PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL *(Lepomis macrochirus)*

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-134
ENVIRONMENTAL LABORATORY REQUEST NUMBER: U2723

U.S. Environmental Protection Agency
Series 850 – Ecological Effects Test Guidelines
OPPTS Number 850.1730
and
OECD Guideline 305

AUTHORS :

Kurt R. Drottar
Raymond L. VanHoven, Ph.D.
Henry O. Krueger, Ph.D..

STUDY INITIATION DATE: October 31, 2000

STUDY COMPLETION DATE: June 21, 2001

AMENDED STUDY COMPLETION DATE: June 6, 2002

SUBMITTED TO :

3M Corporation
Environmental Laboratory
Building 2-3E-09
935 Bush Avenue
St. Paul, Minnesota 55144St. Paul, Minnesota 55144

Wildlife International, Ltd.

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

AMENDED

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR : 3M Corporation

TITLE : Perfluorooctanesulfonate, Potassium Salt (PFOS): A Flow-Through Bioconcentration Test with the Bluegill (*Lepomis machochirus*)

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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.

STUDY DIRECTOR:

Henry Krueger — 6 June 02

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

Date

SPONSOR APPROVAL:

Susan A. Beach — 6/11/02

Sponsor Representative

Date

AMENDED

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO: STUDY DIRECTOR:	MANAGEMENT:
Test SubstancePreparation	November 29, 2000	November 29, 2001	November 30, 2000
Matrix Fortification	December 8, 2000	December 8, 2000	December 11, 2000
Water Chemistry	February 20, 2001	February 20, 2001	February 22, 2001
Analytical Data and DraftReport	May 14 – 17 and May 20 – 24, 2001	May 24, 2001	June 2, 2001
Biological Data and Draft Report	June 11- 13, 2001	June 13, 2001	June 14, 2001
Final Report	June 20, 2001	June 20, 2001	June 20, 2001
Amended Final Report	June 5, 2002	June 5, 2002	June 6, 2002

Marshall T. Hynson
Quality Assurance Program Supervisor

6/6/2002
DATE

AMENDED

REPORT APPROVAL

SPONSOR : 3M Corporation

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Director of Aquatic Toxicology and
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6 June 02
Date

PRINCIPAL INVESTIGATOR:

Mark Jaber
Director of Research

6/6/02
Date

AMENDED

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SUMMARY

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

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER:	454A-134
TEST SUBSTANCE:	Perfluorooctanesulfonate, Potassium Salt (PFOS)
STUDY:	Perfluorooctanesulfonate, Potassium Salt (PFOS): A Flow-Through Bioconcentration Test with the Bluegill *(Lepomis macrochirus)*
NOMINAL TEST CONCENTRATIONS:	Negative Control, 0.10 and 1.0 mg a.i./L
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control, 0.086 and 0.87 mg a.i./L. Exposure to 0.87 mg a.i./L ceased after 35 days due to fish mortality and tissue sampling
TEST DATES:	Experimental Start (OECD) – November 29, 2000 Experimental Start (EPA) - December 5, 2000 Biological Termination – April 2, 2001 Experimental Termination - April 11, 2001
LENGTH OF TEST (0.086 mg a.i./L):	118 Days (62-Day Uptake, 56-Day Depuration)
LENGTH OF TEST (0.87 mg a.i./L):	35 Days of Uptake

TEST ORGANISM:	Bluegill *(Lepomis macrochirus)*
SOURCE OF TEST ORGANISMS:	Osage Catfisheries, Inc. 1170 Nichols Road Osage Beach, Missouri 65065
AGE OF TEST ORGANISMS:	Juveniles
MEASUREMENTS OF 10 NEGATIVE CONTROL FISH COLLECTED AT TEST TERMINATION:	
WEIGHT (g):	Mean = 2.70; Range = 2.03 to 3.32
TOTAL LENGTH (mm):	Mean = 62; Range = 56 to 66

RESULTS: (0.086 mg a.i./L)	Edible	Nonedible	Whole Fish
Kinetic Bioconcentration factor (BCFK):	1124	4013	2796

RESULTS: (0.87 mg a.i./L)	Edible	Nonedible	Whole Fish
DAY 28 BCF*:	136	386	278

*Note: The BCFs were underestimated due to fish mortality prior to achieving steady-state.

AMENDED

INTRODUCTION

A bluegill sunfish, *Lepomis macrochirus*, bioconcentration study was conducted for 3M Corporation at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The in-life phase of the test was conducted from December 5, 2000 to April 2, 2001. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454A-134 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to determine the bioconcentration potential of perfluorooctanesulfonate, potassium salt (PFOS) in the bluegill sunfish.

EXPERIMENTAL DESIGN

The bioconcentration test consisted of a 62-day uptake phase followed by a 56-day depuration phase. During the uptake phase, the test organisms were exposed in one of three groups: 1) A negative (dilution water) control; 2) A nominal concentration of 0.10 mg active ingredient (a.i.)/L; or 3) A nominal concentration of 1.0 mg a.i./L. At the start of the depuration phase, stock flow to the treated groups was stopped and the bluegill were exposed to dilution water without PFOS for the remainder of the test.

Each test chamber contained 90 bluegill at test initiation, and one replicate was tested for each treatment and the negative control. Water samples were collected on Day -4 (pre-test), Day -1 (pre-test) on uptake Days 0 (0 and ~4 hours), 1, 3, 7, 14, 21, 28, 35, 42, 49, 56 and 62 and on depuration Days 14, 28, 42 and 56 during the test and analyzed for PFOS using liquid chromatography-mass spectrometry (LC/MS). Tissue samples were also collected at selected water sample collection periods during the test and analyzed for PFOS by LC/MS. The results of these analyses were used to calculate the BCF values, uptake rates and depuration rates in edible tissue, nonedible tissue and whole fish.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "Perfluorooctanesulfonate, Potassium Salt (PFOS) (Appendix 9): A Flow-Through Bioconcentration Test with the Bluegill *(Lepomis macrochirus)*". The protocol was based on procedures outlined in U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.1730 (1); *ASTM Standard E1022-84 Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs* (2); and OECD Guideline for Testing of Chemicals 305, *Bioconcentration: Flow-Through Fish Test* (3).

Test and Reference Substances

The test substance was received from 3M Corporation on October 29, 1998 and was assigned Wildlife International, Ltd. identification number 4675. The test substance, a white powder, was identified as FC-95, Lot #217. Information provided by the Sponsor indicated a purity of 86.9% and an expiration date of August 31, 2001. The test substance was stored under ambient conditions.

Preparation of Test Solutions

The nominal test concentrations were 0.10 and 1.0 mg a.i./L . Two stock solutions were prepared at concentrations of 10 and 100 mg a.i./L. The appropriate amount of test substance was weighed out and dissolved in dilution water for each stock. The stock solutions were stirred with an electric top-down mixer to aid in the solubilization of the test substance. After mixing, the stock solutions appeared clear and colorless. Stock solutions were prepared at approximately weekly intervals during the uptake phase of the test. The stock solutions were injected into the diluter mixing chambers (at a rate of 3.5 mL/minute) where they were mixed with dilution water (at a rate of 350 mL/minute) to achieve the desired test concentrations. All test solutions appeared clear and colorless.

Test Organism

The bluegill, *Lepomis macrochirus*, was selected as the test species for this study. The bluegill is one of the recommended freshwater fish species for use in bioconcentration tests (1, 2, 3). Bluegill used in the test were obtained from Osage Catfisheries, Inc., Osage Beach, Missouri. The fish were approximately 7 months old at test initiation. Identification of the species was verified by the supplier.

The bluegill were held in Wildlife International, Ltd. well water for 103 days prior to testing. The fish were acclimated to test conditions for approximately 49 hours prior to test initiation. During the holding and acclimation periods the fish showed no signs of disease or stress. During the 14-day holding period preceding the test, water temperatures ranged from 22.3 to 22.7°C. The pH of the water ranged from 7.8 to 8.2 and dissolved oxygen ranged from 7.6 to 8.2 mg/L. Instrumentation used for water measurements are described in the *Environmental Conditions* section of this report. At test initiation, the bluegill were collected from the acclimation tank and indiscriminately distributed 1 to 2 at a time into the test chambers until each chamber contained 90 fish.

During holding and during the test, the bluegill were fed flake food supplied by Zeigler Brothers, Inc., Gardners, Pennsylvania. The bluegill were fed at least once daily during holding and once daily during the test. Feeding and sampling schedules were coordinated so that fish were sampled at least four hours after feeding.

All fish used in the test were from the same source and year class, and the standard length of the longest fish was no more than twice the length of the shortest. The length and weight of fish in the negative control were considered to be representative of all fish used in the test. The mean total length of 10 negative control fish measured at the end of the test was 62 mm with a range of 56 to 66 mm. The average wet weight (blotted dry) was 2.70 grams with a range of 2.03 to 3.32 grams. Loading was defined as the total wet weight of fish per liter of test water that passed through the test chamber in 24 hours, and was determined to be 0.48 g fish/L/day. The loading rate was based on the average weight of the fish at the end of the test and the initial stocking density (90 fish per tank).

Test Apparatus

A continuous-flow diluter was used to deliver each concentration of the test substance and a negative control. A peristaltic pump (Cole-Parmer Instrument Company, Chicago, Illinois) was used to deliver the test substance stock solutions into mixing chambers assigned to each PFOS treatment. The stock solutions were mixed with dilution water in the mixing chambers prior to delivery to the test chambers. The flow of dilution water to the test chambers was controlled by rotameters. The delivery of water from the rotameters was checked prior to the test and at approximately weekly intervals thereafter. Approximately 6.3 volume additions of test water were delivered to the test chambers every 24 hours. The general operation of the diluter was checked at least two times a day during the test and once on the last day of the test.

Test chambers were 104-L stainless steel aquaria filled with approximately 80 L of test solution. The depth of the test water in a representative chamber was approximately 19 cm. Test chambers were indiscriminately positioned in a temperature-controlled water bath designed to maintain a constant temperature. The water bath was enclosed in a plexiglass ventilation hood in order to minimize any potential for cross-contamination. Test chambers were siphoned daily and periodically cleaned during the test to remove excess feed and fecal matter. Test chambers were identified by the project number and test concentration.

Dilution Water

The water used for holding and testing was freshwater obtained from a well approximately 45 meters deep located on the Wildlife International, Ltd. site. The well water is characterized as moderately-hard water. The specific conductance, hardness, alkalinity and pH measurements of the well water during the four-week period immediately preceding the test are presented in Appendix 1.

The well water was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank and aerated with spray nozzles. Prior to use, the water again was filtered (0.45µm) to remove microorganisms and particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International, Ltd. are presented in Appendix 2.

Environmental Conditions

The target temperature range for the test was 22 ±1°C. Temperature was recorded continuously in the negative control with a Fulscope ER/C Recorder (1900 J Series model no. A). Temperature was also measured in all test chambers at the beginning and end of the test and at weekly intervals during the test, with a liquid-in-glass thermometer.

Dissolved oxygen was measured with a Yellow Springs Instrument Company, Inc. Model 51B dissolved oxygen meter. With the exception of uptake Day 24, measurements were made daily in each test chamber. Dissolved oxygen measurements were not made on uptake Day 24 due to biologist oversight. Measurements of pH were made in each test chamber at the beginning and end of the test and at weekly intervals during the test using a Fisher Accumet Model 915 pH meter.

Hardness, alkalinity, conductivity and total organic carbon (TOC) were measured in the negative control at the beginning and end of the test, and at weekly intervals during the test. Hardness and alkalinity were measured by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* (4). Conductivity was measured using a Yellow Springs Instrument Company, Inc. Model 33 Salinity-Conductivity Temperature meter. Total organic carbon was measured using a Shimadzu model TOC-5000 total organic carbon analyzer.

Ambient room light was used to illuminate the test systems. Fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50) were controlled by an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in light intensity. Light intensity at the surface of the water (over the negative control) was 278 lux at test initiation. Light intensity was measured using a SPER Scientific Model 840006 light meter.

Observations

All fish were observed once each day to evaluate the number of mortalities and the number of individuals exhibiting signs of abnormal behavior.

Procedures for Exposure of Fish to PFOS

The test chambers were conditioned by delivering PFOS to the diluter system for approximately 5 days before adding the fish. Water samples were collected three times during the pre-test period to confirm that equilibrium concentrations of test substance in the test chambers were achieved prior to adding the fish.

At the end of the pre-test period, the uptake phase of the test was initiated on December 5, 2000 by placing the fish in the test chambers. Bluegill were impartially removed from the holding tank in groups of 1 to 2. The groups of bluegill were distributed among the test chambers until each test chamber contained 90 fish. The duration of the uptake phase was 62 days for the low exposure concentration (0.1 mg a.i./L, nominal). The uptake phase for the high concentration (1.0 mg a.i./L, nominal) was terminated after 35 days due to fish mortality. All subsequent references in this report to sampling periods after Day 35 of uptake refer to the 0.1 mg a.i./L (nominal) exposure concentration. At the end of the uptake phase, stock flow to the treatment groups was stopped and the bluegill were exposed to dilution water without PFOS for a period of 56 days.

Collection and Analysis of Water Samples

Water samples were collected on Days 0 (0 hours and 4 hours), 1, 3, 7, 14, 21, 28, 35, 42, 49, 56 and 62 of the uptake phase. Water samples were also collected on Days 14, 28, 42 and 56 of the depuration phase. At each water sampling interval, two water samples were collected from the negative control and three samples were collected from each of the two PFOS treatment groups. One negative control sample and two samples from each of the PFOS treatment groups were analyzed for PFOS. The remaining samples were held in reserve as backup samples. All water samples were collected from mid-depth of each test chamber using a glass pipette. The water samples were analyzed for PFOS by liquid chromatography-mass spectrometry (LC/MS). Procedures for analysis of the water samples are provided in Appendix 3. Water samples were analyzed as soon as possible after collection without storage.

Collection and Analysis of Tissue Samples

Tissue samples were collected on Days 0 (4 hours), 1, 3, 7, 14, 21, 28, 35, 42, 49, 56 and 62 of the uptake phase. Tissue samples were also collected on Days 14, 28, 42 and 56 of the depuration phase. At each tissue sampling interval, a sufficient number of fish were collected to provide two replicate samples of negative control fish and four replicate samples of each PFOS treatment group. Fish were impartially removed from the test chambers and euthanized by severing the spinal cord above the opercular region. The fish were blotted dry and measured for total length and wet weight within approximately 15 minutes of collection, when possible. Each fish was then rinsed with dilution water, blotted dry again and dissected into edible and nonedible tissue fractions. Dissection was accomplished by making an incision from just posterior to the base of the pectoral fin dorsally through the spinal cord. The head, fins and viscera were removed from the body and were considered to be nonedible tissue. The remaining tissue (including skin) was considered the edible tissue. Tissue samples were transferred to tared scintillation vials and weighed. Procedures for extraction and analysis of the tissue samples are provided in Appendix. All tissue samples were extracted immediately or stored at approximately -14°C until extraction.

Tissue Lipid Content

Selected fish were collected to determine lipid content (Appendix 3). The determination of percent lipids provides the potential to express BCF values in terms of lipid content. Fish were sampled on Day 0 of uptake, on Day 62 of uptake and on Day 56 of depuration. All fish collected for lipid content were stored at approximately -14°C until analysis. In this study, no attempt was made to express bioconcentration in relation to lipid content.

Data Analysis

Whole fish concentrations were calculated based on the sum of the edible and nonedible parts. The steady-state bioconcentration factor (BCF) values were determined from the tissue concentrations at apparent steady-state divided by the average water concentration. Tissue concentrations were considered to be at apparent steady-state if three or more consecutive sets of tissue concentrations were not significantly different ($p > 0.05$). Tissue concentrations were evaluated for normality and homogeneity of variance using the Shapiro-Wilk's test and Bartlett's test, respectively. If the data did not meet the assumptions, the data was transformed in an attempt to correct the data. Mean tissue concentrations were then compared using analysis of variance and Dunnett's test.

In the original report the tissue values were considered to be at steady state and the computer program BIOFAC was used to estimate the kinetic concentration factor (BCFK), the uptake rate constant (k_1), the depuration rate constant (k_2), the estimated time to reach 90% steady state, and the half-life for clearance. Although tissue concentrations from uptake days 49, 56 and 62 were not significantly different ($p > 0.05$), the concentrations of PFOS in tissues appeared to still be increasing. Therefore, it can be argued that the fish did not reach an apparent steady state and that using the BIOFAC program may not have been the best way to estimate these parameters. Therefore, the data were reanalyzed using the equations and graphical methods outlined in the draft OPPTS 850.1730 Guidance Document (Appendix 11). The recalculated rate constants were used to calculate a BCFK (BCFK = $k1/k2$).

