

PFOS: A Flow-through Life-Cycle Toxicity Test with the
Saltwater Mysid (*Mysidopsis bahia*)

CHRONIC TOXICITY TO THE SALTWATER MYSID

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.1350

Type: Flow-through chronic

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Mysidopsis bahia*

Supplier: In-house cultures, Wildlife International, Ltd., Easton, MD

Analytical monitoring: PFOS measured on days 0, 7, 14, 21, 28, and 35

Exposure period: 35 days

Statistical methods: Survival data was evaluated (prior to pairing and after pairing) using 2 X 2 contingency tables to identify treatment groups that showed a statistically significant difference ($p < 0.05$) from the negative control group. All continuous-variable data (reproduction and growth) were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. Analysis of variance and Dunnett's test were used to evaluate differences between treatment and control means. All statistical tests were performed using a personal computer with SPSS/PC Version 2.0 or "TOXSTAT Release 3.5" statistical software.

Test mysids age: < 24-hours old at test initiation

Pretreatment: None

Test conditions: Natural seawater diluted to 20‰ with well water, 0.45µm filtered.

Dilution water chemistry (during the 4-week period immediately preceding the test):

Salinity: 20 (20-20) ‰

TOC: < 1.0 mg/L

Stock and test solution preparation: Primary stock prepared at 0.0895 mg/L and mixed for approximately 24 hours prior to use. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare five additional stock solutions at concentrations of 0.0447, 0.0224, 0.0112, 0.00559, and 0.00280 mg/L. The six stocks were injected into the diluter mixing chambers (at a rate of 4.60 mL/minute) where they were mixed with dilution water (at a rate of 150 mL/minute) to achieve the desired test concentrations.

Flow through rate: Approximately eleven volume additions of test water every 24- hours

Stability of the test chemical solutions: Extremely stable

Exposure vessels: Prior to pairing, mysids placed in glass beakers with nylon mesh screen attached to two holes on opposite sides. After reaching sexual maturity, pairs placed in glass petri dishes with sides of nylon mesh screen attached with silicone adhesive. Both pre-pairing and post-pairing exposure vessels were placed in 9L glass aquaria filled with approximately 5 L of test solution. The depth was approximately 6.2 cm prior to pairing and 5.5 cm after pairing.

The test chambers for the second generation exposure were 2L beakers with 1L of test solution which was dipped out of a test chamber from the appropriate treatment group.

Number of replicates: four

Number of concentrations: six plus a negative control

Number of fish per replicate: Fifteen juveniles before pairing, 5 pairs (10 adults) when possible after pairing.

Feeding: Fed live brine shrimp nauplii 3 or four times per day. Not fed the last day of the test.

Water chemistry during the study:

Dissolved oxygen range (0 – 35 days):

6.0 – 6.4 mg/L (control exposure)

5.9 – 6.3 mg/L (1.3 mg/L exposure)

pH range (0 – 35 days)

8.2 – 8.4 (control exposure)

8.3 – 8.4 (1.3 mg/L exposure)

Test temperature range (0 – 35 days)

24.5 – 25.2°C (control exposure)

24.4 – 25.1°C (1.3 mg/L exposure)

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Bk control, 0.086, 0.17, 0.34, 0.69, 1.4, 2.7 mg/L

Measured concentrations: <LOQ, 0.057, 0.12, 0.25, 0.55, 1.3, 2.6 mg/L

Element value: 20-day survival (pre-pairing) NOEC = 0.55 mg/L

35-day (post-pairing) survival NOEC = 0.55 mg/L

35-day reproduction NOEC = 0.25 mg/L

35-day growth NOEC = 0.25 mg/L

35-day reprod & growth LOEC = 0.55 mg/L

2nd generation acute survival NOEC = 0.55 mg/L (highest concentration tested)

All element values based on mean measured concentrations

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.0458 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 92.8. Samples collected at pre-test ranged from 57.4 to 99.3% of nominal. Samples at test initiation had measured values from 67.1 to 103% of nominal. Measured values for samples taken at test termination ranged from 59.8 to 90.0% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Duplicate Values at 0, 7, 14, 21, 28, 35, Days, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	-	-
0.086	0.0694, 0.0578, 0.0478, 0.0619, 0.0606, 0.0614, 0.0554, 0.0509, 0.0515, 0.0569, 0.0580, 0.0514	0.057	66
0.17	0.125, 0.114, 0.0778, 0.125, 0.124, 0.127, 0.0970, 0.112, 0.122, 0.128, 0.124, 0.119	0.12	71
0.34	0.289, 0.286, 0.231, 0.197, 0.276, 0.253, 0.227, 0.212, 0.262, 0.271, 0.278, 0.251	0.25	74
0.69	0.562, 0.659, 0.581, 0.450, 0.543, 0.542, 0.516, 0.528, 0.529, 0.544, 0.556, 0.583	0.55	80
1.4	1.23, 1.32, 1.13, 1.20, 1.35, 1.27, 1.23, 1.15, 1.39, 1.39, 1.26, 1.20	1.3	93
2.7	2.56, 2.79, 2.58, 2.30, 2.54, 2.69, all mysids dead after 14-days exposure	2.6	96

Biological observations

Survival: All surviving mysids appeared normal. Survival in the 1.3 and 2.6 mg/L treatments were statistically significantly different from the negative control group.

Reproduction: The day of first brood release in this study was Day 22. Dunnett's test showed that reproduction was significantly reduced in the 0.55 mg/L treatment group when compared to the negative control ($p \leq 0.05$). The 1.3 and 2.6 mg/L treatment groups were not included in the statistical analysis of the reproduction data due to a statistically significant difference in survival.

Growth: Mysids exposed to PFOS at concentrations ≤ 0.25 mg/L showed no statistically significant reductions in length or dry weight ($p \leq 0.05$).

Second Generation Acute Exposure: Survival in all PFOS treatment groups was $\geq 95\%$ and was not statistically different from the controls. All surviving mysids in the second generation exposure appeared normal with no overt signs of toxicity.

Percent Survival

Mean Measured Concentration, mg/L	Juvenile Pre-Pairing Survival, Day 20	Adult Post-Pairing Survival, Day 35
Negative Control	78	92
0.057	92	96
0.12	75	90
0.25	82	97
0.55	83	95
1.3	32	57
2.6	0	-

Second Generation Survival

Mean Measured Concentrations, mg/L	Total Number Exposed	Number Alive after 96-hours	Percent Survival
Negative Control	71	68	96
0.057	65	63	97
0.12	83	79	95
0.25	62	59	95
0.55	13	13	100

Adult Mysid Growth

Mean Measured Concentration, mg/L	Number of Surviving Mysids/Number Exposed	Total Length, Mean $\pm$ SD, mm	Dry Weight, Mean $\pm$ SD, mg
Negative Control	36/39	6.43 $\pm$ 0.0634	0.634 $\pm$ 0.0510
0.057	44/46	6.43 $\pm$ 0.0729	0.599 $\pm$ 0.0276
0.12	36/40	6.56 $\pm$ 0.105	0.641 $\pm$ 0.0241
0.25	36/37	6.40 $\pm$ 0.0548	0.622 $\pm$ 0.0227
0.55	35/37	6.14 $\pm$ 0.0794	0.562 $\pm$ 0.00624
1.3	8/14	5.85 $\pm$ 0.178	0.436 $\pm$ 0.0441

Reproduction

Mean Measured Concentration, mg/L	Replicate	Number of Reproductive Days	Number of Young	Mean Number of Young/Reproductive Day	Overall Mean $\pm$ SD
Negative Control	A	70	18	0.257	0.315 $\pm$ 0.0925
	B	53	14	0.264	
	C	70	20	0.286	
	D	42	19	0.452	
0.057	A	60	17	0.283	0.261 $\pm$ 0.0873
	B	70	14	0.200	
	C	70	13	0.186	
	D	56	21	0.375	
0.12	A	70	21	0.300	0.361 $\pm$ 0.101
	B	46	22	0.478	
	C	54	22	0.407	
	D	70	18	0.257	
0.25	A	70	19	0.271	0.252 $\pm$ 0.0723
	B	56	12	0.214	
	C	61	21	0.344	
	D	56	10	0.179	
0.55	A	54	3	0.0556	0.0559 $\pm$ 0.0376
	B	56	6	0.107	
	C	70	3	0.0429	
	D	56	1	0.0179	
1.3	A	22	0	-	-
	B	14	0	-	
	C	0	0	-	
	D	11	0	-	

CONCLUSIONS

There were no statistically significant effects on survival, reproduction or growth of mysid shrimp exposed to potassium perfluorooctanesulfonate at concentrations ≤ 0.25 mg/L for 35 days. Reproduction, length and dry weight were the most sensitive biological endpoints in this study. Second generation mysids exposed to PFOS during a static 96-hour exposure showed no adverse effects.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

OTHER

Last changed: 5/3/00

PFOS:
A FLOW -THROUGH LIFE-CYCLE TOXICITY TEST WITH
THE SALTWATER MYSID (*Mysidopsis bahia*)

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-107
3M LAB REQUEST NO. U2723

U.S. Environmental Protection Agency
Series 850 – Ecological Effects Test Guidelines
OPPTS Number 850.1350

AUTHORS:

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

STUDY INITIATION DATE: May 21, 1999

STUDY COMPLETION DATE: April 26, 2000

Submitted to

3M Corporation
Environmental Laboratory
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St. Paul, MN 55144

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-107

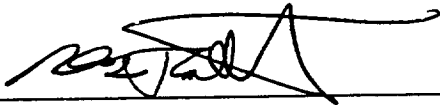
STUDY COMPLETION: April 26, 2000

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:
Protocol	May 24, 1999	May 24, 1999	July 16, 1999
Test Substance Preparation	June 10, 1999	June 14, 1999	June 16, 1999
Light Meter Reading and Analytical Sampling	June 16, 1999	June 17, 1999	June 18, 1999
Salinity Measurements	June 17, 1999	June 18, 1999	June 18, 1999
Calibration Standards Preparation	July 21, 1999	July 21, 1999	July 21, 1999
Biological Data and Draft Report	October 1, 4 and 5, 1999	October 6, 1999	October 7, 1999
Analytical Data and Draft Report	October 4 - 7, 1999	October 7, 1999	October 8, 1999
Final Report	April 20, 2000	April 20, 2000	April 24, 2000

Kimberly A. Hoxter
Kimberly A. Hoxter
Quality Assurance Representative

4-24-00
DATE

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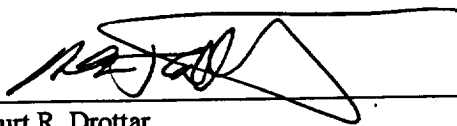
REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid
(*Mysidopsis bahia*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454A-107

STUDY DIRECTOR:


Kurt R. Drott
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-107
TEST SUBSTANCE:	PFOS (Perfluorooctane Sulfonic Acid Potassium Salt)
STUDY:	PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (<i>Mysidopsis bahia</i>)
NOMINAL TEST CONCENTRATIONS:	Negative Control, 0.086, 0.17, 0.34, 0.69, 1.4 and 2.7 mg a.i./L
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control, 0.057, 0.12, 0.25, 0.55, 1.3 and 2.6 mg a.i./L
TEST DATES:	Experimental Start (OECD) – May 26, 1999 Experimental Start (EPA) – June 16, 1999 Biological Termination – July 25, 1999 Experimental Termination – July 25, 1999
LENGTH OF FIRST- GENERATION EXPOSURE:	35 Days

TEST ORGANISM:	Saltwater Mysid (<i>Mysidopsis bahia</i>)
SOURCE OF TEST ORGANISMS:	Wildlife International, Ltd. Cultures Easton, Maryland 21601
AGE OF TEST ORGANISMS:	Juveniles <24 hours old

NOEC:	0.25 mg a.i./L
LOEC:	0.55 mg a.i./L
MATC:	0.37 mg a.i./L

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 June 16, 1999 to July 25, 1999. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454A-107 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to evaluate the effects of Perfluorooctane Sulfonic Acid Potassium Salt (PFOS) on the survival, growth and reproduction of the saltwater mysid (*Mysidopsis bahia*) under flow-through test conditions.

