD.

PROJECT NO.: 454A-109

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AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A SEMI-STATIC LIFE-CYCLE TOXICITY TEST WITH THE
CLADOCERAN (*Daphnia magna*)

PROTOCOL NO.: 454/061099/DAP-LC3/SUB454

AMENDMENT NO.: 1

SPONSOR: 3M Corporation

PROJECT NO.: 454A-109

EFFECTIVE DATE: July 12, 1999

AMENDMENT: Page 2

Add: Experimental Start Date: 7/12/99

Experimental Termination Date: 8/2/99

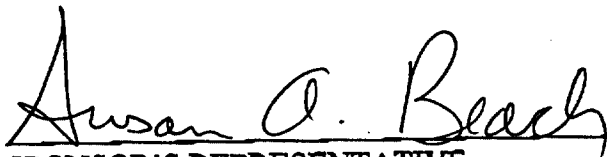
REASON: The above information was not known when the protocol was signed by the Study
Director.

STUDY DIRECTOR

DATE

LABORATORY MANAGEMENT

DATE



SPONSOR'S REPRESENTATIVE

7/12/99
DATE

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