RESULTS AND DISCUSSION

Water Chemistry

Means and ranges of temperature, dissolved oxygen and pH of the water in the test chambers are presented in Table 1. The individual measurements are given in Appendicies 4 and 5. Water temperatures in the test chambers were within the temperature range of 22 ± 1°C established for the test with one exception. On Day 19 of depuration, the continuous temperature recorder measured 20° C. The duration of this temperature deviation was approximately 2 hours. Dissolved oxygen concentrations remained ≥6.4 mg/L (74 percent of saturation) throughout the test. Measurements of pH ranged from 7.9 to 8.2. Weekly measurements of hardness, alkalinity, conductivity and total organic carbon remained consistent throughout the test (Table 1 and Appendix 6).

Observations of Mortality and Clinical Signs

Observations of mortality and clinical signs are presented in Appendix 7. Bluegill in the negative control appeared normal and healthy throughout the test. Two bluegill died in the 0.1 mg a.i./L (nominal) treatment group; all other fish appeared normal and healthy. Bluegill in the 1.0 mg a.i./L (nominal) treatment group started to die on Day 9 of the uptake phase. By Day 35 of the uptake phase, all fish in the 1.0 mg/L treatment group had either died or been sampled for tissue analyses. Any fish found dead in the aquaria were stored frozen.

Concentrations of PFOS in Water

Concentrations of PFOS in the negative control were <LOQ (0.0500 mg a.i./L) (Tables 2 and 3). Measured concentrations of PFOS during the uptake phase in the 0.10 mg a.i./L treatment group ranged from 68 to 113% of the nominal test concentration (Appendix 3). When concentrations measured during the uptake phase were averaged, the mean measured concentration was 0.086 mg a.i./L which represented 86% of the nominal test concentration. Measured concentrations of PFOS during the uptake phase in the 1.0 mg a.i./L treatment group ranged from 73 to 101% of the nominal test concentration. When concentrations measured during the uptake phase were averaged, the mean measured concentration was 0.87 mg a.i./L, which represented 87% of the nominal test concentration. Concentrations of PFOS during the depuration phase were all <LOQ.

Concentrations of PFOS in Fish Tissues

Concentrations of PFOS in the negative control tissue samples contained no quantifiable PFOS concentrations (Appendix 3) The concentrations of PFOS in tissues of fish exposed to 0.086 mg a.i./L are presented in Table 4. Although tissue concentrations from uptake days 49, 56 and 62 were not significantly different ($p > 0.05$), the concentrations of PFOS in tissues appeared to still be increasing. The mean measured tissue concentrations from these last three uptake samples were 41.6, 96.7 and 73.6 mg a.i./Kg for edible, nonedible and whole fish, respectively. Steady-state BCF values calculated from the last three days of uptake ranged from 484 in edible tissue to 1124 in nonedible tissue (Table 5), however, these values were believed to be unreliable since tissue concentrations were still increasing with time. It was believed that better estimates of the bioconcentration factor could be obtained by determining the kinetic concentration factor (BCFK).

In the original report computer program BIOFAC was used to estimate the kinetic concentration factor (BCFK), the uptake rate constant (k_1), the depuration rate constant (k_2), the estimated time to reach 90% steady state, and the half-life for clearance. However, after the report had been finalized it was determined that using the BIOFAC program may not have been the best way to estimate these parameters. Therefore, the data were reanalyzed using the graphical methods outlined in the draft OPPTS 850.1730 Guidance Document (1). These new calculations are described in Appendix 11.

Plots of the tissue concentrations versus time were constructed to evaluate the data. The raw data were plotted as well as a curve connecting the mean values, and a curve through the predicted values from BIOFAC. This graphical approach was a simple way to evaluate how well the curves fit the data. The end of the uptake phase (Day 62) was a key date to evaluate, since data from this day has a pronounced influence on the estimates of the uptake and depuration rate constants (k_1 and k_2). The plotted results in Figures 1-3 show that the BIOFAC model predicted values for Day 62 are lower than the Day 62 means for all three tissue types (whole, edible, and non-edible fish tissue). Thus, the BIOFAC model slightly underestimated the uptake and depuration rate constants, and overestimated the kinetic concentration factor, the estimated time to reach 90% steady state, and the half-life for clearance in the original report. The recalculated BCFK values for edible, nonedible, and whole fish were 1124, 4013, and 2796, respectively (Table 6).

Recalculated estimates of the time to reach 90% of steady state in edible tissue, nonedible tissue and whole fish were 287, 383, and 371 days, respectively (Table 6). During the depuration phase of the test, estimates of the time to reach 50% clearance of PFOS were 86, 116, and 112 days for edible tissue, nonedible tissue and whole fish, respectively (Table 6).

The concentrations of PFOS in tissues of fish exposed to 0.87 mg a.i./L are presented in Table 7. PFOS concentrations in edible, nonedible and whole fish tissues did not appear to reach steady-state by Day 28 (Figure 4). By Day 35, all fish in the 0.87 mg a.i./L treatment group had been sampled or were dead. Consequently, the only information on bioconcentration that could be determined from the 0.87 mg a.i./Ltreatment group was the Day 28 BCF. Day 28 BCF values ranged from 136 in edible tissue to 386 in nonedible tissue (Table 8). Based on the length of time required to reach 90% of steady-state in the 0.086 mg/L treatment group, these values underestimate the bioconcentration potential of PFOS.

CONCLUSIONS

Perfluorooctanesulfonate, potassium salt (PFOS) bioconcentrated in the tissues of bluegill sunfish *(Lepomis macrochirus*). Bluegill exposed to 0.87 mg a.i./L were either dead or had been sampled by Day 35 of the uptake phase. Consequently, no reliable information was gained from this treatment group. Apparent steady-state concentrations were not reached in the 0.086 mg a.i./L treatment group. PFOS concentrations appeared to be increasing up to Day 62, the last day of exposure. BCFK values for edible, nonedible, and whole fish tissues were 1124, 4013, and 2796, respectively. Estimates for time to reach 50% clearance for edible, nonedible and whole fish tissues were 86, 116, and 112 days, respectively.

REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. Series 850 – Ecological Effects Test Guidelines *(draft)*, OPPTS Number 850.1730: *Fish BCF.*

2 **ASTM Standard E1022-84.** 1988. *Standard Practice for Conducting Bioconcentration Tests withFishes and Saltwater Bivalve Molluscs.* American Society for Testing and Materials.

3 **OECD Guideline for Testing of Chemicals 305.** 1996. *Bioconcentration: Flow-Through Fish Test.*

4 **APHA, AWWA, WPCF.** 1985. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, American Public Health Association. American Water Works Association. WaterPollution Control Federation, New York.

5 **BIOFAC.** 1991. September 19, 1991 version. The Dow Chemical Company, Midland, Michigan.

Table 1

Means of Ranges of Water Quality Parameters

Sponsor:	3M Corporation
Test Sustance:	PFOS
Test Organism:	Bluegill, *Lepomis machrochirus*
Dilution Water:	Well Water

Uptake Phase Nominal Concentration (mg a.i./L)	Temperature[1] (°C)	DO[2] (mg/L)	PH	Conductivity (µmhos/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	TOC (mg C/L)
Negative Control	21.9 21.8 – 22.0	7.8 6.8 – 8.6	8.1 7.9 – 8.2	324 310 – 330	180 174 – 185	127 104 – 138	<1 <1 - <1
0.10	21.8 21.7 – 22.0	7.8 6.8 – 8.6	8.1 7.9 – 8.2				
1.0	21.8 21.7 – 21.9	7.5 6.4 – 8.2	8.1 7.9 – 8.2				

[1] Temperature measured continuously in the negative control ranged from 20.0 to 22.0°C.

[2] At a temperature of 22°C, the dissolved oxygen saturation concentration is 8.7 mg/L and 60% saturation is 5.2 mg/L.

Table 2

Concentrations of PFOS in Water Samples During the Uptake Phase

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Nominal Concentration (mg a.i./L)	Day of Uptake													Mean Measured Concentration (mg a.i./L)	Percent of Nominal
	0 Hour	4 Hours	1	3	7	14	21	28	35	42	49	56	62		
Negative Control	<LOQ[1]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--
0.10	0.0717	0.0791	0.0702	0.0751	0.0826	0.0680	0.110	0.0822	0.0915	0.0983	0.103	0.0853	0.0887	0.086	86
	0.0692	0.0741	0.0734	0.0717	0.0781	0.0709	0.113	0.0843	0.0914	0.110	0.103	0.0948	0.0914		
1.0	0.797	0.891	0.867	0.813	0.845	0.900	0.988	0.913	0.838	--[2]	--	--	--	0.87	87
	0.802	0.930	0.820	0.734	0.818	0.875	1.01	0.925	0.871	--[2]	--	--	--		

[1] The Limit of Quantitation (LOQ) was 0.0500 mg a.i./L.
[2] Samples not collected due to 100% mortality.
Note: Values presented for each sampling interval are two replicate samples collected from each test chamber.

Table 3

Concentrations of PFOS in Water Samples During the Depuration Phase

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Uptake Phase Mean Measured Concentration (mg a.i./L)	Day of Depuration (mg a.i./L)			
	14	28	42	56
Negative Control	<LOQ[1]	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ	<LOQ
0.086	<LOQ	<LOQ	<LOQ	<LOQ

[1] The Limit of Quantitation (LOQ) was 0.0500 mg a.i./L.

Table 4

PFOS Concentrations in Edible, Nonedible and Whole Fish Tissues of Bluegill Exposed to 0.086 mg a.i./L

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Sample ID	Uptake Day	Edible Tissue Concentration (mg a.i./Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (mg a.i./Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (mg a.i./Kg)
E4/N4	0 (4 hours)	0.167	2.0545	0.415	2.1153	0.293
E5/N5	0 (4 hours)	0.155	1.8960	0.519	2.2103	0.351
E6/N6	0 (4 hours)	0.144	2.5219	0.417	2.7449	0.286
E7/N7	0 (4 hours)	0.182	1.3561	0.497	1.8377	0.363
E17/N17	1	0.734	1.6517	1.68	2.0826	1.26
E18/N18	1	0.726	1.9269	1.85	2.2925	1.34
E19/N19	1	0.631	1.2074	1.72	1.8419	1.29
E20/N20	1	0.806	1.0777	2.07	1.4272	1.53
E30/N30	3	1.73	1.7643	4.59	1.8836	3.21
E31/N31	3	2.07	1.3794	5.50	1.8505	4.04
E32/N32	3	2.03	1.2621	5.47	2.0994	4.18
E33/N33	3	2.11	1.1883	5.97	1.7014	4.38
E43/N43	7	3.73	2.2291	10.2	2.7943	7.33
E44/N44	7	4.25	1.6419	10.6	1.8984	7.66
E45/N45	7	4.73	1.2994	11.9	1.6429	8.73
E46/N46	7	6.25	1.2686	15.2	1.7110	11.4
E56/N56	14	11.4	2.0423	27.3	2.5002	20.2
E57/N57	14	9.07	2.1548	23.2	2.6582	16.9
E58/N58	14	13.7	1.0709	35.3	1.4046	26.0
E59/N59	14	12.6	1.1804	32.6	1.7455	24.6
E69/N60	21	11.7	2.1161	33.3	2.4712	23.3
E70/N70	21	12.0	1.2984	22.7	1.9396	18.4
E71/N71	21	12.9	1.4092	24.6	1.9910	19.8
E72/N72	21	10.6	1.6268	24.4	2.1872	18.5

[1] Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

Table 4 (Continued)

PFOS Concentrations in Edible, Nonedible and Whole Fish Tissues of Bluegill Exposed to 0.086 mg a.i./L

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Sample ID	Uptake Day	Edible Tissue Concentration (mg a.i./Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (mg a.i./Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (mg a.i./Kg)
E82/N82	28	18.3	1.4636	49.4	1.7564	35.3
E83/N83	28	13.7	1.4113	40.7	1.8903	29.2
E84/N84	28	23.9	1.2081	65.3	1.3062	45.4
E85/N85	28	23.1	0.8830	57.9	1.3512	44.1
E95/N95	35	22.6	1.7888	67.1	2.0434	46.3
E96/N96	35	27.7	1.5897	73.3	2.1281	53.8
E97/N97	35	23.8	1.1136	62.0	1.6454	46.6
E98/N98	35	20.6	1.2226	59.1	1.3627	40.9
E103/N103	42	27.6	1.2037	64.0	1.9474	50.1
E104/N104	42	25.3	1.4942	68.1	1.9266	49.4
E105/N105	42	21.2	1.4367	54.4	2.0920	40.9
E106/N106	42	27.6	1.3033	79.6	1.6100	56.3
E111/N111	49	33.3	1.7249	85.0	2.2873	62.8
E112/N112	49	36.2	1.2185	95.1	1.5993	69.6
E113/N113	49	39.0	1.5506	93.1	2.2184	70.8
E114/N114	49	30.6	1.4650	77.7	1.9337	57.4
E119/N119	56	48.3	1.6058	122	2.1688	90.6
E120/N120	56	38.9	1.0946	94.2	1.5831	71.6
E121/N121	56	44.1	1.0521	73.2	2.0532	63.3
E122/N122	56	38.3	1.8592	106	2.1739	74.8
E127/N127	62	42.4	1.2209	101	1.7596	77.0
E128/N128	62	66.2	1.3902	112	1.9170	92.7
E129/N129	62	42.2	1.0832	105	1.5965	79.6
E130/N130	62	39.2	1.6697	96.4	2.4378	73.1

[1] Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

Table 4 (Continued)

PFOS Concentrations in Edible, Nonedible and Whole Fish Tissues of Bluegill Exposed to 0.086 mg a.i./L

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Sample ID	Uptake Day	Edible Tissue Concentration (mg a.i./Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (mg a.i./Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (mg a.i./Kg)
E135/N135	14	48.5	1.3634	124	1.6928	90.3
E136/N136	14	31.8	1.4503	79.4	2.1909	60.4
E137/N137	14	31.6	1.2626	81.8	1.8708	61.6
E138/N138	14	42.0	0.9340	113	1.4560	85.3
E143/N143	28	26.0	1.7691	85.7	2.0744	58.2
E144/N144	28	33.3	1.1358	95.1	1.6772	70.1
E145/N145	28	38.7	0.8845	85.7	1.4801	68.1
E146/N146	28	55.8	0.6909	94.8	1.2803	81.1
E150/N150	42	24.1	1.7590	71.7	2.3578	51.4
E151/N151	42	31.2	1.1220	80.6	1.7635	61.4
E152/N152	42	30.0	0.8440	78.3	1.5181	61.0
E153/N153	42	33.0	0.8672	82.1	1.2687	62.2
E157/N157	56	21.1	1.5471	57.7	1.9735	41.6
E158/N158	56	37.6	0.5892	80.3	1.2353	66.5
E159/N159	56	32.9	0.6244	85.4	1.0439	65.8
E160/N160	56	31.2	0.7223	84.4	0.9975	62.1

[1] Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

Table 5

Apparent Steady-State BCF Values for Bluegill Exposed to 0.086 mg a.i./L

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Tissue Type	Mean Measured Test Concentration (mg a.i./L)	Uptake Days at Apparent Steady-State	Mean Measured Apparent Steady-State Tissue Concentration (mg a.i./Kg)	Apparent Steady-State BCF
Edible	0.086	49, 56 and 62	41.6	484
Nonedible	0.086	49, 56 and 62	96.7	1124
Whole Fish	0.086	49, 56 and 62	73.6	856

Table 6

BCFK Estimates for Bluegill Exposed to 0.086 mg a.i./L

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Tissue Type	Kinetic Bioconcentration Factor (BCFK)	Uptake Rate Constant (k_1)	Depuration Rate Constant (k_2)	Estimate Time to Reach 90% of Steady State (Days)	Estimated Time to Reach 50% Clearance (Days)
Edible	1124	9.022	0.0080	287	86
Nonedible	4013	24.08	0.0060	383	116
Whole Fish	2796	17.35	0.0062	371	111

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Table 7

PFOS Concentrations in Edible, Nonedible and Whole Fish Tissues of Bluegill Exposed to 0.87 mg a.i./L

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Sample ID	Uptake Day	Edible Tissue Concentration (mg a.i./Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (mg a.i./Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (mg a.i./Kg)
E9/N9	0 (4 hours)	1.46	0.9795	3.52	1.5183	2.71
E10/N10	0 (4 hours)	1.48	1.3226	4.37	1.6397	3.08
E11/N11	0 (4 hours)	1.19	1.6284	4.22	1.9391	2.84
E12/N12	0 (4 hours)	1.39	1.5205	4.06	1.9489	2.89
E22/N22	1	4.68	1.7060	11.1	1.8323	8.00
E23/N23	1	6.59	1.3724	14.2	1.7515	10.9
E24/N24	1	5.56	1.1272	13.3	1.7081	10.2
E25/N25	1	5.64	1.0507	12.1	1.5273	9.47
E35/N35	3	17.3	0.9889	39.3	1.4781	30.5
E36/N36	3	15.8	1.2666	42.0	1.6727	30.7
E37/N37	3	19.0	0.8348	43.8	1.4005	34.5
E38/N38	3	20.8	1.1215	51.8	1.6070	39.1
E48/N48	7	42.0	1.7575	100	2.3064	74.9
E49/N49	7	44.0	1.5527	102	2.0505	77.0
E50/N50	7	57.7	1.3391	102	2.2079	85.3
E51/N51	7	46.8	0.9532	120	1.3562	89.8
E61/N61	14	87.1	1.9813	177	2.9193	141
E62/N62	14	81.6	1.2152	207	1.8260	157
E63/N63	14	90.7	1.1290	245	1.5376	180
E64/N64	14	73.3	1.0811	214	1.6199	158

[1] Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

Table 7 (Continued)

PFOS Concentrations in Edible, Nonedible and Whole Fish Tissues of Bluegill Exposed to 0.87 mg a.i./L

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Sample ID	Uptake Day	Edible Tissue Concentration (mg a.i./Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (mg a.i./Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (mg a.i./Kg)
E74/N74	21	79.4	1.8300	201	2.1803	146
E75/N75	21	117	1.4526	278	1.9786	210
E76/N76	21	104	1.6824	246	2.2130	185
E77/N77	21	102	1.4076	229	1.7558	172
E87/N87	28	102	1.6679	289	2.0549	205
E88/N88	28	131	1.0923	372	1.4045	267
E89/N89	28	107	1.1675	320	1.6595	232
E90/N90	28	133	1.1466	361	1.5186	263

[1] Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

Note: Sampling ended after Day 28. By Day 35 all the fish in this treatment group had died or were previously sampled for tissue analysis.