EXPERIMENTAL DESIGN

Mysidopsis bahia neonates, less than 24 hours old, were exposed to a geometric series of six test concentrations and a negative (saltwater) control for 35 days. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon known toxicity data. Nominal test concentrations were 0.086, 0.17, 0.34, 0.69, 1.4 and 2.7 mg active ingredient (a.i.)/L. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at weekly intervals during the test and at test termination.

Delivery of the test substance was initiated approximately 51 hours prior to the introduction of the neonate mysids to the test water in order to achieve equilibrium of the test substance in the test chambers. Four replicate test chambers, each containing one compartment with 15 mysids, were maintained for each treatment and control group. To begin the test, neonate mysids were impartially distributed in groups of one or two among glass beakers until each beaker contained 15 mysids. The mysids were then transferred to the test compartments. A total of 60 mysids were exposed in each treatment and the control group.

On Day 20 of the test, female and male adults were paired, and the reproduction of the paired mysids was monitored through Day 35. Observations of mortality, clinical signs of toxicity, and reproduction were made

daily. At test termination, the lengths and dry weights of all surviving first-generation mysids were measured. All young produced in the test were removed to a separate test chamber at the same test concentration on a daily basis. The second-generation mysids were exposed for 96 hours under static test conditions.

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 Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)". The protocol was based on procedures outlined in U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines, OPPTS Number 850.1350 (1); and *ASTM Standard E1191-90 Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids* (2).

Test Substance

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

Preparation of Test Concentrations

One stock solution was prepared for each of the six concentrations tested. A primary stock was prepared by dissolving the test substance in dilution water at a concentration of 0.0895 mg a.i./mL. The primary stock was stirred with an electric stainless steel top-down mixer for approximately 24 hours to aid in the solubilization of the test substance. After mixing, the primary stock appeared clear and colorless with a foam on the surface. Aliquots of the primary stock solution were proportionally diluted with dilution water to prepare five additional stocks at concentrations of 0.0447, 0.0224, 0.0112, 0.00559 and 0.00280 mg a.i./mL. The six stocks were injected into the diluter mixing chambers (at a rate of 4.60 mL/minute) where they were mixed with dilution water

(at a rate of 150 mL/minute) to achieve the desired test concentrations. All test solutions appeared clear and colorless.

Test Organism

The saltwater mysid, *Mysidopsis bahia* (recently renamed *Americamysis bahia*), was selected as the test species for this study. The saltwater mysid is representative of an important group of aquatic organisms and was selected for use in the test based upon past history of use and ease of culturing in the laboratory. Mysids used in the test were neonates less than 24 hours old and were obtained from cultures maintained by Wildlife International, Ltd., Easton, Maryland.

Adult mysids were held in water from the same source as used during the test. Adult mysids were fed live brine shrimp (*Artemia* sp.) two or three times daily during holding. Brine shrimp were periodically enriched with a fatty acid supplement (ALGAMAC-2000, Aquafauna Bio-Marine, Inc., Hawthorne, California). During the 14-day holding period preceding the test, water temperatures ranged from 25.0 to 25.4°C. The pH of the water ranged from 8.0 to 8.1, salinity ranged from 20 to 23‰ (parts per thousand), and dissolved oxygen ranged from 6.8 to 7.4 mg/L. Instrumentation used for water measurements is described in the *Environmental Conditions* section of this report.

At test initiation, the neonate mysids were carefully collected from the cultures and transferred one and two at a time to glass beakers. Mysids then were transferred from the beakers to the test compartments. All transfers were made using a wide-bore pipette below the surface of the water. The mysids were fed live brine shrimp nauplii three to four times a day during the test to prevent cannibalism (except on the last day of the test).

Test Apparatus

A continuous-flow diluter was used to deliver each concentration of the test substance and a negative (saltwater) control. A peristaltic pump (Cole-Parmer Instrument Company, Chicago, IL) was used to deliver the six test substance stock solutions into mixing chambers assigned to each treatment group. The stock solutions were diluted with dilution water in the mixing chambers in order to obtain the desired test concentrations. The flow of dilution water to the mixing chambers was controlled by rotameters. Rotameters were calibrated prior to test initiation and at weekly intervals thereafter during the test. The flow of test water from each mixing chamber was split and allowed to flow into replicate test chambers. The proportion of test water that was split into each

replicate was checked prior to the test and at weekly intervals thereafter during the test to ensure that flow rates varied by no more than $\pm 10\%$ of the mean for the four replicates.

The diluter was adjusted so that each test chamber received approximately 11 volume additions of test water every 24 hours. The delivery pump was calibrated before the test and at approximately weekly intervals during the test. The general operation of the diluter was checked visually at least two times per day during the test and once at the end of the test.

Prior to sexual maturity, mysids were held in one compartment placed in each replicate test chamber (15/compartment). The compartments were glass beakers with nylon mesh screen attached to two holes on opposite sides. After mysids attained sexual maturity, reproductive pairs were placed in reproductive compartments (one pair per compartment). The reproductive compartments were glass petri dishes with sides of nylon mesh screen attached with silicone adhesive. The test compartments were placed in 9-L glass aquaria test chambers containing approximately 5 L of test solution. Prior to pairing, the depth of water in a representative test compartment was 6.2 cm. After pairing, the depth of water in a representative test compartment was 5.5 cm. The test chambers were impartially positioned in a temperature-controlled environmental chamber designed to maintain a temperature of $25 \pm 2^\circ\text{C}$. Test compartments were uniquely identified and the test chambers were labeled with the project number, test concentration and replicate. The test chambers for the second generation exposure were 2-L beakers with 1 L of test solution which was dipped out of a test chamber from the appropriate treatment group.

Dilution Water

The water used for culturing and testing was natural seawater collected at Indian River Inlet, Delaware, and was diluted to a salinity of approximately 20‰ with well water. Salinity measurements during the four-week period immediately preceding the test are presented in Appendix I.

The freshly-collected seawater 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 delivery to the diluter system, the water again was filtered (0.45 μm) to remove microorganisms and particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in saltwater 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 ranged from 623 to 815 lux over the surface of the negative control, replicate A test chamber. Light intensity was measured weekly using a SPER Scientific Model 840006C light meter.

Temperature was measured in each test chamber at the beginning and end of the test and at weekly intervals during the test using a liquid-in-glass thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ER/C Recorder. The target test temperature during the study was $25 \pm 2^\circ\text{C}$. Dissolved oxygen and pH measurements were measured in alternate replicates of each treatment and control group at the beginning and end of the test and at weekly intervals during the test. Salinity was measured daily in alternate replicates of the negative control and the highest treatment group with surviving mysids.

Dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter, and measurements of pH were made using a Fisher Accumet Model 915 pH meter. Salinity was measured using a Bio-Marine, Inc., Aquafauna refractometer.

Biological Observations and Measurements

Observations of the survival and behavior of each first-generation mysid were made daily throughout the test. The criteria for death included lack of movement, absence of respiratory movements, and lack of reaction to gentle prodding. At the time of pairing (Day 20), the sex and maturity of each mysid was determined by microscopic examination, and, when possible, 5 male/female pairs were made for each replicate test chamber. Any immature mysids or extra females were discarded at this time. Sexually mature males, which were left over after pairing were maintained in a separate compartment within that replicate.

After mysids were paired, the number of second-generation mysids were counted and recorded daily until test termination. Second-generation mysids were also observed for abnormal development and aberrant behavior. After each observation, second-generation mysids were collected and exposed at the same test concentration under static test conditions for 96 hours. If a male in a male/female pair died, it was replaced with a male, if

available, from the pool of males maintained in the same replicate. At test termination, the sex of each surviving first-generation mysid was confirmed and the length of each mysid was measured using calipers. Each surviving first-generation was then placed in a drying oven at approximately 60°C for approximately 24 hours. The dry weight of each surviving first-generation mysid was then determined using an analytical balance.

Statistical Analyses

Statistical analyses were performed on survival of the first and second-generation mysids, the number of young released per reproductive day, and the length and dry weight of each surviving first-generation mysid. Survival was evaluated prior to pairing (Day 0 through Day 20) and after pairing (Day 20 through Day 35). Survival data were analyzed using 2 x 2 contingency tables and the Chi-square test to identify treatment groups that were statistically different from the control group.

The analyses of reproduction (number of live young produced per reproductive day) and growth (dry weights and lengths) data included those treatments which did not exhibit a statistical reduction in survival. Analyses included the evaluation of homogeneity of variances using Bartlett's test and the assessment of normality using the Shapiro-Wilk's test. When data were deemed normal and homogeneous, an analysis of variance test was used to determine whether or not statistically significant differences existed among experimental groups. Those treatments statistically different from the control group were identified using Dunnett's test. The results of the statistical analyses were used to aid in the determination of the NOEC and LOEC. The MATC was calculated as the geometric mean of the NOEC and LOEC. All statistical tests were performed using a personal computer with SPSS/PC Version 2.0 (3) or "TOXSTAT Release 3.5" statistical software (4).

Analytical Chemistry

Prior to test initiation, two sets of pretest water samples were collected from two replicate test chambers of both the low and high level treatment groups to determine if nominal concentrations had been achieved. Water samples were also collected from two alternating replicates on Days 0, 7, 14, 21, 28 and 35. Water samples were collected from mid-depth of the test chamber and placed in plastic (Nalgene®) bottles. Samples 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

Measurement 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 this study were 0.086, 0.17, 0.34, 0.69, 1.4 and 2.7 mg a.i./L. When measured concentrations of samples collected on Days 0, 7, 14, 21, 28 and 35 were averaged, the mean measured concentrations were 0.057, 0.12, 0.25, 0.55, 1.3 and 2.6 mg a.i./L, which represented 66, 71, 74, 80, 93 and 96% of the nominal concentrations, respectively. Mean measured concentrations were used to express the NOEC, LOEC and MATC. Pretest samples collected to verify diluter performance (Appendix III) were not used in the calculation of mean measured concentrations.

Physical and Chemical Measurements of Water

Measurements of salinity in the negative control and the highest treatment group ranged from 19 to 21‰ throughout the test (Table 2). Measurements of pH ranged from 8.2 to 8.4 (Table 3) and temperature was maintained within the $25 \pm 2^\circ\text{C}$ range established for the test (Table 4). Dissolved oxygen concentrations remained ≥ 5.8 mg/L (79% of saturation) throughout the test (Table 5).

Survival

A summary of survival from test initiation to pairing on Day 20 is presented in Table 6. In general, all surviving mysids appeared normal. After 20 days of exposure, survival in the negative control group was 78%. Survival in the PFOS treatment groups ≤ 0.55 mg a.i./L ranged from 75 to 92% and were not statistically different from the negative control. Survival in the 1.3 and 2.6 mg a.i./L treatment groups was 32 and 0%, respectively, and was statistically different from the negative control ($p \leq 0.05$).

Observations of survival after pairing (from Day 20 to test termination on Day 35) are presented in Table 7. In general, all surviving mysids appeared normal. Survival in the negative control was 92%. Survival percentages in the PFOS treatment groups ≤ 0.55 mg a.i./L ranged from 90 to 97% and were not statistically different from the negative control. Survival in the 1.3 mg a.i./L treatment group was 57% and was statistically different from the negative control ($p \leq 0.05$).

Reproduction

A summary of the mean number of young produced per reproductive day is presented in Table 8. Young production for individual test compartments is presented in Appendix IV. For each female, the number of reproductive days was defined as the number of days that the female was alive from the day of first brood release of any female in the test to the end of the test. The day of first brood release in this study was Day 22. The mean number of young produced per reproductive day in the negative control groups was 0.315. Reproduction rates in the 0.057, 0.12, 0.25 and 0.55 mg a.i./L treatment groups were 0.261, 0.361, 0.252 and 0.0559 young per reproductive day, respectively. Dunnett's test showed that reproduction was significantly reduced in the 0.55 mg a.i./L treatment group when compared to the negative control ($p \leq 0.05$). The 1.3 and 2.6 mg a.i./L treatment groups were not included in the statistical analysis of the reproduction data due to a statistically significant difference in survival.

Growth

Summaries of the lengths and dry weights of the surviving adult mysids are presented in Tables 9 and 10, respectively. Individual measurements are provided in Appendices V and VI. The mean length and mean dry weight in the negative control group were 6.43 mm and 0.63 mg, respectively. Mysids exposed to PFOS at concentrations ≤ 0.25 mg a.i./L showed no statistically significant reductions in length or dry weight ($p > 0.05$). Mysids exposed to 0.55 mg a.i./L showed statistically significant reductions in both length and dry weight ($p \leq 0.05$). The 1.3 and 2.6 mg a.i./L treatment groups were not included in the statistical analyses of growth due to a statistically significant difference in survival.

Second Generation Acute Exposure

The results of the second generation exposure are presented in Table 11. After 96-hours, control survival was 96%. Survival in all PFOS treatment groups was $\geq 95\%$ and was not statistically different from the controls. All surviving mysids in the second generation exposure appeared normal with no overt signs of toxicity.

CONCLUSIONS

There were no statistically significant effects on survival, reproduction or growth of mysid shrimp (*Mysidopsis bahia*) exposed to PFOS at concentrations of ≤ 0.25 mg a.i./L for 35 days. Reproduction, length and

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dry weight were the most sensitive biological endpoints in this study. Mysid shrimp exposed to 1.3 and 2.6 mg a.i./L had significantly reduced survival in comparison to the negative control. Mysids exposed to 0.55 mg a.i./L had significantly reduced reproduction, length and dry weight in comparison to the negative control. Consequently, the LOEC, based on reproduction, length and dry weight, was 0.55 mg a.i./L. The NOEC was 0.25 mg a.i./L and the MATC was calculated to be 0.37 mg a.i./L. Second generation mysids exposed to PFOS during a static 96-hour exposure showed no adverse effects.

REFERENCES

- 1 U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.1350: *Mysid Chronic Toxicity Test*.
- 2 ASTM Standard E1191-90. 1991. *Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids*, American Society for Testing and Materials.
- 3 SPSS Inc. 1988. SPSS/PC+ Version 2.0. Chicago, Illinois.
- 4 West, Inc. and D. D. Gulley. 1996. TOXSTAT Version 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

Summary of Analytical Chemistry Data

Sponsor: 3M Corporation Test Substance: PFOS Test Organism: Saltwater Mysid, <i>Mysidopsis bahia</i> Dilution Water: Filtered Saltwater								
Nominal Test Concentration (mg a.i./L)	Measured Concentration (mg a.i./L)						Mean (mg a.i./L)	Percent of Nominal
	Day 0 ¹	Day 7 ²	Day 14 ¹	Day 21 ²	Day 28 ¹	Day 35 ²		
Negative Control	<LOQ ³ <LOQ	<LOQ <LOQ	<LOQ <LOQ	<LOQ <LOQ	<LOQ <LOQ	<LOQ <LOQ	-	-
0.086	0.0694 0.0578	0.0478 0.0619	0.0606 0.0614	0.0554 0.0509	0.0515 0.0569	0.0580 0.0514	0.057	66
0.17	0.125 0.114	0.0778 0.125	0.124 0.127	0.0970 0.112	0.122 0.128	0.124 0.119	0.12	71
0.34	0.289 0.286	0.231 0.197	0.276 0.253	0.227 0.212	0.262 0.271	0.278 0.251	0.25	74
0.69	0.562 0.659	0.581 0.450	0.543 0.542	0.516 0.528	0.529 0.544	0.556 0.583	0.55	80
1.4	1.23 1.32	1.13 1.20	1.35 1.27	1.23 1.15	1.39 1.39	1.26 1.20	1.3	93
2.7	2.56 2.79	2.58 2.30	2.54 2.69	- ⁴ - ⁴	- -	- -	2.6	96

¹ Replicates A and C measured.
² Replicates B and D measured.
³ LOQ - Limit of quantitation was 0.0458 mg a.i./L.
⁴ Samples not collected due to 100% mortality.

Table 2
Salinity of Water in the Negative Control and Highest Treatment Group Test Chambers

Sponsor:		3M Corporation	
Test Substance:		PFOS	
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>	
Dilution Water:		Filtered Saltwater	
Day	Replicate	Negative Control Salinity (‰)	2.6 mg a.i./L / 1.3 mg a.i./L Salinity (‰)
0	A	20	20
1	B	21	21
2	C	20	20
3	D	20	19
4	A	20	20
5	B	20	20
6	C	20	20
7	D	20	20
8	A	20	20
9	B	20	20
10	C	20	20
11	D	20	20
12	A	20	20
13	B	20	20
14	C	21	20
15 ¹	D	20	20
16	A	20	20
17	B	20	20
18	C	20	20
19	D	20	20
20	A	20	20
21	B	20	20
22	C	20	20
23	D	20	20
24	A	20	20
25	B	20	20
26	C	20	20
27	D	20	20
28	A	20	20
29	B	20	20
30	C	20	20
31	D	20	20
32	A	20	20
33	B	20	20
34	C	20	20
35	D	20	20

¹ On Days 15-35, salinity was measured in the 1.3 mg a.i./L treatment group due to 100% mortality in the 2.6 mg a.i./L treatment group.

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Table 3
pH of Water in the Test Chambers

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>
Dilution Water:	Filtered Saltwater

Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Day					
		0	7	14	21	28	35
Negative Control	A	8.4	—	—	—	8.3	—
	B	—	8.2	—	—	—	8.3
	C	—	—	8.3	—	—	—
	D	—	—	—	8.3	—	—
0.057	A	8.4	—	—	—	8.3	—
	B	—	8.2	—	—	—	8.3
	C	—	—	8.3	—	—	—
	D	—	—	—	8.3	—	—
0.12	A	8.4	—	—	—	8.3	—
	B	—	8.2	—	—	—	8.3
	C	—	—	8.3	—	—	—
	D	—	—	—	8.3	—	—
0.25	A	8.4	—	—	—	8.3	—
	B	—	8.2	—	—	—	8.3
	C	—	—	8.3	—	—	—
	D	—	—	—	8.3	—	—
0.55	A	8.4	—	—	—	8.3	—
	B	—	8.2	—	—	—	8.3
	C	—	—	8.3	—	—	—
	D	—	—	—	8.3	—	—
1.3	A	8.4	—	—	—	8.3	—
	B	—	8.3	—	—	—	8.3
	C	—	—	8.3	—	—	—
	D	—	—	—	8.3	—	—
2.6	A	8.4	—	—	—	—	—
	B	—	8.3	—	—	—	—
	C	—	—	8.3	—	—	—
	D	—	—	—	— ¹	—	—

¹Measurements discontinued due to 100% mortality.

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Table 4

Temperature (°C) of Water in the Test Chambers

Sponsor: 3M Corporation							
Test Substance: PFOS							
Test Organism: Saltwater Mysid, <i>Mysidopsis bahia</i>							
Dilution Water: Filtered Saltwater							
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Day					
		0	7	14	21	28	35
Negative Control	A ¹	25.0	25.2	24.8	24.9	25.2	25.0
	B	25.0	25.1	24.8	24.9	25.2	24.9
	C	25.0	25.0	24.6	24.9	25.2	25.0
	D	25.0	25.1	24.5	24.9	25.1	25.0
0.057	A	25.0	25.2	24.8	25.0	25.1	24.9
	B	25.0	25.3	25.0	25.0	25.1	25.0
	C	25.0	25.2	24.9	25.0	25.1	25.1
	D	25.0	25.2	25.0	25.0	25.2	25.0
0.12	A	25.0	25.1	24.8	25.1	25.1	24.9
	B	24.9	25.1	24.9	25.1	25.0	24.9
	C	25.0	25.2	24.9	25.1	25.0	24.9
	D	25.0	25.2	24.9	25.0	25.1	24.9
0.25	A	24.8	25.1	24.7	25.1	25.0	25.0
	B	24.8	25.0	24.9	25.1	25.1	25.0
	C	24.9	25.0	24.8	25.1	25.0	25.0
	D	24.9	25.0	24.9	25.1	25.1	24.9
0.55	A	24.9	25.0	24.9	25.0	25.1	25.0
	B	24.7	25.0	24.8	25.0	25.0	24.9
	C	24.8	25.0	24.8	25.0	25.0	24.9
	D	24.7	25.0	24.8	25.0	25.0	24.9
1.3	A	24.6	24.8	24.4	24.5	24.8	24.8
	B	24.7	24.9	24.7	24.5	24.8	24.8
	C	24.9	25.0	24.5	24.7	24.8	24.9
	D	25.1	25.1	25.1	25.0	25.0	24.9
2.6	A	24.9	25.1	25.0	— ²	—	—
	B	24.8	25.0	— ²	—	—	—
	C	24.8	25.0	25.1	— ²	—	—
	D	24.8	25.1	25.1	— ²	—	—

¹Temperature measured continuously during the test ranged from 24.5 to 25.5°C.²Measurements discontinued due to 100% mortality.

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Table 5

Dissolved Oxygen Content (mg/L) of Water in the Test Chambers¹

Sponsor:	3M Corporation						
Test Substance:	PFOS						
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>						
Dilution Water:	Filtered Saltwater						
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Day					
		0	7	14	21	28	35
Negative Control	A	6.1	—	—	—	6.0	—
	B	—	6.4	—	—	—	6.3
	C	—	—	6.2	—	—	—
	D	—	—	—	6.0	—	—
0.057	A	6.1	—	—	—	6.0	—
	B	—	6.3	—	—	—	6.2
	C	—	—	6.0	—	—	—
	D	—	—	—	6.0	—	—
0.12	A	6.1	—	—	—	6.0	—
	B	—	6.1	—	—	—	6.3
	C	—	—	6.3	—	—	—
	D	—	—	—	6.0	—	—
0.25	A	6.1	—	—	—	5.8	—
	B	—	6.1	—	—	—	6.3
	C	—	—	6.2	—	—	—
	D	—	—	—	5.8	—	—
0.55	A	6.1	—	—	—	6.0	—
	B	—	6.2	—	—	—	6.2
	C	—	—	6.2	—	—	—
	D	—	—	—	6.0	—	—
1.3	A	6.1	—	—	—	6.0	—
	B	—	6.2	—	—	—	6.3
	C	—	—	6.2	—	—	—
	D	—	—	—	5.9	—	—
2.6	A	6.1	—	—	—	—	—
	B	—	6.2	—	—	—	—
	C	—	—	6.3	—	—	—
	D	—	—	—	— ²	—	—

¹ A dissolved oxygen concentration of 4.4 mg/L represents 60% saturation at 25°C in saltwater with a salinity of 20‰.² Measurements discontinued due to 100% mortality.

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Table 6

Survival of Juvenile Mysids Exposed to PFOS
(Day 0 Through Pairing on Day 20)

Sponsor: 3M Corporation				
Test Substance: PFOS				
Test Organism: Saltwater Mysid, <i>Mysidopsis bahia</i>				
Dilution Water: Filtered Saltwater				
Mean Measured Test Concentration (mg a.i./L)	Replicate	Survival on Day 20 (Pairing)		
		No. Alive/No. Exposed	Total	Percent Survival
Negative Control	A	14/15	47/60	78
	B	10/15		
	C	14/15		
	D	9/15		
0.057	A	14/15	55/60	92
	B	13/15		
	C	15/15		
	D	13/15		
0.12	A	14/15	45/60	75
	B	10/15		
	C	9/15		
	D	12/15		
0.25	A	13/15	49/60	82
	B	10/15		
	C	14/15		
	D	12/15		
0.55	A	11/15	50/60	83
	B	13/15		
	C	13/15		
	D	13/15		
1.3	A	7/15	19/60	32*
	B	4/15		
	C	4/15		
	D	4/15		
2.6	A	0/15	0/60	0*
	B	0/15		
	C	0/15		
	D	0/15		
* Indicates a significant difference from the negative control using 2 X 2 contingency tables ($p \leq 0.05$).				