Table 8

Day 28 BCF Values for Bluegill Exposed to 0.87 mg a.i./L

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Tissue Type	Mean Measured Test Concentration (mg a.i./L)	Mean Day 28 Tissue Concentration (mg a.i./Kg)	Day 28 BCF
Edible	0.87	118	136
Non Edible	0.87	336	386
Whole Fish	0.87	242	278

Note: BCF Values presented here are biased low due to fish mortality.

Figure 1. Concentrations of PFOS in Edible Fish Tissues of Bluegill Exposed to 0.086 mg a.i./L.

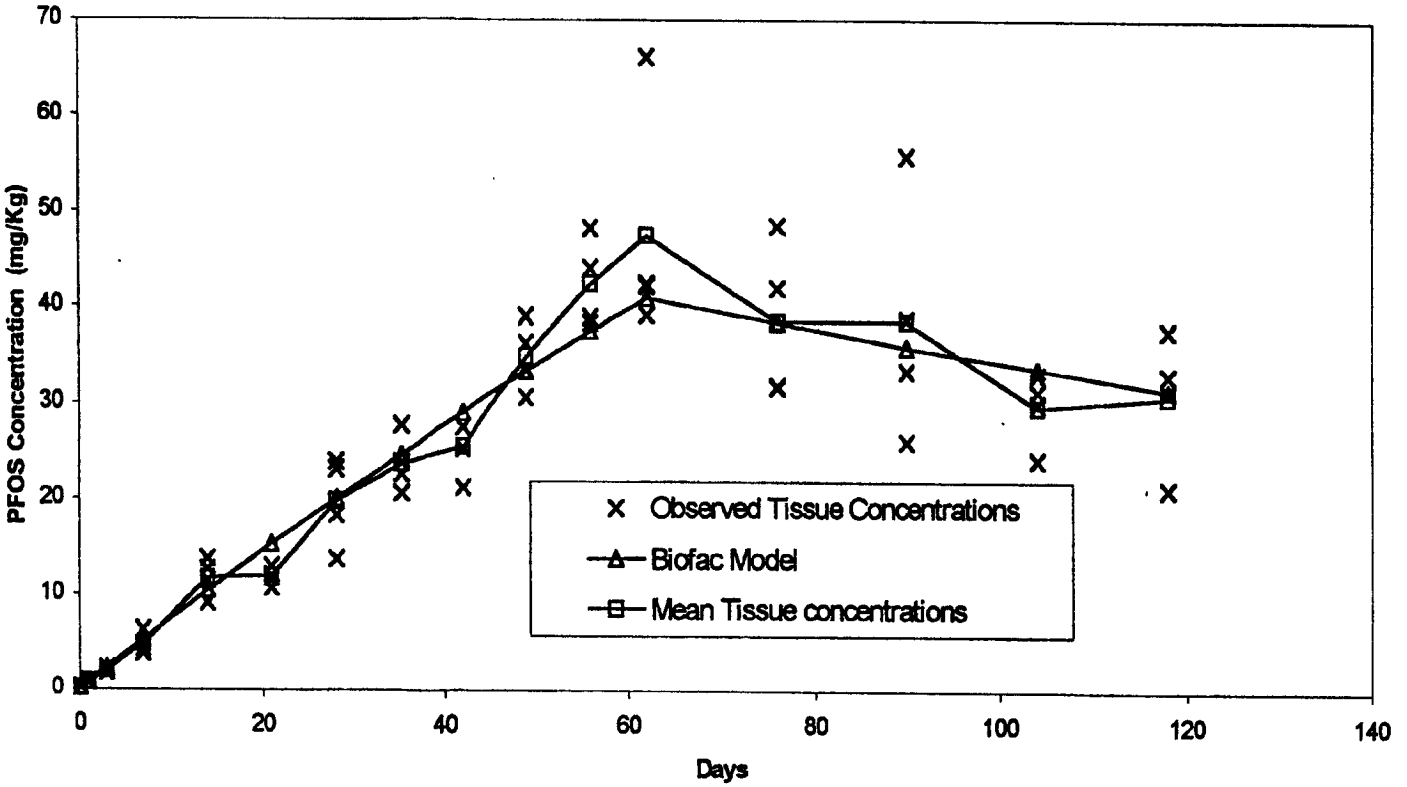

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Figure 2. Concentrations of PFOS in Nonedible Fish Tissues of Bluegill Exposed to 0.086 mg a.i./L.

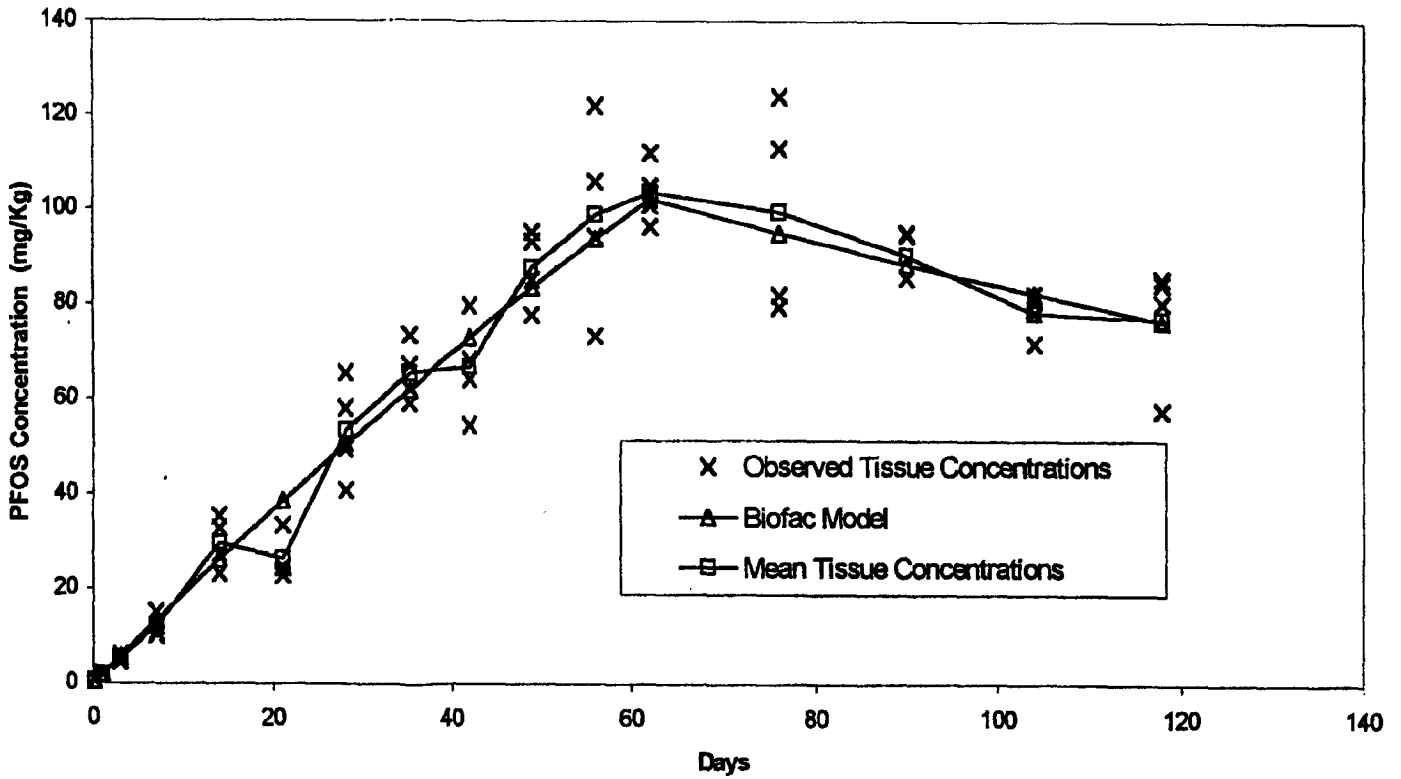

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Figure 3. Concentrations of PFOS in Whole Fish Tissues of Bluegill Exposed to 0.086 mg a.i./L.

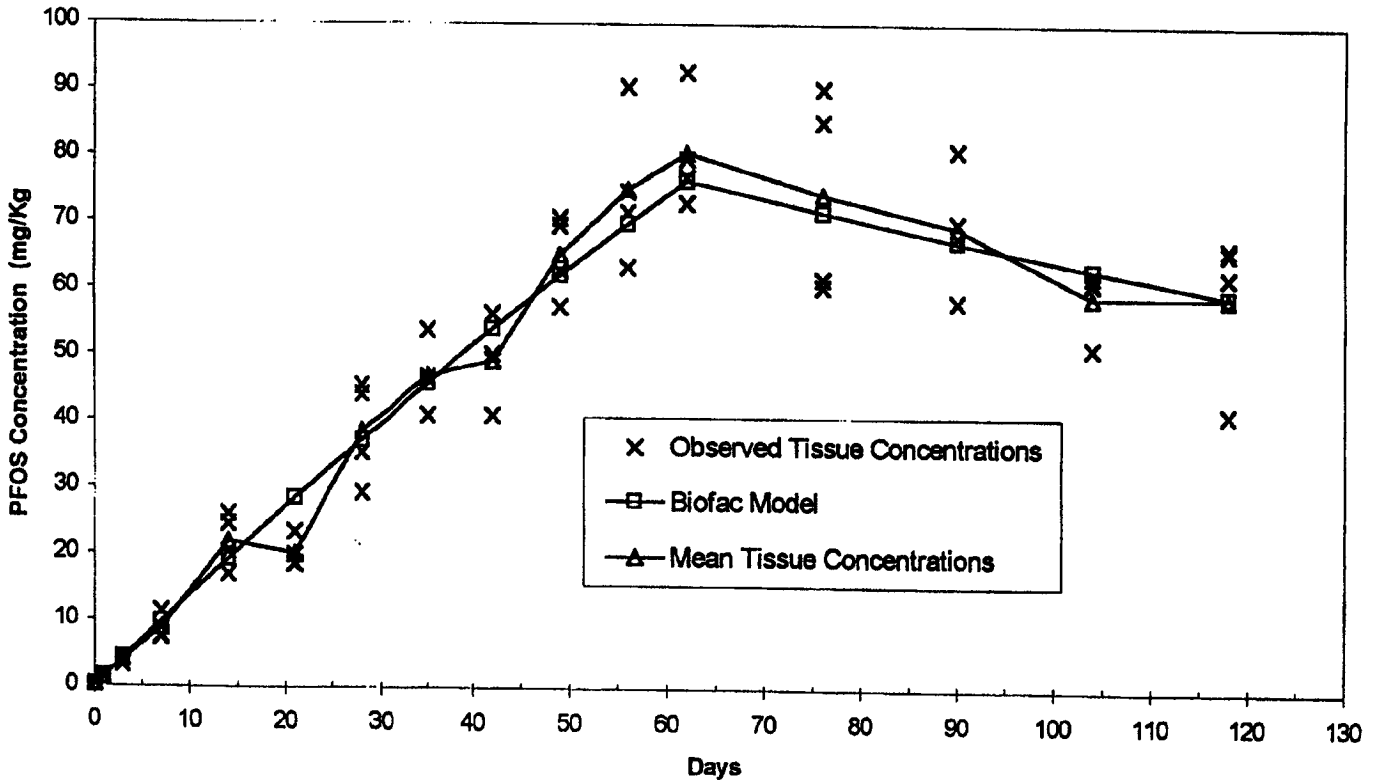

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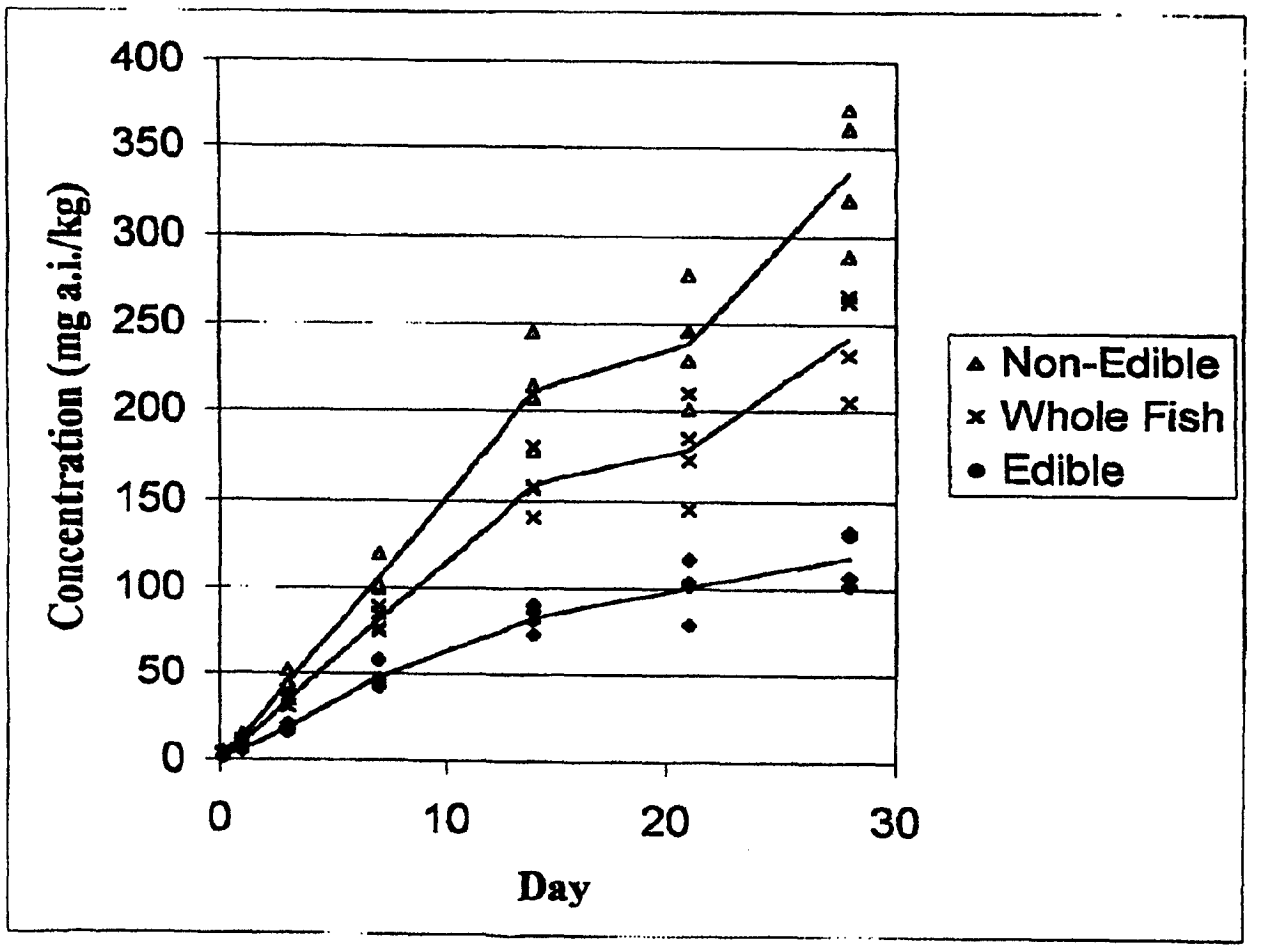
Figure 4. Concentrations of PFOS in Edible, Noneible and Whole Fish Tissues of Bluegill Exposed to 0.87 mg a.i./L.