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Table 7

Survival of Adult Mysids Exposed to PFOS
(Day 20 Through Test Termination on Day 35)

Sponsor:	3M Corporation			
Test Substance:	PFOS			
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water:	Filtered Saltwater			
Mean Measured Test Concentration (mg a.i./L)	Replicate	Survival at Test Termination		
		No. Alive/No. Exposed	Total	Percent
Negative Control	A	11/12	36/39	92
	B	7/8		
	C	10/11		
	D	8/8		
0.057	A	12/13	44/46	96
	B	12/12		
	C	10/11		
	D	10/10		
0.12	A	12/12	36/40	90
	B	7/9		
	C	7/9		
	D	10/10		
0.25	A	10/10	36/37	97
	B	8/8		
	C	9/10		
	D	9/9		
0.55	A	7/8	35/37	95
	B	10/10		
	C	10/10		
	D	8/9		
1.3	A	3/7	8/14	57*
	B	4/4		
	C	0/0		
	D	1/3		
*Indicates a significant difference from the negative control using a 2 X 2 contingency table ($p \leq 0.05$).				

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Table 8

Mean Number of Young Produced Per Reproductive Day

Sponsor: 3M Corporation					
Test Substance: PFOS					
Test Organism: Saltwater Mysid, <i>Mysidopsis bahia</i>					
Dilution Water: Filtered Saltwater					
Mean Measured Test Concentration (mg a.i./L)	Replicate	Number of Reproductive Days	Number of Young Produced	Mean Number of Young/Reproductive Day	Overall Mean $\pm$ s
Negative Control	A	70	18	0.257	0.315 $\pm$ 0.0925
	B	53	14	0.264	
	C	70	20	0.286	
	D	42	19	0.452	
0.057	A	60	17	0.283	0.261 $\pm$ 0.0873
	B	70	14	0.200	
	C	70	13	0.186	
	D	56	21	0.375	
0.12	A	70	21	0.300	0.361 $\pm$ 0.101
	B	46	22	0.478	
	C	54	22	0.407	
	D	70	18	0.257	
0.25	A	70	19	0.271	0.252 $\pm$ 0.0723
	B	56	12	0.214	
	C	61	21	0.344	
	D	56	10	0.179	
0.55	A	54	3	0.0556	0.0559* $\pm$ 0.0376
	B	56	6	0.107	
	C	70	3	0.0429	
	D	56	1	0.0179	
1.3 ¹	A	22	0	0.000	0.000 $\pm$ 0.000
	B	14	0	0.000	
	C	0	0	0.000	
	D	11	0	0.000	

This treatment group was not included in the statistical analyses of reproduction due to a statistically significant difference in survival.

Indicates a significant difference from the negative control using Dunnett's test ($p \leq 0.05$).

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Table 9

Mean Total Length of Adult Mysids at the End of the 35-Day Test Period

Sponsor: 3M Corporation			
Test Substance: PFOS			
Test Organism: Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water: Filtered Saltwater			
Mean Measured Concentration (mg a.i./L)	Replicate	Replicate Mean (mm)	Overall Mean $\pm$ s (mm)
Negative Control	A	6.45	6.43 $\pm$ 0.0634
	B	6.34	
	C	6.46	
	D	6.48	
0.057	A	6.38	6.43 $\pm$ 0.0729
	B	6.45	
	C	6.52	
	D	6.36	
0.12	A	6.55	6.56 $\pm$ 0.105
	B	6.65	
	C	6.62	
	D	6.42	
0.25	A	6.48	6.40 $\pm$ 0.0548
	B	6.38	
	C	6.36	
	D	6.38	
0.55	A	6.05	6.14* $\pm$ 0.0794
	B	6.11	
	C	6.16	
	D	6.24	
1.3 ¹	A	5.93	5.85 $\pm$ 0.178
	B	5.98	
	C	—	
	D	5.65	

¹ This treatment group was not included in the statistical analyses of total length due to a statistically significant difference in survival.

* Indicates a significant difference from the negative control using Dunnett's test ($p \leq 0.05$).

Table 10

Mean Dry Weight of Adult Mysids at the End of the 35-Day Test Period

Sponsor: 3M Corporation			
Test Substance: PFOS			
Test Organism: Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water: Filtered Saltwater			
Mean Measured Concentration (mg a.i./L)	Replicate	Replicate Mean (mg)	Overall Mean $\pm$ s (mg)
Negative Control	A	0.599	0.634 $\pm$ 0.0510
	B	0.706	
	C	0.596	
	D	0.636	
0.057	A	0.616	0.599 $\pm$ 0.0276
	B	0.627	
	C	0.590	
	D	0.565	
0.12	A	0.647	0.641 $\pm$ 0.0241
	B	0.664	
	C	0.644	
	D	0.607	
0.25	A	0.644	0.622 $\pm$ 0.0227
	B	0.601	
	C	0.603	
	D	0.639	
0.55	A	0.556	0.562* $\pm$ 0.00624
	B	0.558	
	C	0.563	
	D	0.570	
1.3 ¹	A	0.440	0.436 $\pm$ 0.0441
	B	0.478	
	C	--	
	D	0.390	

¹ This treatment group was not included in the statistical analyses of dry weight due to a statistically significant difference in survival.

* Indicates a significant difference from the negative control using Dunnett's test ($p \leq 0.05$).

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Table 11

Survival of Second-Generation Mysids Exposed to PFOS
During 96-Hour Static Exposures

Sponsor:	3M Corporation		
Test Substance:	PFOS		
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>		
Dilution Water:	Filtered Saltwater		
Mean Measured Test Concentration (mg a.i./L)	Total No. Exposed	No. Alive After 96 Hours of Exposure (Observations ¹)	Percent Survival
Negative Control	71	68 (AN)	96
0.057	65	63 (AN)	97
0.12	83	79 (AN)	95
0.25	62	59 (AN)	95
0.55	13	13 (AN)	100
¹ AN = Appears Normal.			

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APPENDIX I

Dilution Water Salinity Measured During the
4-Week Period Immediately Preceding the Test

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>
Dilution Water:	Filtered Saltwater

	Mean	Range
Salinity (‰)	20 (N = 4)	20 - 20

APPENDIX II
Analyses of Pesticides, Organics, Metals and Other Inorganics
in Wildlife International, Ltd. Saltwater¹

ANALYSIS		MEASURED CONCENTRATION	
Miscellaneous Measurements			
Total Dissolved Solids		23,500	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		235	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		760	mg/L
Manganese ³	<	4.0	µg/L
Mercury	<	0.20	µg/L
Molybdenum ³	<	2.0	µg/L
Nickel ³	<	20.0	µg/L
Potassium ³		277	mg/L
Selenium ³	<	25.0	µg/L
Silver ³	<	1.0	µg/L
Sodium ³		6,010	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 ANALYSES OF PFOS IN SALTWATER
IN SUPPORT OF
WILDLIFE INTERNATIONAL PROJECT NO.: 454A-107

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REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFOS: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid
(*Mysidopsis bahia*)

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 454A-107

PRINCIPAL INVESTIGATOR:



Jon A. MacGregor, B.S.
Scientist

4/24/00

DATE

MANAGEMENT:



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

4/24/00

DATE

Introduction

Saltwater samples were collected from a flow-through life-cycle toxicity study designed to determine the effects of PFOS (Perfluorooctane Sulfonic Acid Potassium Salt) to the saltwater mysid (*Mysidopsis bahia*). This study was conducted by Wildlife International Ltd. and identified as Project No.: 454A-107. 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 June 15, 1999 and July 21, 1999 and were analyzed between June 15, 1999 and July 22, 1999.

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 saltwater 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 0.000915 to 0.00915 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 0.0458 mg a.i./L calculated as the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

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.

Saltwater was fortified at 0.0823, 0.366 and 3.66 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 92.8%. A representative ion chromatogram of a matrix fortification is presented in Figure 6.

Example Calculations

Sample number 454A-107-3, nominal concentration of 0.086 mg a.i./L in saltwater.

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: 12963

Internal Standard Peak Area: 145150

Peak Area Ratio: 0.08931

Calibration curve equation.

Slope: 5.76026

Intercept: 0.00940

Curve is weighted (1/x).

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

$$= \frac{0.08931 - 0.00940}{5.76026} \times 0.100$$

$$= 0.001387$$

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

$$= 0.001387 \times 50.0$$

$$= 0.06935$$

$$\begin{aligned}\text{Percent of Nominal Concentration} &= \frac{\text{PFOS (mg a.i./L) in sample}}{\text{PFOS (mg a.i./L) nominal}} \times 100 \\ &= \frac{0.06935}{0.086} \times 100 = 80.6\%\end{aligned}$$

RESULTS

Sample Analysis

Saltwater samples were collected from the flow-through life-cycle toxicity study with the saltwater mysid (*Mysidopsis bahia*) at pre-test, June 15, 1999, at test initiation, June 16, 1999 (Day 0), and weekly during the test through test termination on July 21, 1999 (Day 35). The measured concentrations of PFOS in the samples collected at pre-test ranged from 57.3 to 99.3% of the nominal concentrations (Table 4). The measured concentrations of PFOS in the samples collected at initiation of exposure of the test organisms (Hour 0) ranged from 67.1 to 103% of the nominal concentrations (Table 5). Samples collected at Day 7, Day 14, Day 21 and Day 28 had measured concentration ranges of 45.8 to 95.6%, 70.5 to 99.6%, 57.1 to 87.9% and 59.9 to 99.3% of nominal values, respectively. Samples collected at test termination (Day 35) had a measured concentration range of 59.8 to 90.0% 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 3.8 to 4.5 minutes
INTERNAL STANDARD RETENTION TIME:	Approximately 2.6 to 3.0 minutes
PFOS MONITORED MASS:	498.6 amu
INTERNAL STANDARD MONITORED MASS:	426.7 amu

Table 2

Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured Concentration of
Number (454A-107-)	Type	PFOS ¹ (mg a.i./L)
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 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

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Table 3

Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (454A-107-)	Concentrations of PFOS (mg a.i./L)		Percent Recovered
	Fortified	Measured	
MAS-1	0.0823	0.0748	90.9
MAS-4	0.0823	0.0828	101
MAS-7	0.0823	0.0673	81.8
MAS-10	0.0823	0.0662	80.4
MAS-13	0.0823	0.0693	84.2
MAS-16	0.0823	0.0787	95.6
MAS-2	0.366	0.350	95.6
MAS-5	0.366	0.359	98.1
MAS-8	0.366	0.314	85.8
MAS-11	0.366	0.303	82.8
MAS-14	0.366	0.360	98.4
MAS-17	0.366	0.287	78.4
MAS-3	3.66	3.29	89.9
MAS-6	3.66	3.54	96.7
MAS-9	3.66	3.39	92.6
MAS-12	3.66	3.88	106
MAS-15	3.66	3.80	104
MAS-18	3.66	3.98	109
Mean = 92.8			
Standard Deviation = 9.19			
CV = 9.90			
N = 18			

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Table 4

Measured Concentrations of PFOS in Pre-test Diluter Verification Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Hours)	PFOS Measured Concentration (mg a.i./L) ^{1,2}	Percent of Nominal
0.086	PT-1	-24	0.0581	67.6
	PT-2	-24	0.0493	57.3
	PT-5	-24	0.0627	72.9
	PT-6	-24	0.0606	70.5
2.7	PT-3	-24	2.21	81.9
	PT-4	-24	2.34	86.7
	PT-7	-24	2.56	94.8
	PT-8	-24	2.68	99.3

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

² Measured values of matrix blanks and matrix fortification samples analyzed concurrently with pre-test samples were <LOQ, 83.0, 95.6, 100, <LOQ, 93.4, 85.0 and 106 percent of nominal concentrations for pre-test 1 and pre-test 2, respectively.

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Table 5

Measured Concentrations of PFOS in Saltwater Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Day)	PFOS Measured Concentration (mg a.i./L) ¹	Percent of Nominal
0.0	1	0	< LOQ	--
	2	0	< LOQ	--
	15	7	< LOQ	--
	16	7	< LOQ	--
	29	14	< LOQ	--
	30	14	< LOQ	--
	43	21	< LOQ	--
	44	21	< LOQ	--
	55	28	< LOQ	--
	56	28	< LOQ	--
	67	35	< LOQ	--
	68	35	< LOQ	--
0.086	3	0	0.0694	80.7
	4	0	0.0578	67.2
	17	7	0.0478	55.6
	18	7	0.0619	72.0
	31	14	0.0606	70.5
	32	14	0.0614	71.4
	45	21	0.0554	64.4
	46	21	0.0509	59.2
	57	28	0.0515	59.9
	58	28	0.0569	66.2
	69	35	0.0580	67.4
	70	35	0.0514	59.8

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

Table 5 (continued)

Measured Concentrations of PFOS in Saltwater Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Day)	PFOS Measured Concentration (mg a.i./L) ¹	Percent of Nominal
0.17	5	0	0.125	73.5
	6	0	0.114	67.1
	19	7	0.0778	45.8
	20	7	0.125	73.5
	33	14	0.124	72.9
	34	14	0.127	74.7
	47	21	0.0970	57.1
	48	21	0.112	65.9
	59	28	0.122	71.8
	60	28	0.128	75.3
	71	35	0.124	72.9
	72	35	0.119	70.0
0.34	7	0	0.289	85.0
	8	0	0.286	84.1
	21	7	0.231	67.9
	22	7	0.197	57.9
	35	14	0.276	81.2
	36	14	0.253	74.4
	49	21	0.227	66.8
	50	21	0.212	62.4
	61	28	0.262	77.1
	62	28	0.271	79.7
	73	35	0.278	81.8
	74	35	0.251	73.8

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

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Table 5 (continued)

Measured Concentrations of PFOS in Saltwater Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Day)	PFOS Measured Concentration (mg a.i./L) ¹	Percent of Nominal
0.69	9	0	0.562	81.4
	10	0	0.659	95.5
	23	7	0.581	84.2
	24	7	0.450	65.2
	37	14	0.543	78.7
	38	14	0.542	78.6
	51	21	0.516	74.8
	52	21	0.528	76.5
	63	28	0.529	76.7
	64	28	0.544	78.8
	75	35	0.556	80.6
	76	35	0.583	84.5
1.4	11	0	1.23	87.9
	12	0	1.32	94.3
	25	7	1.13	80.7
	26	7	1.20	85.7
	39	14	1.35	96.4
	40	14	1.27	90.7
	53	21	1.23	87.9
	54	21	1.15	82.1
	65	28	1.39	99.3
	66	28	1.39	99.3
	77	35	1.26	90.0
	78	35	1.20	85.7

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

Table 5 (continued)

Measured Concentrations of PFOS in Saltwater Samples from a
Saltwater Mysid Life-Cycle Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-107-)	Sampling Time (Day)	PFOS Measured Concentration (mg a.i./L) ¹	Percent of Nominal
2.7	13	0	2.56	94.8
	14	0	2.79	103
	27	7	2.58	95.6
	28	7	2.30	85.2
	41	14	2.54	94.1
	42	14	2.69	99.6

¹ The limit of quantitation (LOQ) was 0.0458 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.000915 mg a.i./L) and the dilution factor of the matrix blank samples (50.0).

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

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

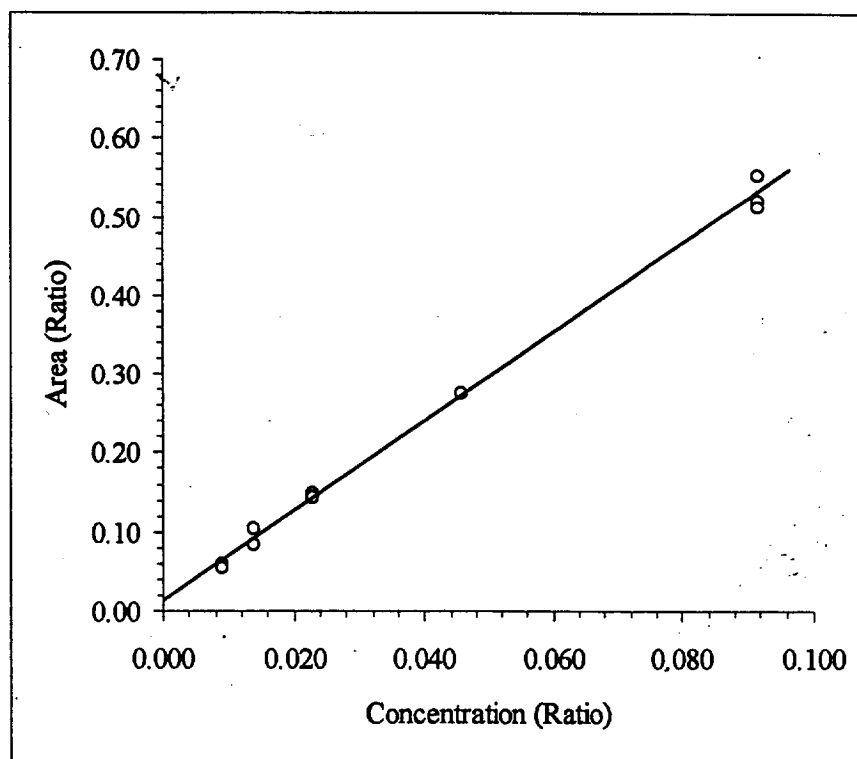


Figure 2. A typical calibration curve for PFOS. Slope = 5.76026; Intercept = 0.00940; $r = 0.9974$. Curve is weighted ($1/x$).

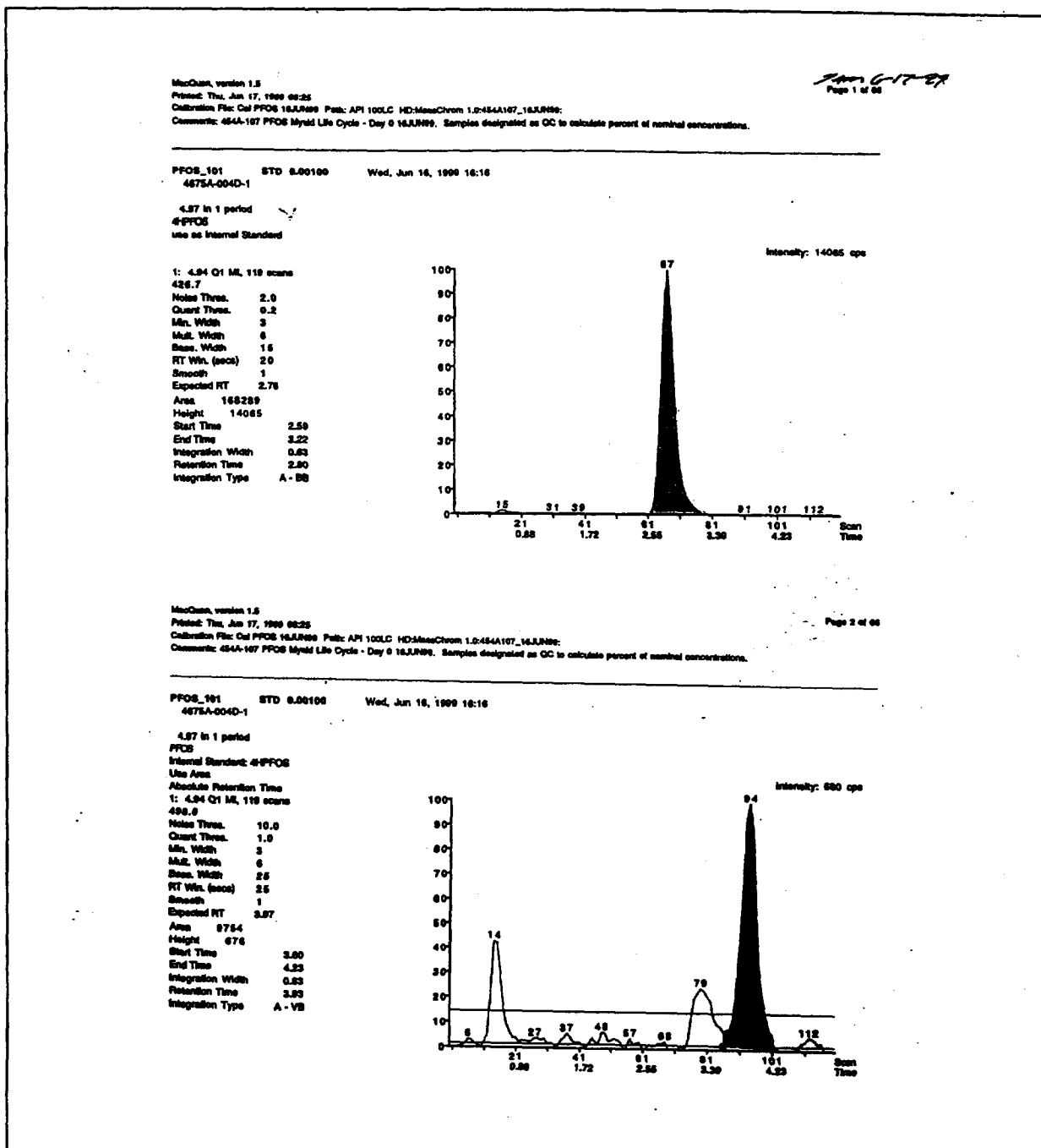


Figure 3. A representative ion chromatogram of a low-level (0.000915 mg a.i./L) PFOS standard.

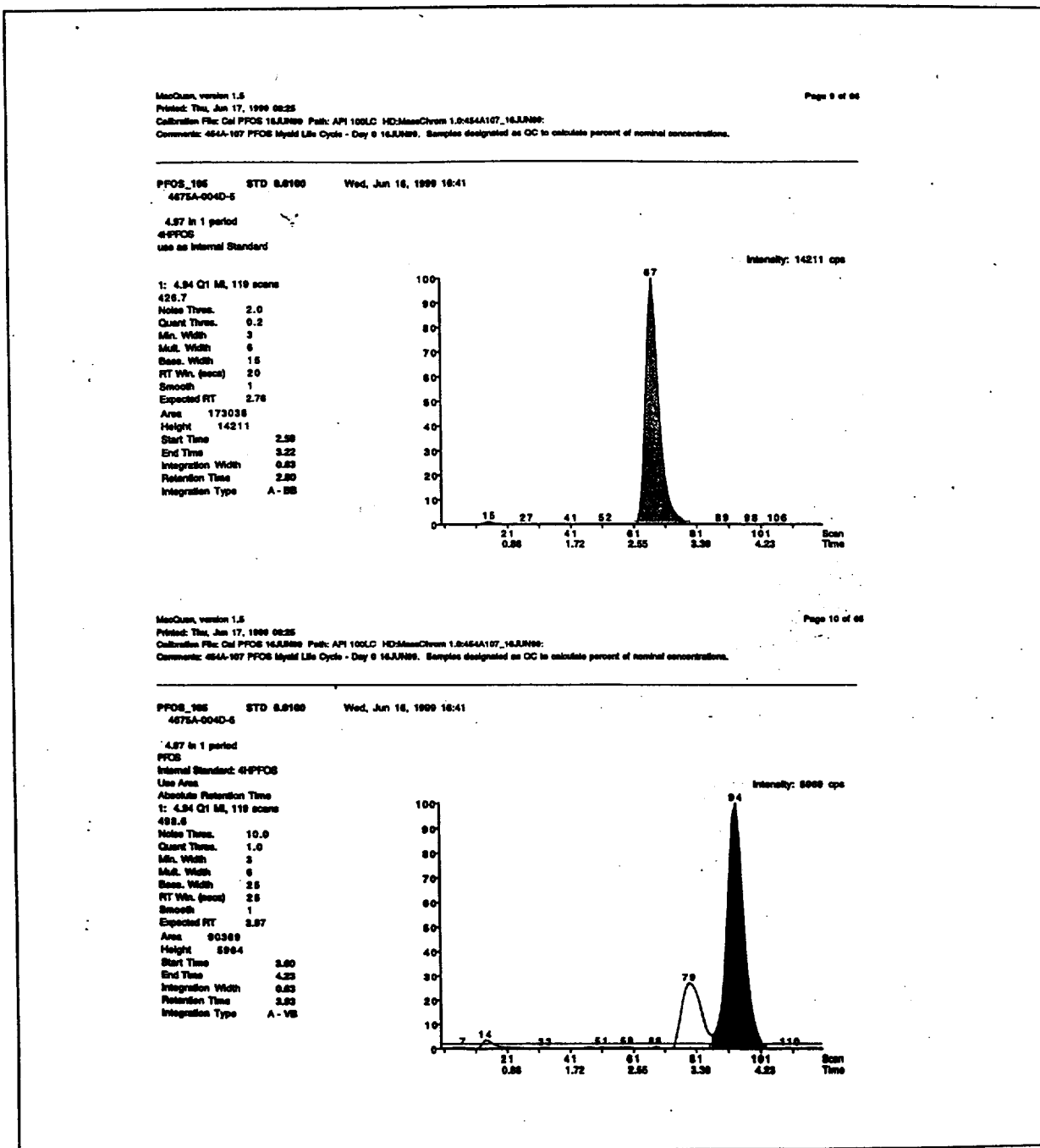


Figure 4. A representative ion chromatogram of a high-level (0.00915 mg a.i./L) PFOS standard.