Appendix 1

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

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

	Mean	Range
Specific Conductance (µmhos/cm)	313 (N = 4)	310 – 315
Hardness (mg/L as $CaCO_3$)	130 (N = 4)	128- 132
Alkalinity (mg/L as $CaCO_3$)	178 (N = 4)	176 – 178
pH	8.1 (N = 4)	8.0 – 8.2

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

Pesticides and Organics

Component	Measured Concentration	Component	Measured Concentration
Aclonifen	<0.03 µg/L	Dichlorvos	<0.01 µg/L
Alachlor	<0.01 µg/L	Dicofol	<0.25 µg/L
Ametryn	<0.01 µg/L	Diethyltoluamide	<0.02 µg/L
Atrazine	<0.01 µg/L	Difenoconazole	<0.03 µg/L
Azinphos-ethyl	<0.04 µg/L	Dimethoate	<0.02 µg/L
Azinphos-methyl	<0.08 µg/L	Dimethomorph	<0.05 µg/L
Azoxystrobin	<0.25 µg/L	Disulfoton	<0.02 µg/L
Bifenthrin	<0.05 µg/L	DMST	<0.05 µg/L
Bioallethrin	<0.05 µg/L	Dodemorph	<0.01 µg/L
Bitertanol	<0.05 µg/L	Endosulfan-α	<0.01 µg/L
Bromacil	<0.05 µg/L	Endosulfan-β	<0.01 µg/L
Bromophos	<0.02 µg/L	Endosulfan-sulfate	<0.02 µg/L
Bromophos-ethyl	<0.02 µg/L	Epoxiconazole	<0.05 µg/L
Bromopropylate	<0.02 µg/L	Eptam	<0.02 µg/L
Bupirimate	<0.05 µg/L	Esfenvalerate	<0.02 µg/L
Carbaryl	<0.05 µg/L	Ethion	<0.05 µg/L
Carbofuran	<0.03 µg/L	Ethofumesate	<0.02 µg/L
Carboxin	<0.02 µg/L	Ethoprophos	<0.01 µg/L
Chlorfenvinphos	<0.02 µg/L	Etridiazole	<0.02 µg/L
Chloridazon	<0.05 µg/L	Etrimfos	<0.05 µg/L
Chlorpropham	<0.02 µg/L	Fenarimol	<0.05 µg/L
Chlorpyrifos	<0.01 µg/L	Fenchlorphos	<0.01 µg/L
Chlorpyrifos-methyl	<0.01 µg/L	Fenitrothion	<0.03 µg/L
Chlorothalonil	<0.04 µg/L	Fenoxycarb	<0.03 µg/L
Coumaphos	<0.02 µg/L	Fenpiclonil	<0.05 µg/L
Cyanazine	<0.05 µg/L	Fenpropathrin	<0.25 µg/L
Cyfluthrin	<0.05 µg/L	Fenpropimorph	<0.01 µg/L
Cypermethrin	<0.25 µg/L	Fenthion	<0.01 µg/L
Cyproconazole	<0.05 µg/L	Fenvalerate	<0.02 µg/L
Deltamethrin	<0.02 µg/L	Fluazifop-butyl	<0.02 µg/L
Demeton	<0.02 µg/L	Fluoroglycofen-ethyl	<0.02 µg/L
Demeton-O	<0.02 µg/L	Fluroxypyr-meptyl	<0.05 µg/L
Desethylatrazine	<0.01 µg/L	Flutolanil	<0.02 µg/L
Desisopropylatrazine	<0.02 µg/L	Fonophos	<0.01 µg/L
Desmetryn	<0.01 µg/L	Furalaxyl	<0.02 µg/L
Diazinon	<0.01 µg/L	Heptenophos	<0.02 µg/L
Dichlobenil	<0.01 µg/L	Imazalil	<0.01 µg/L
Dichloran	<0.03 µg/L	Iprodion	<0.05 µg/L
Dichlorbenzamide	<0.02 µg/L	Kresoxim-methyl	<0.02 µg/L
Dichlorfenthion	<0.01 µg/L	Lenacil	<0.05 µg/L
Dichlorfluanid	<0.03 µg/L	Lindane	<0.02 µg/L

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on November 15, 2000.

Continued

Appendix 2 (Continued)
Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Well Water[1]

Pesticides And Organics (Page 2)

Component	Measured Concentration	Component	Measured Concentration
Malathion	<0.02 µg/L	Methoxychlor	<0.01 µg/L
Metalaxyl	<0.05 µg/L	Metolachlor	<0.01 µg/L
Metamitron	<0.05 µg/L	Metribuzin	<0.02 µg/L
Metazachlor	<0.02 µg/L	Mevinphos	<0.01 µg/L
Methidathion	<0.02 µg/L	Nitrothal-Isopropyl	<0.05 µg/L
Paclobutazole	<0.05 µg/L	Pyrifenox-1	<0.01 µg/L
Parathion	<0.01 µg/L	Pyrifenox-2	<0.01 µg/L
Parathion-methyl	<0.01 µg/L	Pyrimethanil	<0.01 µg/L
Penconazole	<0.05 µg/L	Quizalofop-ethyl	<0.02 µg/L
Pendimethalin	<0.03 µg/L	Simazine	<0.01 µg/L
Permethrin-cis	<0.01 µg/L	Sulfotep	<0.02 µg/L
Permethrin-trans	<0.01 µg/L	Tebuconazole	<0.05 µg/L
Phosalone	<0.05 µg/L	Tebufenpyrad	<0.05 µg/L
Phosmet	<0.02 µg/L	Terbutryn	<0.01 µg/L
Phosphamidon-cis	<0.05 µg/L	Terbuthylazine	<0.01 µg/L
Pirimicarb	<0.01 µg/L	Tetrachlorvinphos	<0.01 µg/L
Pirimiphos-ethyl	<0.01 µg/L	Tetrahydroftalimide	<0.05 µg/L
Pirimiphos-methyl	<0.01 µg/L	Tetramethrin	<0.01 µg/L
Prochloraz	<0.02 µg/L	Thiabendazole	<0.05 µg/L
Procymidon	<0.01 µg/L	Thiometon	<0.04 µg/L
Prometryn	<0.01 µg/L	Tolclofos-methyl	<0.01 µg/L
Propachlor	<0.01 µg/L	Tolylfluanid	<0.04 µg/L
Propazine	<0.01 µg/L	Triadimefon	<0.05 µg/L
Propham	<0.02 µg/L	Triadimenol	<0.05 µg/L
Propiconazole	<0.05 µg/L	Triallate	<0.02 µg/L
Propoxur	<0.03 µg/L	Triazophos	<0.02 µg/L
Propyzamide	<0.02 µg/L	Trifluralin	<0.02 µg/L
Prosulfocarb	<0.02 µg/L	Vamidothion	<0.01 µg/L
Pyrazophos	<0.03 µg/L	Vinclozolin	<0.01 µg/L

Metals

Magnesium	13.2 mg/L	Nickel	<1.4 µg/L
Sodium	21 mg/L	Copper	<1.0 µg/L
Calcium	35 mg/L	Zinc	<2.3 µg/L
Iron	<0.02 mg/L	Molybdenum	<0.7 µg/L
Potassium	6.2 mg/L	Silver	<0.3 µg/L
Aluminum	<0.09 mg/L	Cadmium	<0.3 µg/L
Manganese	0.72 µg/L	Arsenic	<0.25 µg/L
Beryllium	<0.3 µg/L	Mercury	<0.025 µg/L
Chromium	<0.6 µg/L	Selenium	<1 µg/L
Cobalt	<0.4 µg/L		

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on November 15, 2000.

Appendix 3

THE ANALYSIS OF PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS) CONCENTRATIONS IN FRESHWATER AND BLUEGILL SUNFISH TISSUE

IN SUPPORT OF

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454A-134

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: Perfluorooctanesulfonate, Potassium Salt (PFOS): A Flow-Through Bioconcentration Test with the Bluegill (*Lepomis macrochirus*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-134

PRINCIPAL INVESTIGATOR:

Raymond L. VanHoven, Ph.D.
Scientist

6/6/02
Date

WILDLIFE INTERNATIONAL, LTD. MANAGEMENT

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

6/6/02
Date

AMENDED

Introduction

Freshwater samples and tissue samples were collected from a flow-through aquatic test to determine the bioconcentration potential of perfluorooctanesulfonate, potassium salt (PFOS) in the bluegill *(Lepomis macrochirus)*. The study was conducted by Wildlife International, Ltd. and identified as Project Number 454A-134. The analyses of freshwater and tissue samples were performed at Wildlife International, Ltd. by high performance liquid chromatography (HPLC) with mass spectrometric detection. Water samples were diluted and analyzed by HPLC with single quadrupole mass spectrometric detection (LC/MS). Tissue samples were homogenized, extracted, diluted, and analyzed by HPLC with triple quadrupole mass spectrometric detection (LC/MS/MS). Freshwater samples were collected and analyzed from December 1, 2000 to April 2, 2001. Tissue samples were collected and analyzed from December 5, 2000 to April 2, 2001. Additional tissue samples were collected and analyzed gravimetrically for lipid content. Samples for lipid content were collected on December 5, 2000, February 5, 2001 and April 2, 2001 and analyzed between April 4 and April 11, 2001.

Test Substance and Internal Standard

The test substance, PFOS, was used to prepare calibration standards and matrix fortification samples and was identified as Wildlife International, Ltd. identification number 4675.

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 internal standard, referred to hereafter as 4HPFOS, was stored under ambient conditions.

Analytical Method

Water and tissue samples were analyzed for PFOS using high performance liquid chromatography (HPLC) with mass spectrometric detection. Water samples were analyzed according to the method entitled "Analytical Method Validation for the Determination of Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS) in Freshwater, Saltwater and Algal Media" (Wildlife International, Ltd. Project No. 454C-109). Tissue samples were analyzed according to the method entitled "Analytical Method Validation for the Determination of Perfluorooctane Sulfonic Acid,

Potassium Salt (PFOS) in Fish Tissues" (Wildlife International, Ltd. Project No. 454C-119). For water analyses, one minor modification from the validation was incorporated into the present study. This change was use of 10 ppb in place of 100 ppb 4HPFOS internal standard concentration in all calibration standards and study sample dilutions. The lower internal standard nominal concentration was closer to the range of PFOS calibration used in the present work. The analytical methodology implemented for the determination of lipid content in fish tissue is presented on page 42.

Freshwater Samples

A method flow chart for the analysis of PFOS in freshwater is presented in Figure 1. Samples were diluted in a 50% methanol (HPLC grade, 99.9+%): 50% NANOpure®. water solution containing 10.0 µg 4H PFOS/L and 0.05% formic acid (v/v) so that they fell within the calibration range of the LCMS methodology. Aliquots of the dilutions were transferred to autosampler vials and submitted for analysis by direct injection. Concentrations of PFOS in freshwater samples were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) interfaced with a Perkin-Elmer API 3000LC mass spectrometer operated in selective ion monitoring (SIM) detection mode. The mass spectrometer was equipped with a Perkin-Elmer TurboIonSpray ion source. Chromatographic separations were achieved using a Keystone Betasil C_{18} analytical column (50 mm x 2 mm I.D., 3 µm particle size) fitted with a Keystone Javelin C_{18} Guard Cartridge (20 mm x 2 mm). The instrument parameters are summarized in Table 1.

Freshwater quality control (QC) samples (matrix blanks and fortifications) were processed in the same manner as the test samples. Freshwater was fortified with the appropriate PFOS in methanol stock solution using a gas-tight syringe. Matrix blank samples were not fortified with the test substance.

Tissue QC Samples

Bluegill sunfish *(Lepomis macrochirus)* tissues were obtained from a breeding stock maintained at Wildlife International, Ltd. Approximately 200 individual fish (ages 3 to 5 months) were removed from a breeding tank and euthanized by severing the spinal cord above the opercular region. Heads, fins and viscera were removed from the body and were considered to be nonedible tissue. The balance of the tissue was considered to be edible tissue. The edible and nonedible tissues were separately

homogenized with a Waring stainless steel blender. An appropriate number of 1-gram aliquots of edible and nonedible homogenate were weighed into separate, 20-mL glass scintillation vials. Each vial was uniquely identified and labeled with a facility log identification number. The tissue QC samples were stored frozen until they were used in the preparation of matrix blank and matrix fortification samples, or storage stability samples.

Tissue Samples

A method flow chart for the analysis of PFOS in fish tissues is presented in Figure 2. Edible and nonedible fish tissue samples were collected from the test in 20-mL glass scintillation vials and stored frozen, if necessary, until analysis. Upon analysis, tissue samples were batched by sampling interval and tissue type. At that time, three of the appropriate tissue QC samples also were removed from the freezer. Any frozen samples were allowed to thaw. One tissue QC sample was designated as the matrix blank sample. The other tissue QC samples were designated as matrix fortification samples and were fortified with the appropriate PFOS stock solution(s) in methanol using gas-tight syringes.

Test and QC tissue homogenates were extracted as follows. Ten milliliters of methanol were added to each vial. The samples were homogenized with a tissue shredder for approximately one minute. The samples were then sonicated with a sonic dismembrator for approximately five minutes. The samples were capped, shaken, and centrifuged at approximately 2000 rpm for approximately 5 minutes. Aliquots of the extract were then volumetrically diluted into the calibration range of the LCMS methodology with 50:50 methanol: NANOpure®. water dilution solvent. Aliquots of the diluted extracts were transferred to autosampler vials and submitted for analysis.

Back-up tissue samples were collected and stored frozen. If necessary, the back-up tissue samples were removed from the freezer, allowed to thaw and processed using the same procedures described above.

Tissue Storage Stability Samples

Stability samples were prepared at test initiation to establish test substance stability in fish tissues stored frozen during the study. Two tissue QC samples of each fish tissue type (edible and nonedible) were removed from the freezer and allowed to thaw. The edible and nonedible fish tissues

were each fortified at 0.100 and 10.0 mg a.i./Kg using the appropriate PFOS stock solution and a gas-tight syringe. The stability samples were returned to the freezer. Following 119 days of frozen storage, the stability samples were removed from the freezer and analyzed. Fresh fortification and matrix blank samples also were prepared and analyzed at this time. The samples (new and old) were processed using the same procedures described for tissue sample analyses.

Concentrations of PFOS in fish tissue were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 HPLC interfaced with a Perkin-Elmer API 3000LC Mass Spectrometer operated in multiple ion reaction monitoring (MRM) detection mode. The mass spectrometer was equipped with a Perkin-Elmer TurboIonSpray ion source. Chromatographic separations were achieved using a Keystone Betasil C18 analytical column (50 mm x 2 mm I.D., 3 µm particle size) fitted with a Keystone Javelin C_{18} Guard Cartridge (20 mm x 2 mm). The instrument parameters are summarized in Table 2.

Tissue Samples for Lipid Content

A method flow-chart for the analysis of lipid content in fish tissue is presented in Figure 3. Edible and nonedible fish tissue samples to be analyzed for lipid content were collected from the test at initiation, on Day 62 of the uptake phase (Day 0 depuration), and at termination (Day 56 depuration). Only fish from the Negative Control and 0.10 mg a.i./L nominal test level were sampled for lipid content. Samples were collected in 20-mL glass scintillation vials and stored frozen until analysis. Sample weights were recorded at the time of collection. Upon analysis, tissue samples designated for lipid content determination were removed from the freezer and allowed to thaw. For each sample, 10 mL of NANOpure® water was added to the fish tissue in the vial and the sample was homogenized for approximately one minute using a tissue shredder. Each homogenate was transferred to a 250-mL separatory funnel that contained 25 mL of chloroform and 50 mL of methanol. Each sample vial was rinsed with an additional 10 mL of NANOpure® water and the rinse was poured into the respective separatory funnel. The separatory funnels were shaken with venting for approximately one minute. Fifty milliliters of chloroform followed by 50 mL of saturated sodium chloride were added to each separatory funnel. The separatory funnels were briefly swirled with venting. The phases were allowed to separate. For each sample, the chloroform layer was drained through a powder funnel packed with

glass wool and anhydrous sodium sulfate into a 250-mL round- bottom flask. An additional 50-mL aliquot of chloroform was added to each separatory funnel and the extraction and draining procedures were repeated. The extracts were rotary evaporated in a waterbath maintained at approximately 40° C to near dryness. Each extract was transferred to a pre-weighed labeled scintillation vial. Each 250-mL round-bottom flask was rinsed with a small volume of chloroform and the rinse was transferred to the respective scintillation vial. The remaining solvent in each vial was evaporated under a gentle stream of nitrogen or clean dry air. The vials were reweighed and the weights were recorded. Lipid content was calculated for each sample as the ratio of lipid to fish tissue weights (mg/Kg).