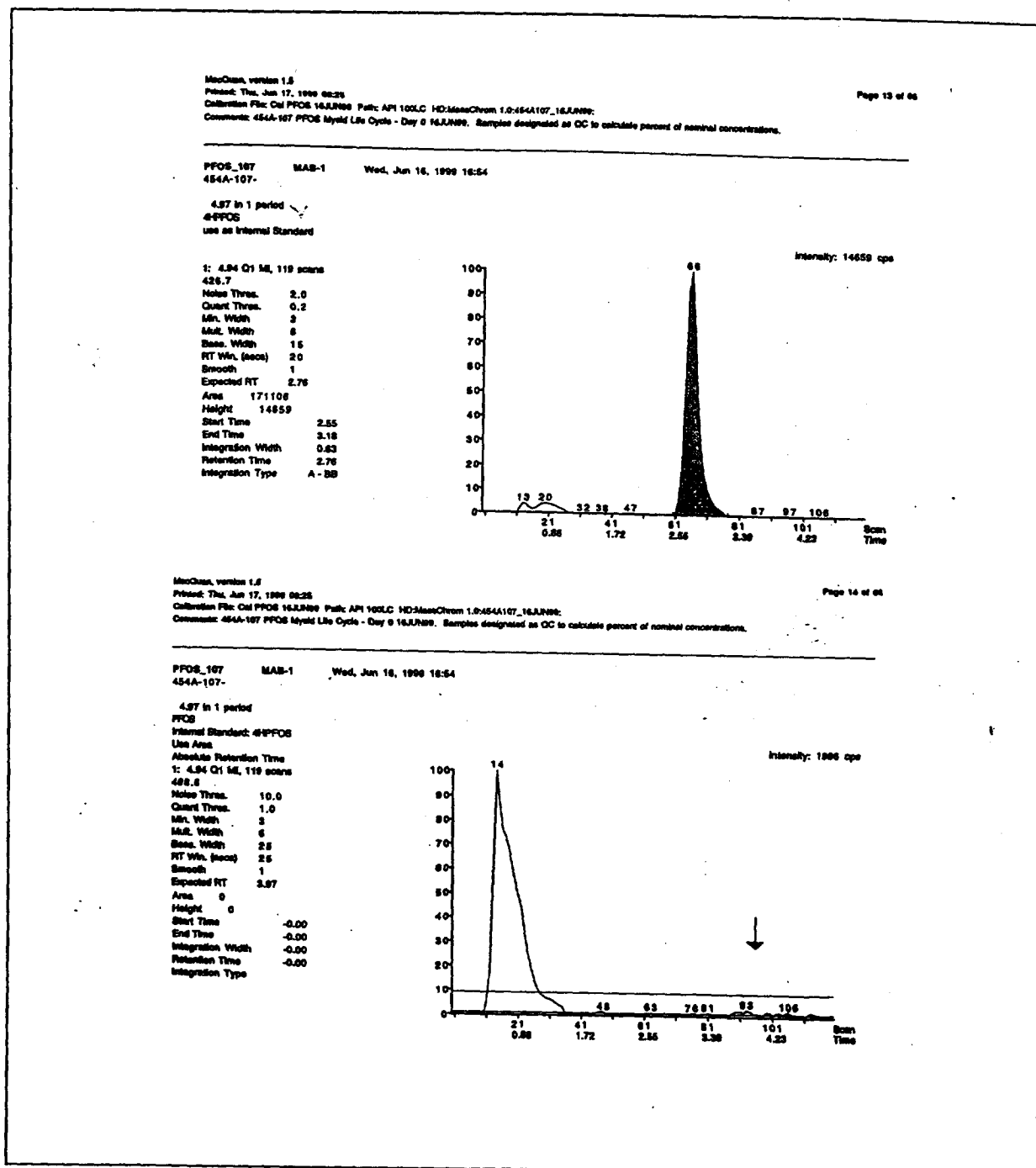


Figure 5. A representative ion chromatogram of a matrix blank sample (454A-107-MAB-1). The arrow indicates the retention time of PFOS.

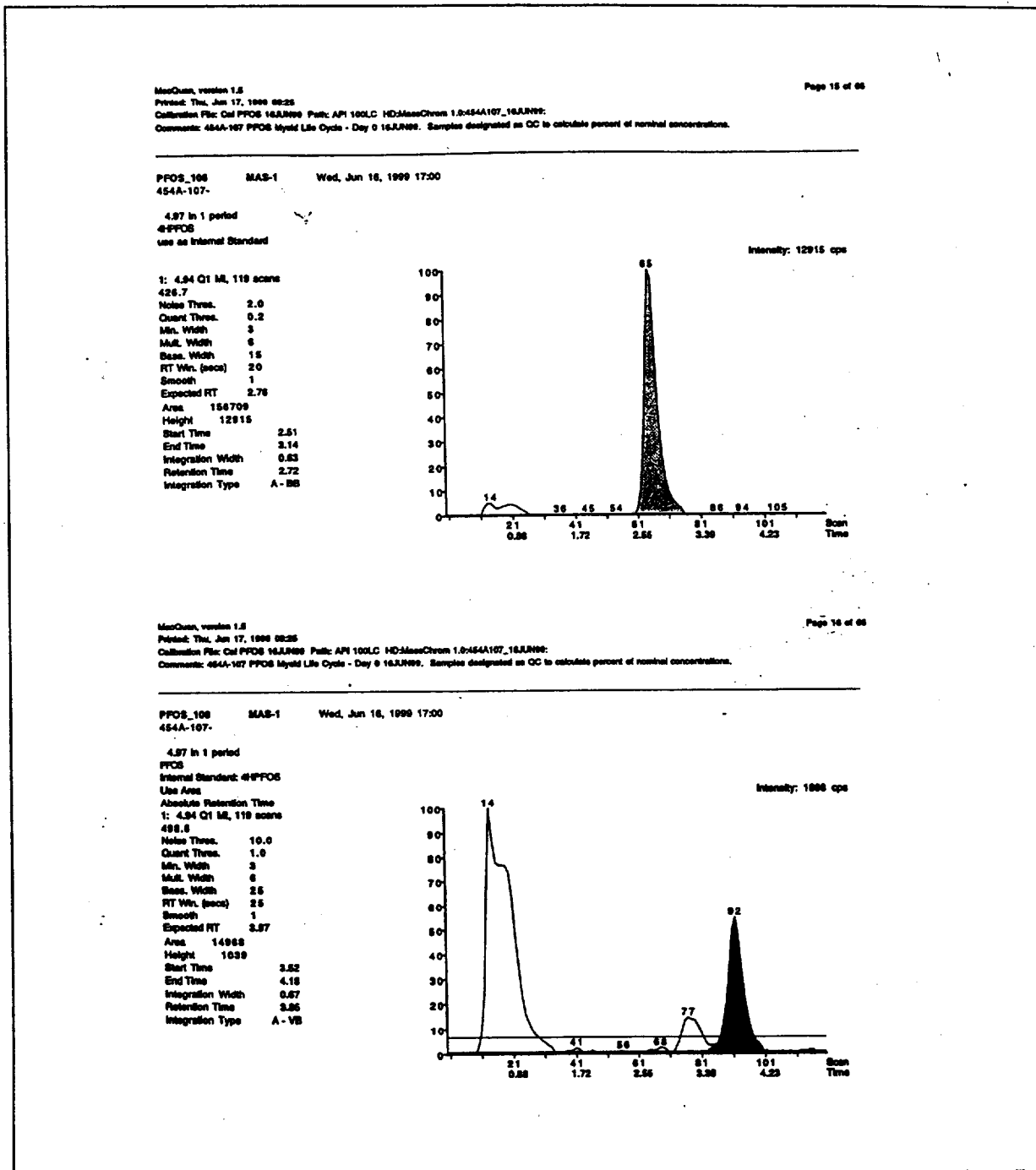


Figure 6. A representative ion chromatogram of a matrix fortification sample (454A-107-MAS-1).

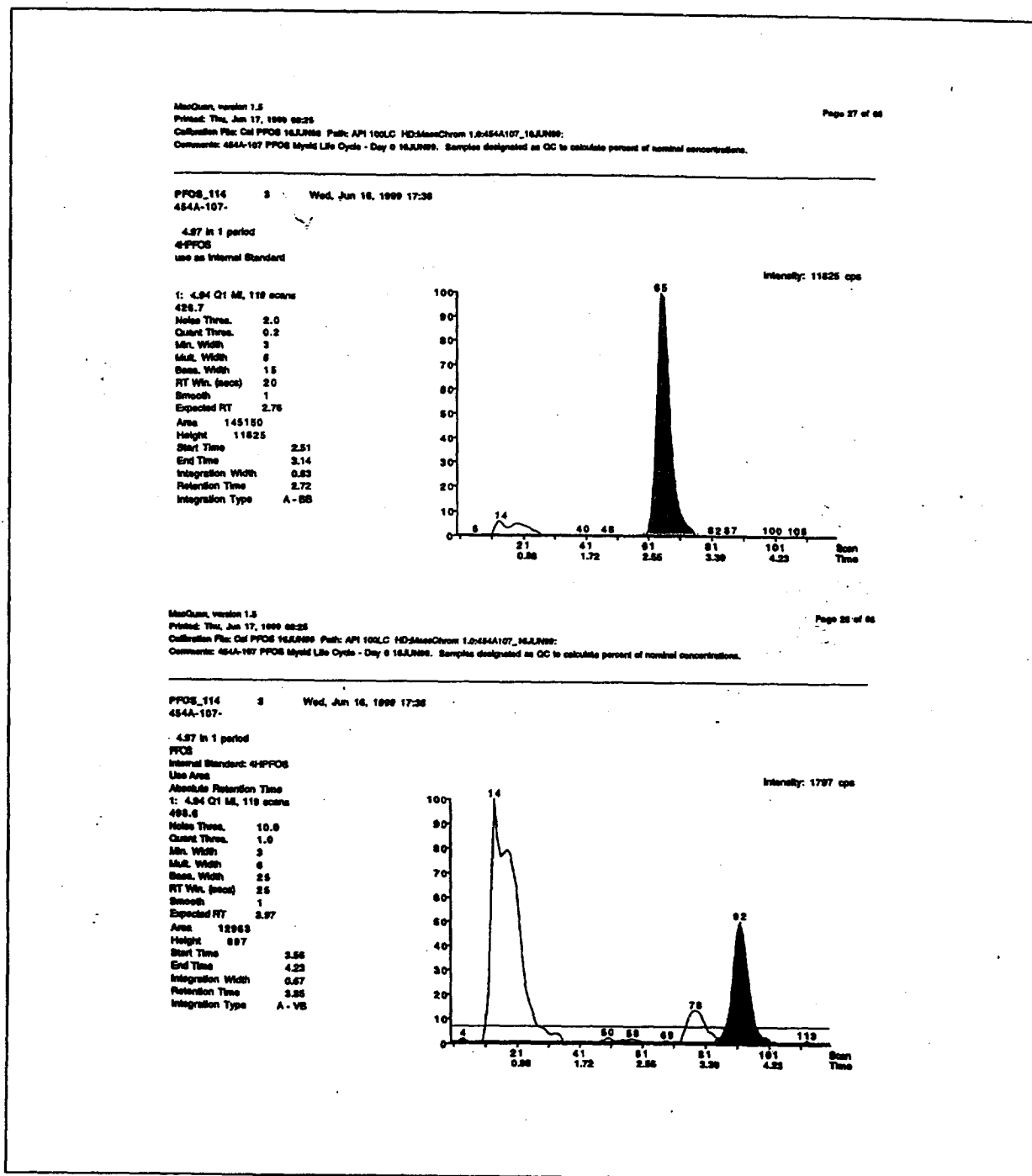


Figure 7. A representative ion chromatogram of a test sample (454A-107-3).