Calibration Standards

For water analyses, calibration standards of PFOS prepared in a 50% methanol : 50% NANOpure® water solution containing 10.0 µg 4H PFOS/L and 0.05% formic acid (v/v), ranging in concentration from 0.500 to 5.00 µ a.i./L, were analyzed with the each sample set. PFOS was fortified into the standards by appropriate dilutions of a 1.00 mg a.i./L stock solution of PFOS in methanol. Five calibration standards (different concentrations) were analyzed with each sample set. The calibration standard series was injected at the beginning and end of each run, and one standard was injected, at a minimum, after every five samples. Linear regression equations were generated using peak area response ratios (PFOS : internal standard) versus the respective concentration ratios (PFOS: internal standard) of the calibration standards. A typical calibration curve from water analyses is presented in Figure 4. The concentration of PFOS in the freshwater 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 used for freshwater analyses are presented in Figures 5 and 6, respectively.

For tissue analyses, calibration standards were prepared in 50:50 methanol: NANOpure® water by appropriate dilutions of either a 1.00 mg a.i./L or a 10.0 mg a.i./L stock solution of PFOS in methanol. Two separate PFOS calibration standard sets were employed in the study. For Days 0 through 7 of the uptake phase, and for stability sample analyses, PFOS calibration standards ranging in concentration from 0.500 to 5.00 µg a.i./L, were analyzed with each sample set. For all subsequent sampling intervals, a higher (5.00 to 50.0 PFOS µg a.i./L) calibration range was required to minimize dilution of the sample extracts. For each sample set, five calibration standards (different

concentrations) were analyzed. The appropriate calibration standard series was injected at the beginning and end of each run, and one standard was injected, at a minimum, after every five samples. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. A typical calibration curve from tissue analyses is presented in Figure 7. Concentration of PFOS in fish tissue samples was determined by substituting the peak area response into the applicable linear regression equation. Representative ion chromatograms of low and high calibration standards used for tissue analyses are presented in Figures 8 and 9, respectively.

For both water and tissue analyses for PFOS, 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.

Fortification Stocks

Freshwater and fish tissue homogenates were fortified with the appropriate stock solution of PFOS prepared in methanol. Each stock solution was assigned a unique identification code that was recorded on a stock preparation log sheet.

Limits of Quantitation

The method limit of quantitation (LOQ) for PFOS in freshwater samples was 0.0500 mg a.i./L, calculated as the product of the lowest PFOS calibration standard (0.0005 mg a.i./L) and the dilution factor of the matrix blank sample (100) analyzed concurrently with the test samples.

The LOQ was calculated on an individual basis for each tissue sample since each entire submitted sample (of differing weight) was extracted without an adjustment to a constant weight. The LOQ (mg a.i./Kg) for a given tissue analysis was calculated as the product of the lowest PFOS calibration standard (0.0005 or 0.005 mg a.i./L) and the overall dilution factor (L/Kg) of the tissue sample. To illustrate for a 1.207 gram sample analyzed with the low-level calibration standard set, extraction with 10 mLs of methanol followed by a 10x volumetric dilution (overall dilution factor = 82.85 L/Kg), gives an LOQ = 0.0414 mg a.i./Kg.

Freshwater Matrix Blank and Fortification Samples

Along with the actual freshwater sample analyses, 20 freshwater matrix blank samples were analyzed at periods throughout the study to determine possible interferences (Table 3). No matrix interferences were observed at or above the limit of quantitation (0.0500 mg a.i./L). A representative ion chromatogram of a freshwater matrix blank sample is presented in Figure 10.

Freshwater samples were fortified at two levels (0.0750 and 0.500 mg a.i./L) or three levels (0.0750, 0.500 and 2.00 mg a.i./L) at each sampling interval using appropriate stock solutions of PFOS prepared in methanol. Freshwater matrix fortifications were analyzed concurrently with each sample set and the analytical results were not corrected for mean procedural recovery. Recoveries ranged from 84.9 to 120% of nominal concentration (Table 3). The mean and standard deviation of fortification recoveries at the 0.0750, 0.500 and 2.00 mg a.i./L fortification levels were 94.7% ± 5.12% (n = 20), 98.7% ± 6.60% (n = 20), and 96.9% ± 2.95% (n = 12), respectively. A representative ion chromatogram of a freshwater matrix fortification is presented in Figure 11.

Tissue Matrix Blank and Fortification Samples

Along with the actual tissue sample analyses, 16 edible and 16 nonedible fish tissue matrix blank samples were analyzed to determine possible interferences (Tables 4 and 5, respectively). No interferences were observed at or above the applicable limits of quantitation during the test. Representative ion chromatograms of edible and nonedible fish tissue matrix blank samples Are presented in Figures 12 and 13, respectively.

Edible and nonedible fish tissue homogenates were fortified with the appropriate stock solution(s) of PFOS prepared in methanol. Tissue matrix fortifications were prepared and analyzed concurrently with each sample set and the analytical results were not corrected for mean procedural recovery. The levels of fortification were chosen to bracket the expected concentration of test samples. Because measured concentrations increased with time for the duration of the uptake phase, the fortification levels were frequently revised. Low-level fortification levels ranged from 0.100 to 5.00 mg a.i./L and high-level fortification levels ranged from 10.0 to 500 mg a.i./L. Edible fish tissue recoveries ranged from 94.8 to 111% of nominal concentration for the low-level fortifications, and

from 92.0 to 107% of nominal concentration for the high-level fortifications (Table 4). The mean and standard deviation of fortification recoveries at the low- and high-level fortifications (n = 16) for the edible fish tissue were 102% ± 4.61% and 100% ± 4.40%, respectively. Nonedible fish tissue recoveries ranged from 93.9 to 116% and from 90.1 to 122% of nominal concentration for low- and high-level fortifications, respectively (Table 5). The mean and standard deviation of fortification recoveries at the low- and high-level fortifications (n = 16) for the nonedible fish tissue were 106% ± 5.55% and 103% ± 8.49%, respectively. Representative ion chromatograms of edible and nonedible fish tissue matrix fortification samples are presented in Figures 14 and 15, respectively.

Example Calculations

The analytical result and percent recovery for freshwater sample 454A-134-68, from the 0.10 mg a.i./L nominal PFOS treatment group, was calculated using the following equations:

Peak Area Ratio = Analyte Peak Area/Internal Standard Peak Area
PFOS Area = 593240
Internal Standard Area = 1295042
Peak area ratio = 0.4581
Internal Standard Concentration: 10.0 µg a.i./L
Y-intercept = -0.0187
Slope = 5.2182
Initial volume (Vi) = 0.100 mL
Final volume (Vf) = 10.0 mL
Dilution factor (Vf/Vi) = 100

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

$$= \frac{0.4581 - (-0.0187)}{5.2182} \times 10.0$$

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

$$= 0.0009137 \times 100$$
$$= 0.09137$$

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

$$= \frac{0.09137 \text{ mg a.i./L}}{0.10 \text{ mg a.i./L}} \times 100$$

$$= 91.4\%$$

The analytical result for edible fish tissue sample 454A-134-E-43, from the 0.10 mg a.i./L nominal PFOS treatment group, was calculated using the following equations:

Peak Area = 11192
Y-intercept = 713.81
Slope = 6287.33
Primary Dilution:
Initial Weight (Wi) = 2.2291 g
Extraction Volume (Ve) = 10.0 mL
Secondary Dilution:
Initial Volume (Vi) = 0.0500 mL
Final Volume (Vf) = 25.0 mL
Overall Dilution Factor (Ve/Wi x Vf/Vi) = 2240 mL/g = 2240 L/Kg

$$\text{PFOS at instrument } (\mu g \text{ a.i./L}) = \frac{(\text{peak area - y - intercept})}{\text{slope}}$$

$$= \frac{(11192 - 713.81)}{6287.33}$$

$$= 1667 \ \mu g \text{ a.i./L}$$

PFOS in sample (mg a.i./Kg) = PFOS at instrument (µg a.i./L) x overall dilution factor x unit conversion factor

$$\text{PFOS in sample (mg a.i./Kg)} = \frac{1.667 \mu g \text{ a.i.}}{L} \times \frac{2440 L}{Kg} \times \frac{1 mg}{1000 \mu g}$$

$$= 3.73 \text{ mg a.i./Kg}$$

RESULTS

Freshwater Sample Analysis

Freshwater samples were collected and analyzed for PFOS concentrations on Days -4 and -1 (twice) during the pre-uptake phase of the test and Days 0 (0 and 4 hours), 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, and 62 during the uptake phase of the test. Uptake was suspended on Day 62 of the test and depuration began. During the depuration phase of the test, freshwater samples were collected and analyzed for PFOS concentrations on Days 14, 28, 42, and 56. Measured concentrations of PFOS in the pre-test diluter verification (pre-uptake phase) samples were <LOQ in the negative controls. Measured concentrations in pre-test samples ranged from 64.6 to 95.4% of the nominal concentration

in the 0.10 mg a.i./L treatment group and from 71.8 to 111% of the nominal concentration in the 1.0 mg a.i./L treatment group (Table 6). During the uptake phase of the test, measured concentrations of PFOS in the negative control freshwater samples were <LOQ. Freshwater samples collected from the 0.10 and 1.0 mg a.i./L treatment groups ranged from 68.0 to 113% and from 73.4 to 101% of the nominal concentration, respectively (Table 7). During the depuration phase of the test, measured concentrations of PFOS in all freshwater samples were <LOQ. A representative ion chromatogram of a freshwater sample is presented in Figure 16.

Tissue Sample Analysis

Tissue samples were collected on uptake Days 0 (4 hours), 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, and 62 and depuration Days 14, 28, 42, and 56 of the test. Measured concentrations of PFOS in all negative control edible and nonedible fish tissue samples were <LOQ (Tables 8 and 9). The results of analyses of edible and nonedible fish tissue samples collected from the 0.100 and 1.00 mg a.i./L treatment groups are presented in Tables 8 and 9, respectively. Representative ion chromatograms of edible and nonedible tissue samples from the same study fish are presented in Figures 17 and 18, respectively.

Stability Sample Analysis

Stability samples were prepared at test initiation (uptake Day 0) and stored frozen. The results of stability sample analyses are presented in Table 10. These data indicate that the test substance was stable during relatively long term (119 days) frozen storage at the 0.100 and 10.0 mg a.i./Kg concentration levels. Definitive tissue sample storage did not exceed 7 days during the conduct of the study.

Tissue Sample Analysis for Lipid Content

Tissue samples were collected on uptake Days 0 (0 hour) and 62 and depuration Day 56 to determine lipid content in fish tissues on a wet-weight basis. The results of lipid analyses in edible and nonedible fish tissues are presented in Tables 11 and 12, respectively.

Table 1

Typical HPLC/MS Operational Parameters for Analysis of Aqueous Samples

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with a Perkin-Elmer API 3000 Mass Spectrometer equipped with a Perkin-Elmer TurbolonSpray ion source. Operated in selective ion monitoring mode (SIM).
ANALYTICAL COLUMN:	Keystone Betasil C_{18} column (50 mm x 2 mm I.D., 2-µ3m particle size)
GUARD COLUMN:	Keystone Javelin C_{18} column (20 mm x 2 mm I.D.)
OVEN TEMPERATURE:	40° C
STOP TIME:	5.00 minutes
FLOW RATE:	250 µL/minute
MOBILE PHASE:	70.0% Methanol: 30.0% NANOpure® Water containing 0.1% Formic Acid
INJECTION VOLUME:	10 µL
PFOS RETENTION TIME:	Approximately 3.7 minutes
INTERNAL STANDARD RETENTION TME:	Approximately 2.5 minutes
PFOS MONITORED MASS:	499 amux
INTERNAL STANDARD MONITORED MASS:	427 amu

Table 2

Typical HPLC/MS/MS Operational Parameters for Analysis of Tissue Samples

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromato-graph (HPLC) with a Perkin-Elmer API 3000 Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in multiple ion reaction monitoring (MRM) mode.
ANALYTICAL COLUMN:	Keystone Betasil C_{18} column (50 mm x 2 mm I.D., 2-µ3m particle size)
GUARD COLUMN:	Keystone Javelin C_{18} column (20 mm x 2 mm I.D.)
OVEN TEMPERATURE:	40° C
STOP TIME:	5.00 minutes
FLOW RATE:	250 µL/minute
MOBILE PHASE:	70.0% Methanol: 30.0% NANOpure® Water containing 0.1% Formic Acid
INJECTION VOLUME:	10.0 µL
PFOS RETENTION TIME:	Approximately 4.1 minutes
PFOS MONITORED MASS:	499.0 AMU
PFOS MONITORED MASS:	499.0 amu →99.1 amu

Table 3

Matrix Blanks and Fortifications Analyzed Concurrently with Freshwater Samples

Sample Number (454A-134-)		Sampling Interval (Day)	Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS) (mg a.i./L)		Percent Recovery[2]
			Fortified	Measured[1]	
PT-MAB-1	-4	Pretest	0.00	<LOQ	–
PT-MAS-1	-4		0.0750	0.0707	94.3
PT-MAS-2	-4		0.500	0.493	98.6
PT-MAS-3	-4		2.00	1.98	99.0
PT-MAB-2	-1	Pretest	0.00	<LOQ	–
PT-MAS-4	-1		0.750	0.0779	104
PT-MAS-5	-1		0.500	0.464	92.9
PT-MAS-6	-1		2.00	1.91	95.3
PT-MAB-2	-1	Pretest	0.00	<LOQ	–
PT-MAS-7	-1		0.0750	0.0724	96.5
PT-MAS-8	-1		0.500	0.483	96.5
PT-MAS-9	-1		2.00	1.86	93.2
MAB-1	0	Hour 0 – Uptake	0.00	<LOQ	–
MAS-1	0		0.0750	0.0712	95.0
MAS-2	0		0.500	0.488	97.5
MAS-3	0		2.00	2.00	99.9
MAB-2	0	Hour 4 – Uptake	0.00	<LOQ	–
MAS-4	0		0.0750	0.0728	97.1
MAS-5	0		0.500	0.490	98.0
MAS-6	0		2.00	1.97	98.6
MAB-3	1	Uptake	0.00	<LOQ	–
MAS-7	1		0.0750	0.0745	99.3
MAS-8	1		0.500	0.498	99.7
MAS-9	1		2.00	1.95	97.6
MAB-4	3	Uptake	0.00	<LOQ	–
MAS-10	3		0.0750	0.0666	88.9
MAS-11	3		0.500	0.509	102
MAS-12	3		2.00	1.86	92.8
MAB-5	7	Uptake	0.00	<LOQ	–
MAS-13	7		0.0750	0.0753	100
MAS-14	7		0.500	0.508	102
MAS-15	7		2.00	1.96	98.2
MAB-6	14	Uptake	0.00	<LOQ	–
MAS-16	14		0.0750	0.0671	89.5
MAS-17	14		0.500	0.450	90.0
MAS-18	14		2.00	1.85	92.7

[1]The limit of quantitation(LOQ) was 0.0500 mg a.i./L, calculated as the product of the lowest calibration standard (0.0005 mg a.i./L) and the dilution factor of the matrix blank sample (100).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 3 (Continued)

Matrix Blanks and Fortifications Analyzed Concurrently with Freshwater Samples

Sample Number (454A-134-)		Sampling Interval (Day)	Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS) (mg a.i./L)		Percent Recovery[2]
			Fortified	Measured[1]	
MAB-7	21	Uptake	0.00	<LOQ	–
MAS-19	21		0.0750	0.0710	94.6
MAS-20	21		0.500	0.600	120
MAS-21	21		2.00	1.99	99.6
MAB-8	28	Uptake	0.00	<LOQ	–
MAS-22	28		0.750	0.0659	87.6
MAS-23	28		0.500	0.517	103
MAS-24	28		2.00	1.91	95.4
MAB-9	35	Uptake	0.00	<LOQ	–
MAS-25	35		0.0750	0.0727	96.9
MAS-26	35		0.500	0.470	94.0
MAS-27	35		2.00	2.01	101
MAB-10	42	Uptake	0.00	<LOQ	–
MAS-28	42		0.0750	0.0734	97.9
MAS-29	42		0.500	0.517	104
MAB-11	49	Uptake	0.00	<LOQ	–
MAS-30	49		0.0750	0.0712	95.0
MAS-31	49		0.500	0.463	92.7
MAB-12	56	Uptake	0.00	<LOQ	–
MAS-32	56		0.0750	0.0701	93.4
MAS-33	56		0.500	0.477	95.3
MAB-13	62	Uptake	0.00	<LOQ	–
MAS-34	62		0.0750	0.0745	99.3
MAS-35	62		0.500	0.481	96.1
MAB-14	14	Depuration	0.00	<LOQ	–
MAS-36	14		*0.0750*	*0.0751*	*100*
MAS-37	14		0.500	0.494	98.7
MAB-15	28	Depuration	0.00	<LOQ	–
MAS-38	28		0.0750	0.0644	85.8
MAS-39	28		0.500	0.525	105

[1]The limit of quantitation(LOQ) was 0.0500 mg a.i./L, calculated as the product of the lowest calibration standard (0.0005 mg a.i./L) and the dilution factor of the matrix blank sample (100).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 3 (Continued)

Matrix Blanks and Fortifications Analyzed Concurrently with Freshwater Samples

Sample Number (454A-134-)		Sampling Interval (Day)	Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS) (mg a.i./L)		Percent Recovery[2]
			Fortified	Measured[1]	
MAB-16	42	Depuration	0.00	<LOQ	–
MAS-40	42		0.0750	0.0637	84.9
MAS-41	42		0.500	0.452	90.4
MAB-17	56	Depuration	0.00	<LOQ	–
MAS-42	56		0.750	0.0712	94.9
MAS-43	56		0.500	0.484	96.9

[1]The limit of quantitation(LOQ) was 0.0500 mg a.i./L, calculated as the product of the lowest calibration standard (0.0005 mg a.i./L) and the dilution factor of the matrix blank sample (100).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 4

Matrix Blanks and Fortifications Analyzed Concurrently with Edible Fish Tissue Samples

Sample Number (454A-134-)		Sampling Interval (Day)	Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS) (mg a.i./Kg)		Percent Recovery[2]
			Fortified	Measured[1]	
E-MAB-1	0	Hour 4 – Uptake	0.00	<0.0500	--
E-MAS-1	0		0.100	0.0970	97.0
E-MAS-2	0		10.0	9.65	96.5
E-MAB-2	1	Uptake	0.00	<0.0500	--
E-MAS-3	1		0.100	0.0993	99.3
E-MAS-4	1		10.0	9.77	97.7
E-MAB-3	3	Uptake	0.00	<0.100	--
E-MAS-5	3		0.500	0.494	98.8
E-MAS-6	3		50.0	48.6	97.2
E-MAB-4	7	Uptake	0.00	<0.200	--
E-MAS-7	7		1.00	0.948	94.8
E-MAS-8	7		100	95.0	95.0
E-MAB-5	14	Uptake	0.00	<1.00	--
E-MAS-9	14		5.00	5.31	106
E-MAS-10	14		250	268	107
E-MAB-6	21	Uptake	0.00	<1.00	--
E-MAS-11	21		5.00	5.21	104
E-MAS-12	21		250	251	101
E-MAB-7	28	Uptake	0.00	<1.00	--
E-MAS-13	28		5.00	5.27	105
E-MAS-14	28		250	249	99.5
E-MAB-8	35	Uptake	0.00	<1.00	--
E-MAS-15	35		5.00	4.92	98.3
E-MAS-16	35		100	104	104
E-MAB-10	42	Uptake	0.00	<1.00	--
E-MAS-19	42		5.00	4.97	99.4
E-MAS-20	42		100	105	105
E-MAB-11	49	Uptake	0.00	<1.00	--
E-MAS-21	49		5.00	5.27	106
E-MAS-22	49		100	106	106
E-MAB-12	56	Uptake	0.00	<1.00	--
E-MAS-23	56		5.00	5.31	106
E-MAS-24	56		200	208	104

[1]The limit of quantitation (LOQ) was 0.0500 mg a.i./L, calculated as the product of the lowest calibration standard (0.0005 mg a.i./L) and the dilution factor of the matrix blank sample (100).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 4 (Continued)

Matrix Blanks and Fortifications Analyzed Concurrently with Edible Fish Tissue Samples

Sample Number (454A-134-)		Sampling Interval (Day)	Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS) (mg a.i./Kg)		Percent Recovery[2]
			Fortified	Measured[1]	
E-MAB-13	62	Uptake	0.00	<1.00	--
E-MAS-25	62		5.00	5.54	111
E-MAS-26	62		200	192	96.0
E-MAB-14	14	Depuration	0.00	<1.00	--
E-MAS-27	14		5.00	5.37	107
E-MAS-28	14		200	205	102
E-MAB-15	28	Depuration	0.00	<1.00	--
E-MAS-29	28		5.00	5.15	103
E-MAS-30	28		100	104	104
E-MAB-16	42	Depuration	0.00	<1.00	--
E-MAS-31	42		5.00	4.82	96.5
E-MAS-32	42		100	92.0	92.0
E-MAB-17	56	Depuration	0.00	<1.00	--
E-MAS-33	56		5.00	4.99	99.7
E-MAS-34	56		100	101	101

[1]Less than values correspond to limit of quantitation (LOQ). For each analysis, the LOQ was calculated as the product of the lowest calibration standard and the overall dilution factor of the matrix blank sample. All sample weights = 1.00 gram.

[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 5

Matrix Blanks and Fortifications Analyzed Concurrently with Nonedible Fish Tissue Samples

Sample Number (454A-134-)		Sampling Interval (Day)	Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS) (mg a.i./Kg)		Percent Recovery[2]
			Fortified	Measured[1]	
N-MAB-1	0	Hour 4 - Uptake	0.00	<0.0500	--
N-MAS-1	0		0.100	0.109	109
N-MAS-2	0		10.0	9.26	92.6
N-MAB-2	1	Uptake	0.00	<0.0500	--
N-MAS-3	1		0.100	0.106	106
N-MAS-4	1		10.0	9.36	93.6
N-MAB-3	3	Uptake	0.00	<0.100	--
N-MAS-5	3		0.500	0.470	93.9
N-MAS-6	3		50.0	45.8	91.6
N-MAB-4	7	Uptake	0.00	<0.200	--
N-MAS-7	7		1.00	0.975	97.5
N-MAS-8	7		100	90.1	90.1
N-MAB-5	14	Uptake	0.00	<1.00	--
N-MAS-9	14		5.00	5.25	105
N-MAS-10	14		250	255	102
N-MAB-6	21	Uptake	0.00	<1.00	--
N-MAS-11	21		5.00	4.98	99.7
N-MAS-12	21		500	505	101
N-MAB-7	28	Uptake	0.00	<1.00	--
N-MAS-13	28		5.00	5.25	105
N-MAS-14	28		500	5.16	103
N-MAB-8	35	Uptake	0.00	<1.00	--
N-MAS-15	35		5.00	5.15	103
N-MAS-16	35		100	108	108
N-MAB-10	42	Uptake	0.00	<1.00	--
N-MAS-19	42		5.00	5.26	105
N-MAS-20	42		100	104	104
N-MAB-11	49	Uptake	0.00	<1.00	--
N-MAS-21	49		5.00	5.40	108
N-MAS-21	49		100	108	108
N-MAB-12	56	Uptake	0.00	<1.00	--
N-MAS-23	56		5.00	5.41	108
N-MAS-24	56		200	245	122

[1]Less than values correspond to limit of quantitation (LOQ). For each analysis, the LOQ was calculated as the product of the lowest calibration standard and the overall dilution factor of the matrix blank sample. All sample weights = 1.00 gram.

[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 5 (Continued)

Matrix Blanks and Fortifications Analyzed Concurrently with Nonedible Fish Tissue Samples

Sample Number (454A-134-)		Sampling Interval (Day)	Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS) (mg a.i./Kg)		Percent Recovery[2]
			Fortified	Measured[1]	
N-MAB-13	62	Uptake	0.00	<1.00	--
N-MAS-25	62		5.00	5.68	114
N-MAS-26	62		200	212	106
N-MAB-14	14	Depuration	0.00	<1.00	--
N-MAS-27	14		5.00	5.29	106
N-MAS-28	14		200	207	104
N-MAB-15	28	Depuration	0.00	<1.00	--
N-MAS-29	28		5.00	5.45	109
N-MAS-30	28		100	111	111
N-MAB-16	42	Depuration	0.00	<1.00	--
N-MAS-31	42		5.00	5.19	104
N-MAS-32	42		100	98.9	98.9
N-MAB-17	56	Depuration	0.00	<1.00	--
N-MAS-33	56		5.00	5.80	116
N-MAS-34	56		100	112	112

[1]Less than values correspond to limit of quantitation (LOQ). For each analysis, the LOQ was calculated as the product of the lowest calibration standard and the overall dilution factor of the matrix blank sample. All sample weights = 1.00 gram.

[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 6

Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Pre-Test Diluter Verification Samples

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS)[1] (mg a.i./L)	Percent of Nominal[2]
0.00 (Negative Control)	PT-1	Pre-Uptake	-4	<LOQ	--
	PT-9		-1	<LOQ	--
	PT-17		-1	<LOQ	--
0.10	PT-3		-4	0.0850	85.0
	PT-4		-4	0.0844	84.4
	PT-11		-1	0.0695	69.5
	PT-12		-1	0.0646	64.6
	PT-19		-1	0.0954	95.4
	PT-20		-1	0.0937	93.7
1.0	PT-6		-4	0.917	91.7
	PT-7		-4	0.949	94.9
	PT-14		-1	0.718	71.8
	PT-15		-1	0.738	73.8
	PT-22		-1	1.11	111
	PT-23		-1	1.01	101

[1]The limit of quantitation (LOQ) was 0.0500 mg a.i./L, calculated as the product of the lowest calibration standard (0.0005 mg a.i./L) and the dilution factor of the matrix blank sample (100).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 7

Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Freshwater Samples from a Bluegill Sunfish Bioconcentration Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS)[1] (mg a.i./L)	Percent of Nominal[2]
0.0	1	Uptake	0, 0 hours	<LOQ	--
(Negative	9		0, 4 hours	<LOQ	--
	17		1	<LOQ	--
	25		3	<LOQ	--
	33		7	<LOQ	--
	41		14	<LOQ	--
	49		21	<LOQ	--
	57		28	<LOQ	--
	65		35	<LOQ	--
	73		42	<LOQ	--
	78		49	<LOQ	--
	83		56	<LOQ	--
	88		62	<LOQ	--
	93	Depuration	14	<LOQ	--
	98		28	<LOQ	--
	103		42	<LOQ	--
	108		56	<LOQ	--
0.10	3	Uptake	0, 0 hours	0.0717	71.7
	4		0, 0 hours	0.0692	69.2
	11		0, 4 hours	0.0791	79.1
	12		0, 4 hours	0.0741	74.1
	19		1	0.0702	70.2
	20		1	0.0734	73.4
	27		3	0.0751	75.1
	28		3	0.0717	71.7
	35		7	0.0826	82.6
	36		7	0.0781	78.1
	43		14	0.0680	68.0
	44		14	0.0709	70.9
	51		21	0.110	110
	52		21	0.113	113
	59		28	0.0822	82.2
	60		28	0.0843	84.3
	67		35	0.0915	91.5
	68		35	0.0914	91.4
	75		42	0.0983	98.3
	76		42	0.110	110
	80		49	0.103	103
	81		49	0.103	103
	85		56	0.0853	85.3
	86		56	0.0948	94.8
	90		62	0.0887	88.7
	91		62	0.0914	91.4

[1]The limit of quantitation (LOQ) was 0.0500 mg a.i./L, calculated as the product of the lowest calibration standard (0.0005 mg a.i./L) and the dilution factor of the matrix blank sample (100).

[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 7 (Continued)

Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Freshwater Samples from a Bluegill Sunfish Bioconcentration Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS)[1] (mg a.i./L)	Percent of Nominal[2]
0.10	95	Depuration	14	<LOQ	--
	96		14	<LOQ	--
	100		28	<LOQ	--
	101		28	<LOQ	--
	105		42	<LOQ	--
	106		42	<LOQ	--
	110		56	<LOQ	--
	111		56	<LOQ	--
1.0	6	Uptake	0, 0 hours	0.797	79.7
	7		0, 0 hours	0.802	80.2
	14		0, 4 hours	0.891	89.1
	15		0, 4 hours	0.930	93.0
	22		1	0.867	86.7
	23		1	0.820	82.0
	30		3	0.813	81.3
	31		3	0.734	73.4
	38		7	0.845	84.5
	39		7	0.818	81.8
	46		14	0.900	90.0
	47		14	0.875	87.5
	54		21	0.988	98.8
	55		21	1.01	101
	62		28	0.913	91.3
	63		28	0.925	92.5
	70		35[3]	0.838	83.8
	71		35[3]	0.871	87.1

[1]The limit of quantitation (LOQ) was 0.0500 mg a.i./L, calculated as the product of the lowest calibration standard (0.0005 mg a.i./L) and the dilution factor of the matrix blank sample (100).

[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

[3]Sampling was suspended after Day 35 Uptake due to 100% mortality at the 1.0 mg a.i./L nominal test level.

Table 8

Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Edible Fish Tissue Samples from a Bluegill Sunfish Bioconcentration Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS)[1] (mg a.i./L)
0.0	E-1	Uptake	0, 4 hours	<0.0510
(Negative	E-2		0, 4 hours	<0.0665
	E-14		1	<0.0960
	E-15		1	<0.0835
	E-27		3	<0.114
	E-28		3	<0.0910
	E-40		7	<0.0965
	E-41		7	<0.162
	E-53		14	<0.468
	E-54		14	<0.560
	E-66		21	<0.730
	E-67		21	<0.710
	E-79		28	<0.740
	E-80		28	<0.800
	E-92		35	<0.970
	E-93		35	<0.575
	E-100		42	<0.399
	E-101		42	<0.590
	E-108		49	<0.575
	E-109		49	<0.520
	E-116		56	<0.775
	E-117		56	<0.820
	E-124		62	<0.660
	E-125		62	<0.615
	E-132	Depuration	14	<0.685
	E-133		14	<0.710
	E-140		28	<0.585
	E-141		28	<0.590
	E-147		42	<0.830
	E-148		42	<0.825
	E-154		56	<0.755
	E-155		56	<1.07
0.10	E-4	Uptake	0,4 hours	0.167
	E-5		0, 4 hours	0.155
	E-6		0, 4 hours	0.144
	E-7		0, 4 hours	0.182
	E-17		1	0.734
	E-18		1	0.726
	E-19		1	0.631
	E-20		1	0.806
	E-30		3	1.73
	E-31		3	2.07
	E-32		3	2.03
	E-33		3	2.11

[1]Less than values correspond to limit of quantitation (LOQ). For each analysis, the LOQ was calculated as the product of the lowest calibration standard and the overall dilution factor of the sample (L/Kg).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 8 (Continued)
Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Edible Fish Tissue Samples from a Bluegill Sunfish Bioconcentration Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS)[1] (mg a.i./L)
0	E-43	Uptake	7	3.73
	E-44		7	4.25
	E-45		7	4.73
	E-46		7	6.25
	E-56		14	11.4
	E-57		14	9.07
	E-58		14	13.7
	E-59		14	12.6
	E-69		21	11.7
	E-70		21	12.0
	E-71		21	12.9
	E-72		21	10.6
	E-82		28	18.3
	E-83		28	13.7
	E-84		28	23.9
	E-85		28	23.1
	E-95		35	22.6
	E-96		35	27.7
	E-97		35	23.8
	E-98		35	20.6
	E-103		42	27.6
	E-104		42	25.3
	E-105		42	21.2
	E-106		42	27.6
	E-111		49	33.3
	E-112		49	36.2
	E-113		49	39.0
	E-114		49	30.6
	E-119		56	48.3
	E-120		56	38.9
	E-121		56	44.1
	E-122		56	38.3
	E-127		62	42.4
	E-128		62	66.2
	E-129		62	42.2
	E-130		62	39.2
	E-135	Depuration	14	48.5
	E-136		14	31.8
	E-137		14	31.6
	E-138		14	42.0
	E-143		28	26.0
	E-144		28	33.3
	E-145		28	38.7
	E-146		28	55.8
	E-150		42	24.1
	E-151		42	31.2
	E-152		42	30.0
	E-153		42	33.0

[1]Less than values correspond to limit of quantitation (LOQ). For each analysis, the LOQ was calculated as the product of the lowest calibration standard and the overall dilution factor of the sample (L/Kg).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 8 (Continued)

Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Edible Fish Tissue Samples from a Bluegill Sunfish Bioconcentration Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS)[1] (mg a.i./L)
0.10	E-157	Depuration	56	21.1
	E-158		56	37.6
	E-159		56	32.9
	E-160		56	31.2
1.0	E-9	Uptake	0, 4 hours	1.46
	E-10		0, 4 hours	1.48
	E-11		0, 4 hours	1.19
	E-12		0, 4 hours	1.39
	E-22		1	4.68
	E-23		1	6.59
	E-24		1	5.56
	E-25		1	5.64
	E-35		3	17.3
	E-36		3	15.8
	E-37		3	19.0
	E-38		3	20.8
	E-48		7	42.0
	E-49		7	44.0
	E-50		7	57.7
	E-51		7	46.8
	E-61		14	87.1
	E-62		14	81.6
	E-63		14	90.7
	E-64		14	73.3
	E-74		21	79.4
	E-75		21	117
	E-76		21	104
	E-77		21	102
	E-87		28	102
	E-88		28	131
	E-89		28	107
	E-90		28	133