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APPENDIX IV

Mysid Reproduction

Sponsor:		3M Corporation			
Test Substance:		PFOS			
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water:		Filtered Saltwater			
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Compartment	Number of Reproductive Days ¹	Number of Young Produced	Number of Young/Reproductive Day
Negative Control	A	1	14	3	0.257
		2	14	2	
		3	14	6	
		4	14	5	
		5	14	2	
	B	1	14	4	0.264
		2	14	8	
		3	14	2	
		4	11	0	
	C	1	14	6	0.286
		2	14	3	
		3	14	4	
		4	14	5	
		5	14	2	
	D	1	14	9	0.452
		2	14	6	
		3	14	4	
0.057	A	1	14	3	0.283
		2	14	6	
		3	14	3	
		4	14	5	
		5	4	0	
	B	1	14	2	0.200
		2	14	3	
		3	14	5	
		4	14	0	
		5	14	4	
	C	1	14	3	0.186
		2	14	3	
		3	14	3	
		4	14	0	
		5	14	4	
	D	1	14	2	0.375
		2	14	10	
		3	14	7	
		4	14	2	

¹ The number of reproductive days is the number of days that the female was alive from first brood release to the end of the test.

APPENDIX IV (Continued)

Mysid Reproduction

Sponsor:		3M Corporation			
Test Substance:		PFOS			
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>			
Dilution Water:		Filtered Saltwater			
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Compartment	Number of Reproductive Days	Number of Young Produced	Number of Young/Reproductive Day
0.12	A	1	14	6	0.300
		2	14	0	
		3	14	8	
		4	14	6	
		5	14	1	
	B	1	14	7	0.478
		2	14	8	
		3	14	5	
		4	4	2	
	C	1	14	7	0.407
		2	14	9	
		3	14	4	
		4	12	2	
	D	1	14	9	0.257
		2	14	2	
		3	14	0	
		4	14	5	
		5	14	2	
0.25	A	1	14	2	0.271
		2	14	4	
		3	14	2	
		4	14	6	
		5	14	5	
	B	1	14	4	0.214
		2	14	1	
		3	14	7	
		4	14	0	
	C	1	14	2	0.344
		2	5	2	
		3	14	7	
		4	14	6	
		5	14	4	
	D	1	14	1	0.179
		2	14	0	
		3	14	3	
		4	14	6	
		5			

¹ The number of reproductive days is the number of days that the female was alive from first brood release to the end of the test.

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APPENDIX IV (Continued)

Mysid Reproduction

Sponsor:	3M Corporation				
Test Substance:	PFOS				
Test Organism:	Saltwater Mysid, <i>Mysidopsis bahia</i>				
Dilution Water:	Filtered Saltwater				
Mean Measured Test Concentration (mg a.i./L)	Replicate	Test Compartment	Number of Reproductive Days ¹	Number of Young Produced	Number of Young/Reproductive Day
0.55	A	1	14	0	0.0556
		2	14	3	
		3	12	0	
		4	14	0	
	B	1	14	0	0.107
		2	14	0	
		3	14	2	
		4	14	4	
	C	1	14	0	0.0429
		2	14	0	
		3	14	0	
		4	14	3	
		5	14	0	
	D	1	14	0	0.0179
		2	14	0	
		3	14	0	
		4	14	1	
1.3	A	1	3	0	0.000
		2	11	0	
		3	8	0	
	B	1	14	0	0.000
	C	--	--	--	--
	D	1	11	0	0.000

¹ The number of reproductive days is the number of days that the female was alive from pairing to the end of the test.

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APPENDIX V

Mysid Total Length (mm)

Mysid Number	Negative Control				0.57 mg a.i./L				0.12 mg a.i./L			
	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D
1	6.80	6.20	6.50	6.55	6.15	6.35	6.05	6.30	6.25	6.75	6.90	6.30
2	6.85	6.65	6.85	7.10	6.30	6.20	6.80	6.45	6.70	6.60	6.70	6.75
3	6.30	6.55	6.20	6.35	6.35	6.15	6.55	6.40	6.40	6.55	6.45	6.40
4	6.45	6.95	6.60	6.10	6.15	6.70	6.70	6.90	6.55	6.80	7.15	6.40
5	6.30	5.90	6.25	6.40	6.55	6.45	6.45	6.55	6.60	6.45	6.35	6.10
6	6.75	6.10	6.40	6.45	6.90	6.60	6.65	6.45	7.10	6.90	6.60	6.15
7	6.15	6.00	6.15	6.30	6.20	6.30	6.50	5.90	6.35	6.50	6.20	6.30
8	6.65	—	6.30	6.55	6.65	6.80	6.70	6.35	6.40	—	—	6.80
9	6.30	—	6.70	—	6.25	6.30	6.30	5.95	6.25	—	—	6.40
10	6.50	—	6.65	—	6.40	6.40	6.50	6.35	7.00	—	—	6.55
11	5.85	—	—	—	6.15	6.50	—	—	6.55	—	—	—
12	—	—	—	—	6.50	6.65	—	—	6.40	—	—	—
Mean	6.45	6.34	6.46	6.48	6.38	6.45	6.52	6.36	6.55	6.65	6.62	6.42

APPENDIX V (Continued)

Mysid Total Length (mm)

Mysid Number	0.25 mg a.i./L				0.55 mg a.i./L				1.3 mg a.i./L			
	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D
1	6.55	6.25	5.95	6.20	6.05	6.05	6.20	6.20	5.85	5.75	—	5.65
2	6.40	6.40	6.35	6.45	5.85	6.25	6.20	6.45	6.05	6.25	—	—
3	6.35	6.30	6.80	6.50	6.10	6.35	5.85	5.95	5.90	5.85	—	—
4	6.45	6.15	6.10	6.35	5.90	6.20	6.00	6.10	—	6.05	—	—
5	6.50	6.45	6.75	6.75	5.80	6.00	6.10	6.25	—	—	—	—
6	6.25	6.90	6.20	6.55	6.35	6.20	6.35	6.40	—	—	—	—
7	6.55	6.35	6.40	6.05	6.30	6.15	6.25	6.10	—	—	—	—
8	6.70	6.20	6.35	6.80	—	6.60	6.40	6.45	—	—	—	—
9	6.45	—	6.35	5.75	—	5.05	6.25	—	—	—	—	—
10	6.60	—	—	—	—	6.25	6.00	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—
Mean	6.48	6.38	6.36	6.38	6.05	6.11	6.16	6.24	5.93	5.98	—	5.65

APPENDIX VI
Mysid Dry Weight (mg)

Sponsor:		3M Corporation											
Test Substance:		PFOS											
Test Organism:		Saltwater Mysid, <i>Mysidops ts bahia</i>											
Dilution Water:		Filtered Saltwater											
Mysid Number	Negative Control				0.057 mg a.i./L				0.12 mg a.i./L				
	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	
1	0.51	0.57	0.49	0.68	0.53	0.65	0.45	0.59	0.52	0.87	0.78	0.54	
2	0.70	0.77	0.78	0.86	0.62	0.50	0.78	0.51	0.71	0.62	0.52	0.68	
3	0.52	0.70	0.60	0.54	0.58	0.55	0.60	0.46	0.65	0.55	0.56	0.52	
4	0.64	0.86	0.57	0.59	0.64	0.69	0.56	0.80	0.70	0.68	0.88	0.67	
5	0.56	0.61	0.51	0.53	0.82	0.47	0.63	0.57	0.72	0.64	0.55	0.46	
6	0.75	0.75	0.72	0.67	0.79	0.84	0.59	0.52	0.80	0.71	0.72	0.55	
7	0.44	0.68	0.50	0.60	0.51	0.52	0.54	0.46	0.54	0.58	0.50	0.64	
8	0.79	--	0.67	0.62	0.66	0.73	0.71	0.67	0.58	--	--	0.71	
9	0.58	--	0.52	--	0.49	0.61	0.49	0.49	0.59	--	--	0.74	
10	0.69	--	0.60	--	0.57	0.72	0.55	0.58	0.83	--	--	0.56	
11	0.41	--	--	--	0.50	0.66	--	--	0.49	--	--	--	
12	--	--	--	--	0.68	0.58	--	--	0.63	--	--	--	
Mean	0.599	0.706	0.596	0.636	0.616	0.627	0.590	0.565	0.647	0.664	0.644	0.607	

APPENDIX VI (Continued)

Mysid Dry Weight (mg)

Sponsor:		3M Corporation										
Test Substance:		PFOS										
Test Organism:		Saltwater Mysid, <i>Mysidopsis bahia</i>										
Dilution Water:		Filtered Saltwater										
Mysid Number	0.25 mg a.i./L				0.55 mg a.i./L				1.3 mg a.i./L			
	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D	Rep A	Rep B	Rep C	Rep D
1	0.72	0.45	0.50	0.61	0.62	0.50	0.57	0.54	0.36	0.40	--	0.39
2	0.60	0.68	0.62	0.67	0.51	0.48	0.42	0.66	0.52	0.57	--	--
3	0.54	0.62	0.86	0.59	0.54	0.67	0.39	0.52	0.44	0.43	--	--
4	0.55	0.53	0.45	0.53	0.45	0.62	0.61	0.68	--	0.51	--	--
5	0.63	0.58	0.71	0.84	0.43	0.57	0.51	0.55	--	--	--	--
6	0.48	0.79	0.55	0.73	0.71	0.61	0.60	0.60	--	--	--	--
7	0.79	0.60	0.51	0.52	0.63	0.54	0.81	0.49	--	--	--	--
8	0.71	0.56	0.67	0.80	--	0.59	0.65	0.52	--	--	--	--
9	0.67	--	0.56	0.46	--	0.36	0.48	--	--	--	--	--
10	0.75	--	--	--	--	0.64	0.59	--	--	--	--	--
11	--	--	--	--	--	--	--	--	--	--	--	--
Mean	0.644	0.601	0.603	0.639	0.556	0.558	0.563	0.570	0.440	0.478	--	0.39

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. Analysis of feed for PFOS was deleted by amendment.
3. The proportion of water split to each replicate was not checked at the end of the test.
4. Temperature was not measured in the B replicate of the 2.7 mg a.i./L treatment group on Day 14 of the test.
5. Nominal test concentrations were 0.086, 0.17, 0.34, 0.69, 1.4 and 2.7 mg a.i./L.

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. Drott, Senior Biologist

PROTOCOL

PFOS: A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST WITH THE SALTWATER MYSID (*Mysidopsis bahia*)

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.1350

3M Lab Request No. U2723

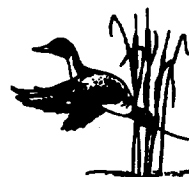
Submitted to

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



WILDLIFE INTERNATIONAL LTD.

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



May 13, 1999

- 2 -

PFOS: A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST
WITH THE SALTWATER MYSID (*Mysidopsis bahia*)

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: Negative Control, 0.094, 0.19, 0.38, 0.75, 1.5 and 3.0 mg a.i./L

Test Substance No.: 4675 Reference Substance No. (if applicable): 4526

PROTOCOL APPROVAL

STUDY DIRECTOR

DATE

LABORATORY MANAGEMENT

DATE

Susan A. Beach
SPONSOR'S REPRESENTATIVE

5/18/99
DATE

INTRODUCTION

Wildlife International, Ltd. will conduct a flow-through life-cycle toxicity test with the saltwater mysid, *Mysidopsis bahia*, for the Sponsor at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.1350 (1); and *ASTM Standard E1191-90 Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids* (2). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

PURPOSE

The purpose of this study is to determine the effects of a test substance on the saltwater mysid, *Mysidopsis bahia*, during chronic exposure. Effects on growth, survival, and reproduction will be evaluated.

EXPERIMENTAL DESIGN

Mysids will be exposed to a series of six test concentrations, a negative (dilution water) control beginning at the juvenile stage (<24 hours old) and continuing for at least 7 days after the median time of first brood release in the control treatment. The test will not be terminated until at least 28 days and may be continued at the request of the Sponsor. Test concentrations will be chosen in consultation with the Sponsor and will be based upon known toxicity data. Each test concentration will be 50% of the next higher treatment.