[1]Less than values correspond to limit of quantitation (LOQ). For each analysis, the LOQ was calculated as the product of the lowest calibration standard and the overall dilution factor of the sample (L/Kg).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 9

Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Nonedible Fish Tissue Samples from a Bluegill Sunfish Bioconcentration Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS)[1] (mg a.i./L)
0.0	N-1	Uptake	0, 4 hours	<0.0458
(Negative	N-2		0, 4 hours	<0.0575
	N-14		1	<0.0820
	N-15		1	<0.0645
	N-27		3	<0.0820
	N-28		3	<0.0795
	N-40		7	<0.0805
	N-41		7	<0.0965
	N-53		14	<0.434
	N-54		14	<0.457
	N-66		21	<0.560
	N-67		21	<0.560
	N-79		28	<0.580
	N-80		28	<0.620
	N-92		35	<0.590
	N-93		35	<0.417
	N-100		42	<0.363
	N-101		42	<0.480
	N-108		49	<0.555
	N-109		49	<0.375
	N-116		56	<0.540
	N-117		56	<0.520
	N-124		62	<0.505
	N-125		62	<0.505
	N-132	Depuration	14	<0.470
	N-133		14	<.0472
	N-140		28	<0.449
	N-141		28	<0.505
	N-147		42	<0.590
	N-148		42	<0.570
	N-154		56	<0.630
	N-155		56	<0.780
0.10	N-4	Uptake	0, 4 hours	0.415
	N-5		0, 4 hours	0.519
	N-6		0, 4 hours	0.417
	N-7		0, 4 hours	0.497
	N-17		1	1.68
	N-18		1	1.85
	N-19		1	1.72
	N-20		1	2.07
	N-30		3	4.59
	N-31		3	5.50
	N-32		3	5.47
	N-33		3	5.97

[1]Less than values correspond to limit of quantitation (LOQ). For each analysis, the LOQ was calculated as the product of the lowest calibration standard and the overall dilution factor of the sample (L/Kg).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 9 (Continued)
Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Nonedible Fish Tissue Samples from a Bluegill Sunfish Bioconcentration Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS)[1] (mg a.i./L)
0.10	N-43	Uptake	7	10.2
	N-44		7	10.6
	N-45		7	11.9
	N-46		7	15.2
	N-56		14	27.3
	N-57		14	23.2
	N-58		14	35.3
	N-59		14	32.6
	N-69		21	33.3
	N-70		21	22.7
	N-71		21	24.6
	N-72		21	24.4
	N-82		28	49.4
	N-83		28	40.7
	N-84		28	65.3
	N-85		28	57.9
	N-95		35	67.1
	N-96		35	73.3
	N-97		35	62.0
	N-98		35	59.1
	N-103		42	64.0
	N-104		42	68.1
	N-105		42	54.4
	N-106		42	79.6
	N-111		49	85.0
	N-112		49	95.1
	N-113		49	93.1
	N-114		49	77.7
	N-119		56	122
	N-120		56	94.2
	N-121		56	73.2
	N-122		56	106
	N-127		62	101
	N-128		62	112
	N-129		62	105
	N-130		62	96.4
	N-135	Depuration	14	124
	N-136		14	79.4
	N-137		14	81.8
	N-138		14	113
	N-143		28	85.7
	N-144		28	95.1
	N-145		28	85.7
	N-146		28	94.8
	N-150		42	71.7
	N-151		42	80.6
	N-152		42	78.3
	N-153		42	82.1

[1]Less than values correspond to limit of quantitation (LOQ). For each analysis, the LOQ was calculated as the product of the lowest calibration standard and the overall dilution factor of the sample (L/Kg).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 9 (Continued)

Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Nonedible Fish Tissue Samples from a Bluegill Sunfish Bioconcentration Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS)[1] (mg a.i./L)
0.10	N-157	Depuration	56	57.7
	N-158		56	80.3
	N-159		56	85.4
	N-160		56	84.4
1.0	N-9	Uptake	0,4 hours	3.52
	N-10		0,4 hours	4.37
	N-11		0,4 hours	4.22
	N-12		0,4 hours	4.06
	N-22		1	11.1
	N-23		1	14.2
	N-24		1	13.3
	N-25		1	12.1
	N-35		3	39.3
	N-36		3	42.0
	N-37		3	43.8
	N-38		3	51.8
	N-48		7	100
	N-49		7	102
	N-50		7	102
	N-51		7	120
	N-61		14	177
	N-62		14	207
	N-63		14	245
	N-64		14	214
	N-74		21	201
	N-75		21	278
	N-76		21	246
	N-77		21	229
	N-87		28	289
	N-88		28	372
	N-89		28	320
	N-90		28	361

[1]Less than values correspond to limit of quantitation (LOQ). For each analysis, the LOQ was calculated as the product of the lowest calibration standard and the overall dilution factor of the sample (L/Kg).
[2]Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table 10

Measured Concentrations of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Tissue Storage Stability Samples from a Bluegill Sunfish Bioconcentration Test

Nominal Concentration (mg a.i./Kg)	Sample Number (454A-134-)	Tissue Type	Measured Concentration of Perfluorooctanesulfonate, Potassium Salt (PFOS) (mg a.i./Kg)	Percent of Nominal[2,5]
Negative Control	E-MAB-18[4]	Edible	<LOQ[1]	--
	N-MAB-18[4]	Nonedible	<LOQ1	--
0.100	E-STMAS-1[3]	Edible	0.109	109
	E-MAS-35[4]	Edible	0.111	111
	N-STMAS-1[3]	Nonedible	0.114	114
	N-MAS-35[4]	Nonedible	0.112	112
10.0	E-STMAS-2[3]	Edible	8.43	84.3
	E-MAS-36[4]	Edible	11.3	113
	N-STMAS-2[3]	Nonedible	9.30	93.0
	N-MAS-36[4]	Nonedible	11.3	113

[1] Less than values correspond to limit of quantitation (LOQ). The LOQ was 0.0500 mg a.i./Kg, calculated as the product of the lowest calibration standard (0.0005 mg a.i./:)) and the overall dilution factor of the matrix blank sample (100 L/Kg). All sample weights = 1.00 gram.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

[3] The stability samples were fortified on December 5, 2000 and stored in the freezer. The samples were removed from the freezer on April 3, 2001 (after 119 days of frozen storage) and analyzed.

[4] These samples were prepared on April 3, 2001 and the results were compared to the stability sample results.

[5] The criterion for storage was Percent of Nominal between 80 and 120%.

Table 11

Lipid Content in Edible Fish Tissue

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Lipid Weight (g)	Fish Tissue Weight (g)	Lipid/Fish Tissue Weight[1] (mg/Kg)
0.0	EL1	Uptake	0, 0 hour	0.0314	1.7499	17,900
(Negative Control)	EL2		0, 0 hour	0.0453	2.1445	21,100
	EL3		0, 0 hour	0.0453	1.8577	24,400
	EL4		0, 0 hour	0.0463	1.8335	25,300
	EL6	Uptake	62	0.0231	1.5122	15,300
	EL7		62	0.0135	1.0670	12,700
	EL8		62	0.0134	0.9726	13,800
	EL9		62	0.0162	1.0650	15,200
	EL16	Depuration	56	0.0254	1.3549	18,700
	EL17		56	0.0163	1.0890	15,000
	EL18		56	0.0158	1.1234	14,100
	EL19		56	0.0256	1.3777	18,600
0.10	EL11	Uptake	62	0.0162	1.0411	15,600
	EL12		62	0.0327	1.3551	24,100
	EL13		62	0.0329	1.4893	22,100
	EL14		62	0.0188	0.9054	20,800
	EL21	Depuration	56	0.0172	1.2362	13,900
	EL22		56	0.0190	1.3483	14,100
	EL23		56	0.0250	1.3303	18,800
	EL24		56	0.0252	1.1040	22,800

[1]Ratio calculated as [lipid weight (g) ÷ fish tissue weight (g)] x 1000 mg/g x 1000 g/Kg.

Table 12

Lipid Content in Nonedible Fish Tissue

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-134-)	Phase	Sampling Time (Day)	Lipid Weight (g)	Fish Tissue Weight (g)	Lipid/Fish Tissue Weight[1] (mg/Kg)
0.0 (Negative Control)	NL1	Uptake	0, 0 hour	0.1363	2.0649	66,000
	NL2		0, 0 hour	0.2383	2.6355	90,400
	NL3		0, 0 hour	0.2258	1.9568	115,400
	NL4		0, 0 hour	0.1992	2.0812	95,700
	NL6	Uptake	62	0.0506	1.8821	26,900
	NL7		62	0.0274	1.5901	17,200
	NL8		62	0.0298	1.7596	16,900
	NL9		62	0.0518	1.7302	29,900
	NL16	Depuration	56	0.0616	1.7263	35,700
	NL17		56	0.0318	1.6746	19,000
	NL18		56	0.0360	1.5847	22,700
	NL19		56	0.0966	1.8165	53,200
0.10	NL11	Uptake	62	0.0503	1.5484	32,500
	NL12		62	0.1370	1.7642	77,700
	NL13		62	0.1276	2.0113	63,400
	NL14		62	0.1221	1.2898	94,700
	NL21	Depuration	56	0.0343	1.7085	20,100
	NL22		56	0.0404	1.8394	22,000
	NL23		56	0.0710	1.7652	40,200
	NL24		56	0.0714	1.5682	45,500

[1]Ratio calculated as [lipid weight (g) ÷ fish tissue weight (g)] x 1000 mg/g x 1000 g/Kg.

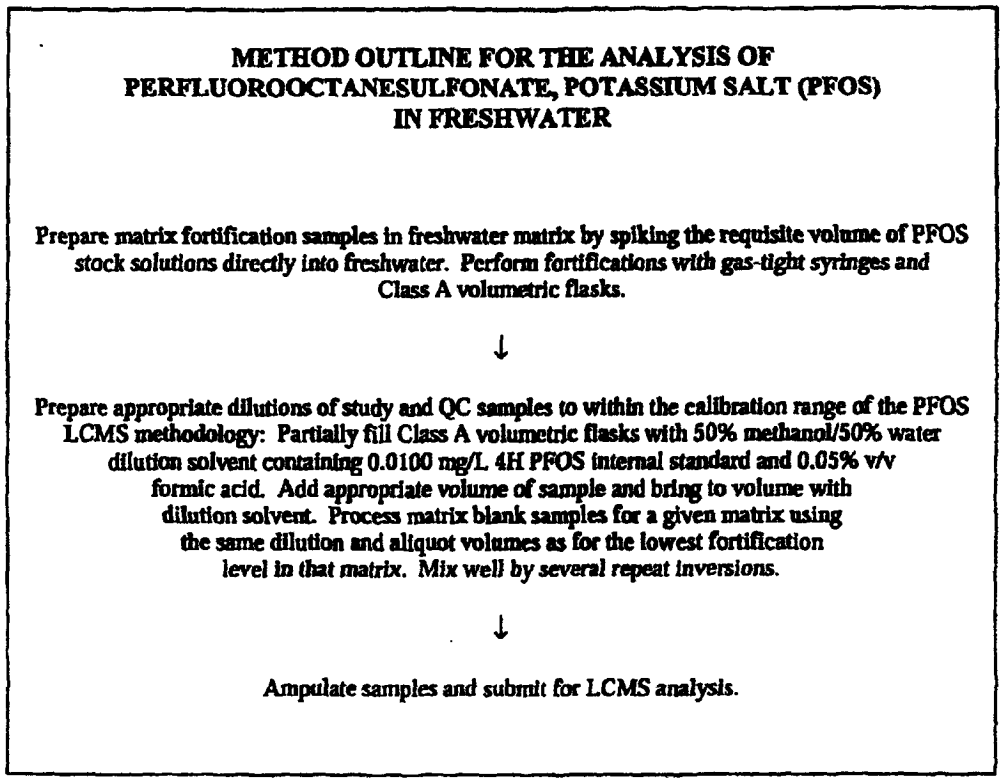


Figure 1. Method flow chart for the analysis of Perfluorooctanesulfonate, Potassium Salt (PFOS) in freshwater.

METHOD OUTLINE FOR THE ANALYSIS OF PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS) IN FISH TISSUES

Quality control samples are prepared from aliquots (approximately 1 g) of bulk control fish tissue homogenate. Remove appropriate (edible or non-edible) aliquots from frozen storage and allow to thaw. Prepare fortification samples with the appropriate PFOS stock solution using gas-tight syringe(s). The matrix blank sample will be unfortified fish tissue homogenate.

↓

Add 10.0 mL of methanol to each sample with a glass Class A volumetric pipette. Homogenize each test sample for approximately 1 minute using a tissue shredder. Rinse the homogenizer with the appropriate solvent(s) in between samples.

↓

Sonicate each sample for approximately 5 minutes with a sonic dismembrator

↓

Cap the vials and shake well. Centrifuge the vials at approximately 2000 rpm for approximately 5 minutes.

↓

Prepare appropriate dilutions of study and QC samples to within the calibration range of the PFOS LCMS methodology. Partially fill Class A volumetric flasks with 50% : 50% methanol/NANOpure® water dilution solvent. Add appropriate volume of sample and bring to volume with dilution solvent. Process matrix blank samples for a given matrix using the same dilution and aliquot volumes as for the lowest fortification level in that matrix. Mix well by several repeat inversions.

↓

Transfer an aliquot of each sample to an autosampler vial and submit for LC/MS/MS analysis.

Figure 2. Method flow chart for the analysis of Perfluorooctanesulfonate, Potassium Salt (PFOS) in fish tissues.

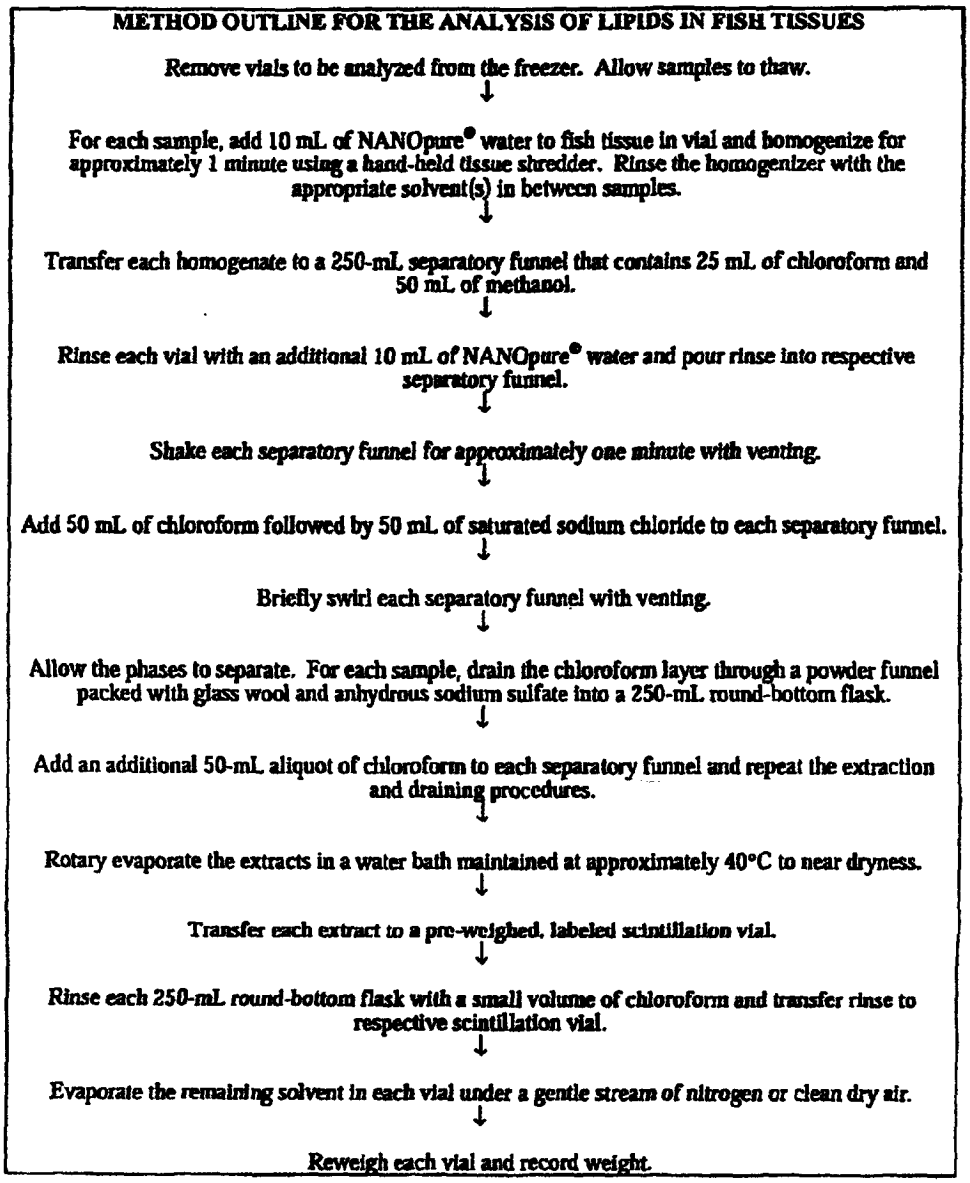


Figure 3. Method flow chart for the analysis of lipids in fish tissues.