Four replicate test chambers will be used for each treatment and control group, and each test chamber will contain one compartment holding 15 mysids. In order to control bias, mysids will be indiscriminately assigned to exposure chambers at test initiation. No other potential sources of bias are expected to affect the results of the study. Mysids will be indiscriminately distributed in groups of one to three among glass beakers representing each test compartment until each beaker contains 15 mysids. The mysids will then be gently transferred into the test compartments. Thus, a total of 60 mysids will be exposed in each treatment and control group. After mysids attain sexual maturity (usually 10 to 14 days after the beginning of the test), female and male adult mysids will be paired, and the reproduction of the paired mysids will be monitored.

Observations for mortality and clinical signs of toxicity will be made daily. The number of live young produced by each pair will be counted, recorded, and removed to a separate test compartment at the same test concentration on a daily basis. The young will be exposed under static conditions for 96 hours. The dry weight and length of first-generation mysids alive at the end of the test will be determined. Data on survival, growth, and reproduction will be analyzed to determine the effects of the test substance on these parameters.

MATERIALS AND METHODS

Test Substance

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

The Sponsor is responsible for all information related to the test substance including the retention of a reserve sample of the lot or batch of the test substance used in this study. The Sponsor also 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 administered to the test organism in water. This route of administration was selected because it represents the most likely route of exposure to aquatic organisms.

Test Organism

The mysid, *Mysidopsis bahia*, will be used in this test. This species is representative of an important group of organisms and was selected for use in the test based upon past use and ease of handling in the laboratory. Mysids will be obtained from Wildlife International, Ltd. cultures. All mysids used in the test will be from the same brood stock.

Tests will be initiated with mysids less than 24-hours old (juveniles). Cultures will be maintained in a recirculating saltwater system kept at approximately 25°C. Saltwater will be filtered through a biofilter and UV sterilized prior to entering the recirculating system. All mysids will be held in the laboratory for at least 14 days before juveniles are collected for testing. Mysids will not be subjected to more than a 3°C change in temperature or a 3 ‰ change in salinity in any 12-hour period during the 14 days before test initiation. Mysids will be fed live brine shrimp nauplii (*Artemia sp.*) *ad libitum* daily. Brine shrimp will be periodically enriched with ALGAMAC-2000 (fatty acid supplement). Feed (brine shrimp) provided to the mysids will be analyzed for PFOS. Excess food will be removed from the test chambers daily.

Mysids will be handled as infrequently as possible but when handling is necessary it will be done gently, carefully, and quickly. A siphon collector system will be used to collect neonate mysids directly from brood tanks. Young mysids will be transferred using large-bore pipettes.

Dilution Water

Natural seawater collected at Indian River Inlet, Delaware will be the source of the dilution water used in the culture facility and test system. The seawater will be filtered through a sand filter prior to its delivery to a 37,800-L holding tank. The salinity of the seawater will be diluted to approximately 20 ‰ (parts per thousand) with Wildlife International Ltd. wellwater in the holding tank. The 20 ‰ saltwater will be aerated using spray nozzles and filtered (0.45 µm) prior to its delivery to the test system.

Salinity will be measured weekly to monitor the consistency of the saltwater. 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 to determine the concentrations of selected organic and inorganic constituents of the seawater and results of the analyses will be summarized in the final report.

Test Apparatus

A continuous-flow diluter will be used to provide each concentration of the test substance and a negative (dilution water) control. A syringe pump, peristaltic pump, or a similar device will be used to deliver the test substance to mixing chambers where the test substance will be mixed with dilution water. The flow of dilution water into each mixing chamber will be controlled using rotameters. The rotameters will be calibrated prior to the test and verified and/or calibrated at least once a week during the test. After mixing,

test solutions will be split to each replicate chamber. The proportion of water split to each replicate will be checked prior to the test, at approximately weekly thereafter and at the end of the test to ensure that these flow rates vary by no more than $\pm 10\%$ of the mean.

The diluter will be adjusted so that each test chamber receives at least 5 volume additions of test solution every 24 hours. Peristaltic pumps will be calibrated prior to test initiation and at approximately weekly intervals thereafter and syringe pumps will be calibrated prior to test initiation. The delivery of the test substance to the test chambers will begin at least 48 hours prior to the test in order to establish equilibrium concentrations of the test substance. The general operation of the diluter will be checked visually at least two times per day during the test.

Test chambers will be 9-L glass aquaria filled with approximately 5 L of test solution. Each treatment and control group will have four replicate test chambers. Test chambers will be impartially positioned in an environmental chamber designed to maintain a temperature of $25 \pm 2^\circ\text{C}$. Test chambers will be labelled with the project number, test concentration and replicate.

Prior to sexual maturity, mysids will be held in one compartment placed in each replicate test chamber (15/compartment). The compartments consist of glass beakers with nylon mesh screen attached to two holes on opposite sides of the beaker. All attachments of the nylon mesh screen and glass will be made with silicone adhesive.

After mysids attain sexual maturity (usually 10 to 14 days after the beginning of the test), up to 5 male and 5 female adult mysids will be paired per replicate test chamber. Individual pairs will be held in reproductive compartments (one pair per compartment) placed in each test chamber. The reproductive compartments will be glass petri dishes with sides of nylon mesh screen attached with silicone adhesive. Immature and additional females will be discarded. Additional male mysids will be maintained in a separate test compartment of each replicate test chamber. Additional males will be used to replace dead males in the same replicate during the exposure. The test will not be terminated before 7 days past the median time of first brood release in the control(s). The test will continue for at least 28 days and may be extended longer at the request of the Sponsor.

Environmental Conditions

Ambient room light will be used to illuminate the test systems. Fluorescent light tubes (Colortone® 50 or equivalent) that emit wavelengths similar to natural sunlight will be controlled by an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. A 15-to 30-minute transition period will be provided when lights go on and off to avoid sudden changes in light intensity. Light intensity will be measured at test initiation and at weekly intervals thereafter with a SPER Scientific Ltd. light meter or equivalent.

The target test temperature will be $25 \pm 2^{\circ}\text{C}$. Temperature will be monitored and recorded continuously in a negative control using a Fulscope ER/C Recorder (1900 J Series Model A) or equivalent during the entire test. Recorder measurements will be verified with a liquid-in-glass thermometer prior to test initiation and at least weekly thereafter. Temperature will also be measured in each test chamber at the beginning and end of the test and at weekly intervals during the test using a liquid-in-glass thermometer.

Dissolved oxygen and pH will be measured at the beginning and the end of the test and at weekly intervals during the test in alternating replicate test chambers of each treatment level. In the event that dissolved oxygen levels fall below 60% saturation, appropriate actions will be taken after consultation with the Sponsor. Salinity will be measured daily in alternating replicates of the negative control treatment and the highest PFOS treatment group. If 100% mortality occurs in any treatment, then dissolved oxygen, pH and temperature measurements will be made at that time and then discontinued. Dissolved oxygen will be measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter or equivalent, water pH will be measured using a Fisher Accumet Model 915 pH meter or equivalent, and salinity will be measured using a refractometer or equivalent instrument.

Biological Measurements

Observations of mortality and clinical signs of toxicity in first-generation mysids will be made daily. The criteria for death include lack of movement, absence of respiratory movements, and lack of reaction to gentle prodding. Live young produced in each compartment will be counted, recorded, and removed daily. Live young will be exposed to the same concentration of the test substance under static test conditions for approximately 96 hours. At the end of the test, the dry weight and total body length of each first-generation

mysid will be determined. Observations of abnormal development and aberrant behavior will be made for first-and second-generation mysids throughout the test.

Sampling for Analytical Measurements

Water samples will be collected from two replicate test chambers of both the low and high level concentrations prior to exposure. Water samples also will be collected from two alternating replicate test chambers of each treatment group and the control at the beginning of the test, at weekly intervals during the test, and at the end of the test to determine concentrations of the test substance. 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 from each test chamber, and analyzed immediately. The sample scheme is summarized below:

PROPOSED NUMBERS OF VERIFICATION SAMPLES

Experimental Group	Pretest ^a	Day 0	Day 7	Day 14	Day 21	Day 28	End
Control	-	2	2	2	2	2	2
Level 1-Low Concentration	2 ^b	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	2
Totals	4	14	14	14	14	14	14

^aPre-test samples will be collected after conditioning of the diluter. More than one pre-test sampling interval may be required, depending upon the Sponsor's needs, and additional sampling and results will be documented in the raw data and included in the final report.

^bOne sample from the A and B replicate test chambers.

Total Number of Verification Samples = 88

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. At the discretion of the Study Director, Water samples also will be collected from at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system.

Analytical Measurements

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

Data to be analyzed include the survival of first-generation mysids, the number of young released per reproductive day, the dry weight and length of each first-generation mysid alive at the end of the test. If deemed necessary, clinical signs of toxicity in the adult mysids may also be analyzed statistically. Concentration-response curves will be fitted to the adult survival, reproduction and growth data.

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 mysids). Analyses of reproduction (number of young produced per surviving adult) 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 test 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. All statistical evaluations will be done using commercially available computer software programs such as TOXSTAT (3) OR SPSS/PC+ (4).

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by 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.
3. Dates of initiation and termination of the test.
4. History of the test organism.
5. Test organism weight and length measurements at the end of the test.
6. Stock solution calculation and preparation.
7. Daily observations.
8. Water chemistry results (e.g., salinity and pH).
9. If applicable, the methods used to analyze test substance concentrations and the results of analytical measurements.
10. Statistical calculations.
11. Test conditions (light intensity, photoperiod, etc.).
12. Calculation and preparation of test concentrations.
13. Copy of final report.

FINAL REPORT

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

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 any changes in the original protocol.
5. The test substance identification, including name, chemical abstract number or code number, strength, purity, composition, and other characteristics provided by the Sponsor.
6. Stability and solubility of the test substance under the conditions of administration, if provided by the Sponsor.
7. A description of the methods used to conduct the test.
8. A description of the test organisms, including the source of the test organisms, scientific name, age, life stage, means and ranges of weights and lengths, observed diseases, treatments, holding procedures, and feeding regime.

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

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (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.

REFERENCES

- 1 U.S. Environmental Protection Agency. 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1350: *Mysid Chronic Toxicity Test*.
- 2 ASTM Standard E1191-90. 1991. *Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids*, American Society for Testing and Materials.
- 3 West, Inc. and D. D. Gulley. 1996. *TOXSTAT Release 3.5*. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.
- 4 Norusis, M.J. 1988. SPSS/PC+ V2.0 base manual for the IBM PC/XT/AT and PS/2.

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)
	<u>Daphnia magna: 27 mg/L</u>	<u>Rainbow Trout: 11 mg/L</u>
	<u>Daphnia magna: 50 mg/L</u>	<u>Fathead Minnow: 38 mg/L</u>

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

Please see MSDS

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.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST WITH THE
SALTWATER MYSID (*Mysidopsis bahia*)

PROTOCOL NO.: 454/051399/MYS-LC/SUB454

AMENDMENT NO.: 1

SPONSOR: 3M Corporation

PROJECT NO.: 454A-107

EFFECTIVE DATE: June 16, 1999

AMENDMENT: Page 2

Add: Proposed Dates:

Experimental Start Date: 6/16/99

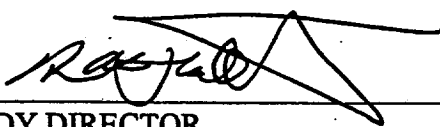
Experimental Termination Date: 7/21/99

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

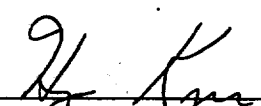
AMENDMENT: Test Organism, Page 5

Delete: Feed (brine shrimp) provided to the mysids will be analyzed for PFOS.

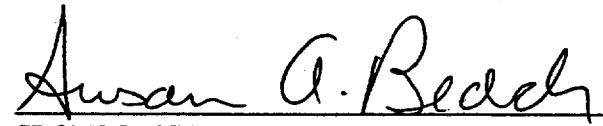
REASON: The feed has already been screened for PFOS.


STUDY DIRECTOR

7/19/99
DATE


LABORATORY MANAGEMENT

7/19/99
DATE


SPONSOR'S REPRESENTATIVE

7/27/99
DATE