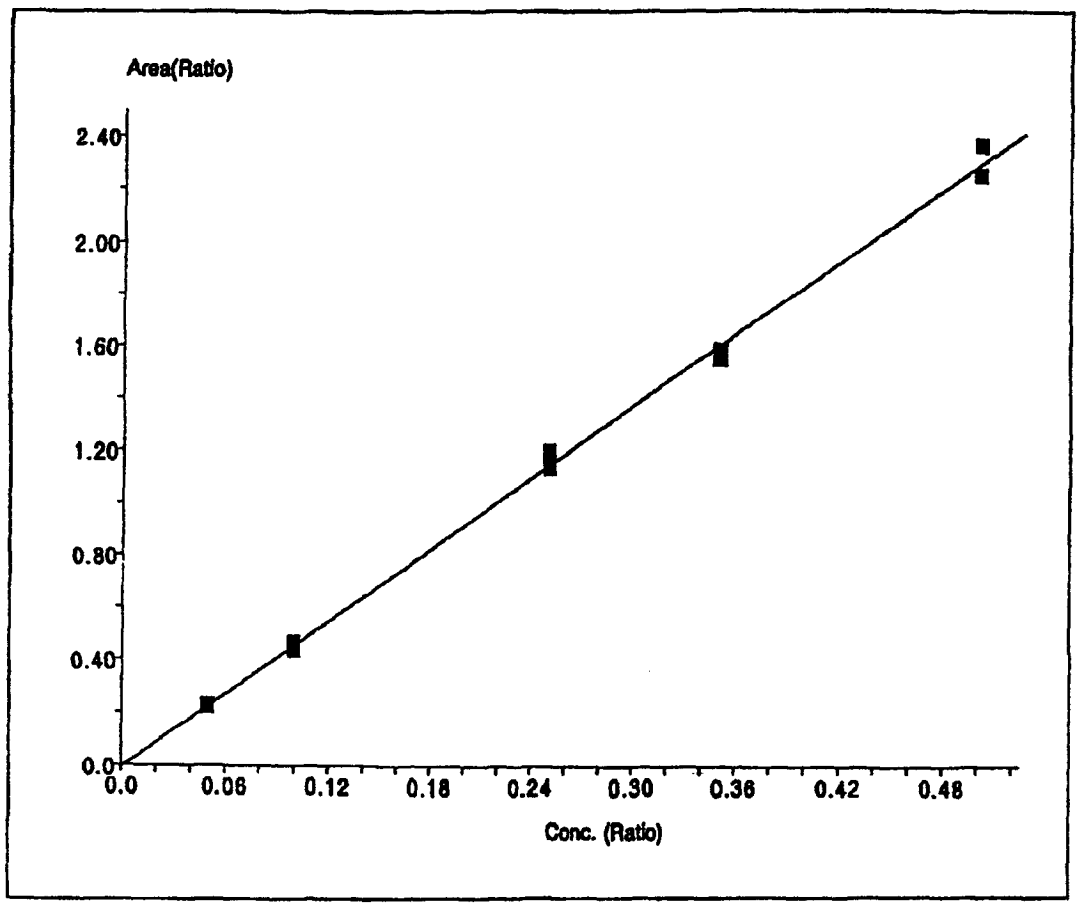


Figure 4. A typical calibration curve for Perfluorooctanesulfonate, Potassium Salt (PFOS) in freshwater. Slope = 4.64; Intercept = -0.01; r = 0.99932. Curve is weighted (1/x). (monitored masses = 499 amu (PFOS) and 427 amu (4HPFOS internal standard)).

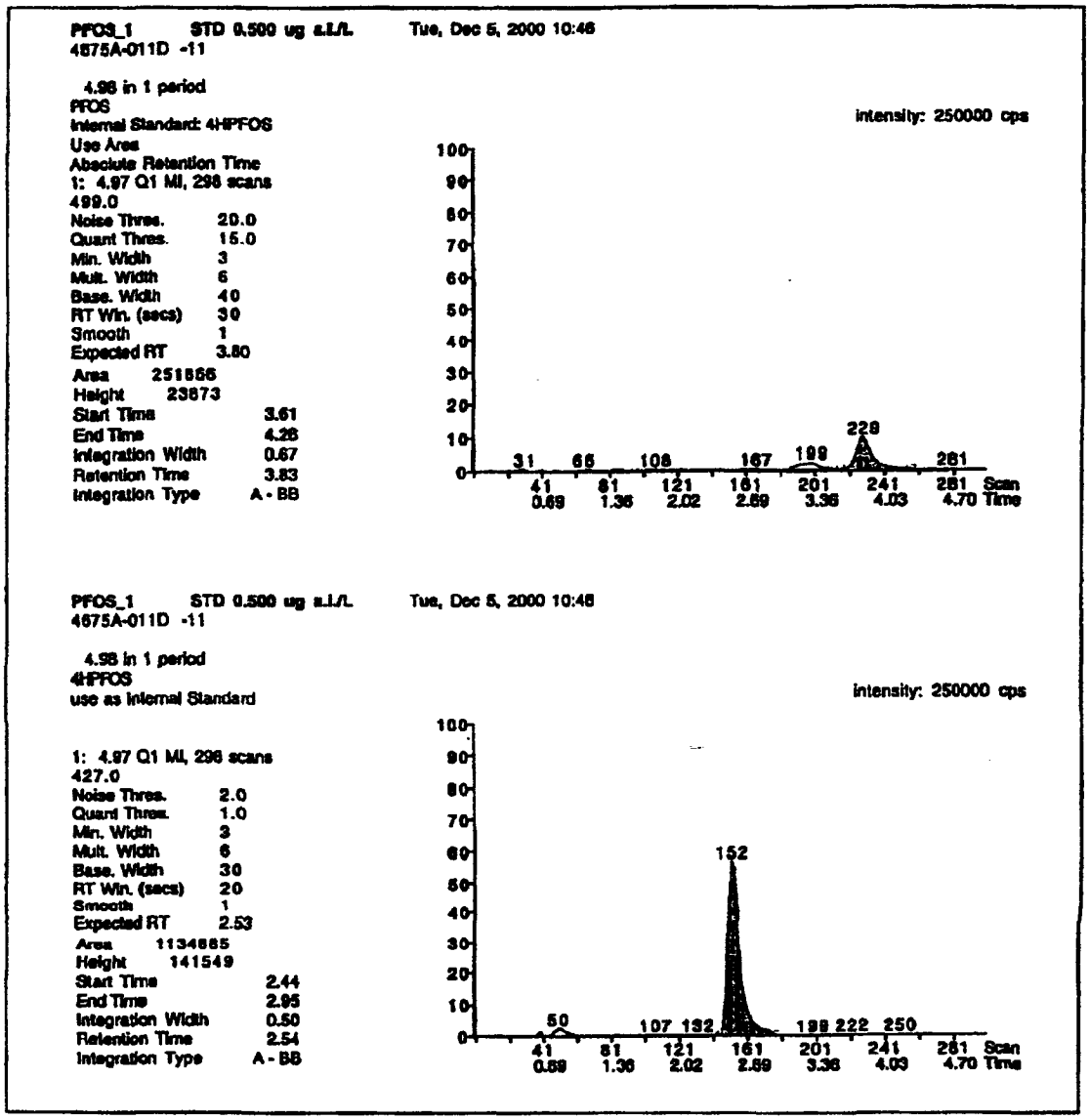


Figure 5. A representative ion chromatogram of a low-level (0.500 µg a.i./L) Perfluorooctanesulfonate, Potassium Salt (PFOS) standard for freshwater analyses. (monitored masses = 499 amu (PFOS – top) and 427 amu (4HPFOS internal standard – bottom)).

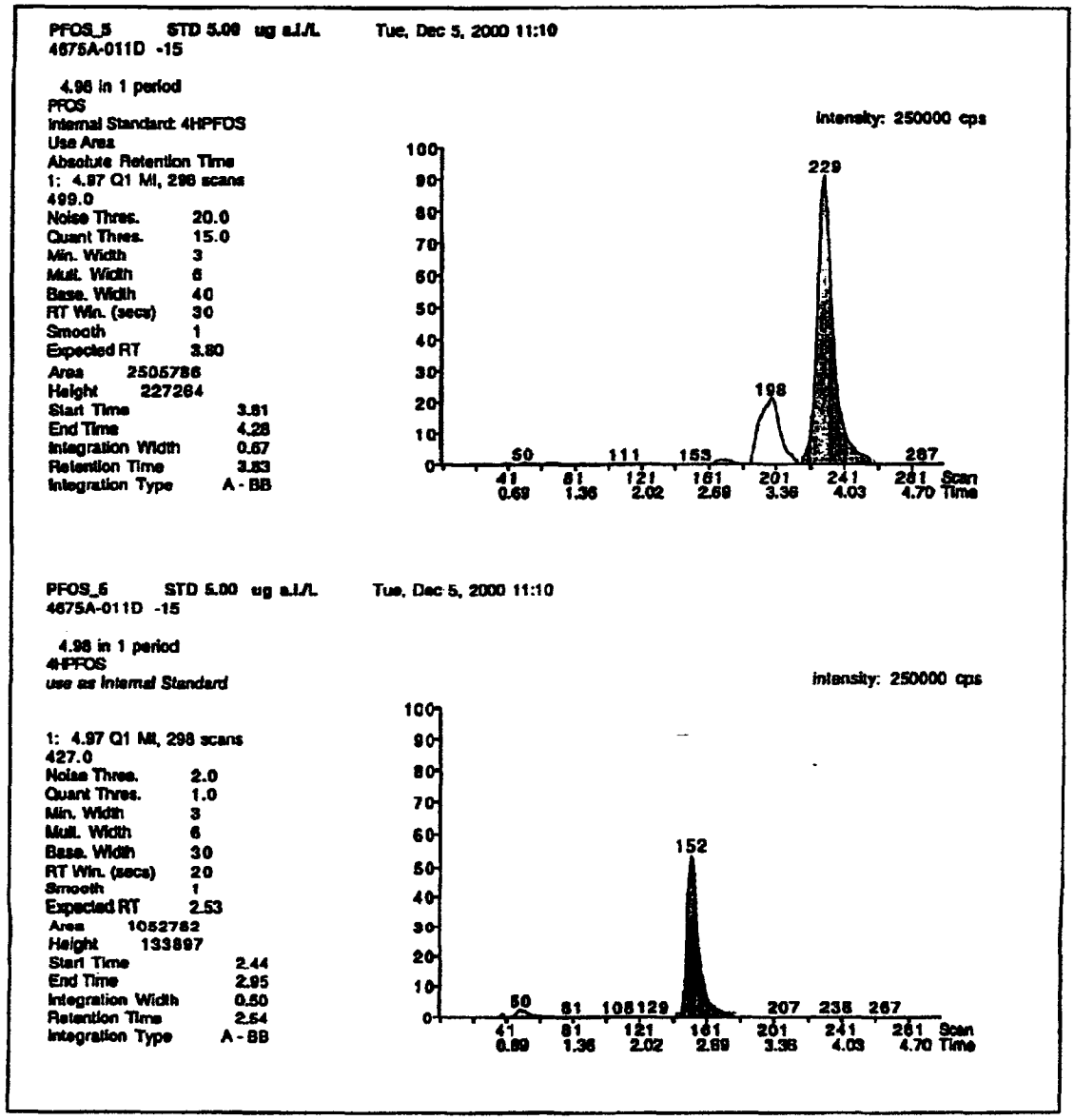


Figure 6. A representative ion chromatogram of a high-level (5.00 µg a.i./L) Perfluorooctanesulfonate, Potassium Salt (PFOS) standard for freshwater analyses. (monitored masses = 499 amu (PFOS - top) and 427 amu (4HPFOS internal standard - bottom)).

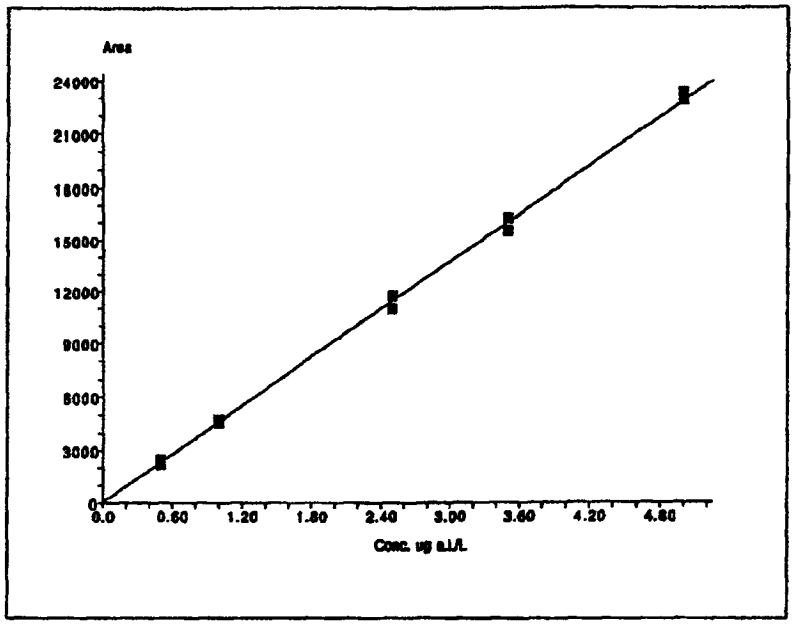


Figure 7. A typical calibration curve for Perfluorooctanesulfonate, Potassium Salt (PFOS) in fish tissue. Slope = 4552.41; Intercept = 69.58; r = 0.99940. Curve is weighted (1/x). (monitored mass = 499 amu →99.1 amu).

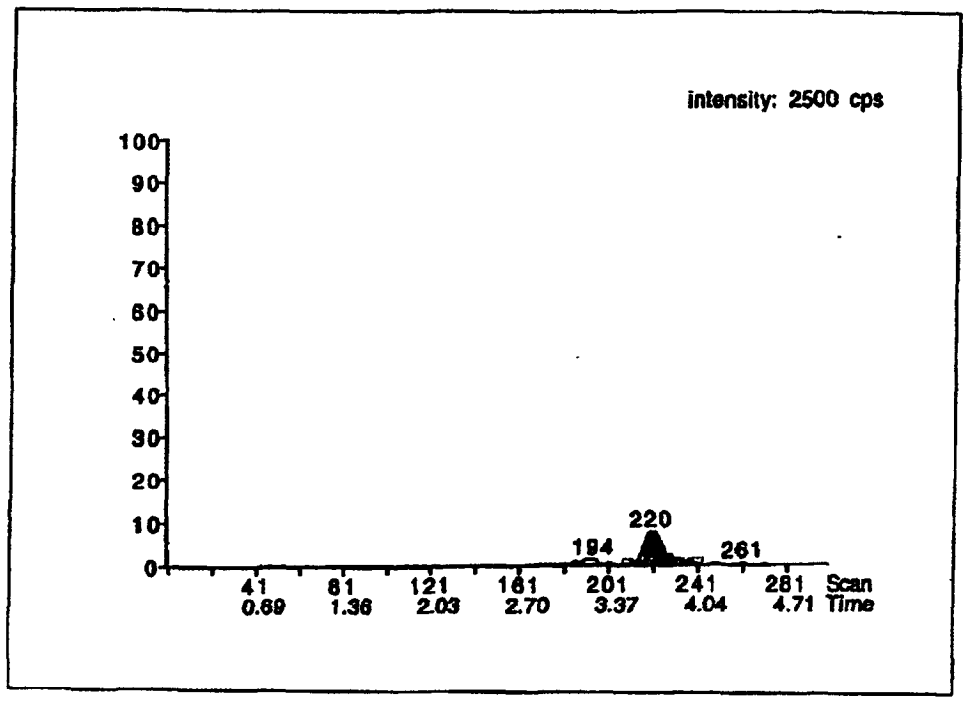


Figure 8. A representative ion chromatogram of a low-level (0.500 µg a.i./L) Perfluorooctanesulfonate, Potassium Salt (PFOS) standard for fish tissue analyses. From low-range (0.500 to 5.00 µg a.i./L) calibration set. (monitored mass = 499 amu →99.1 amu).

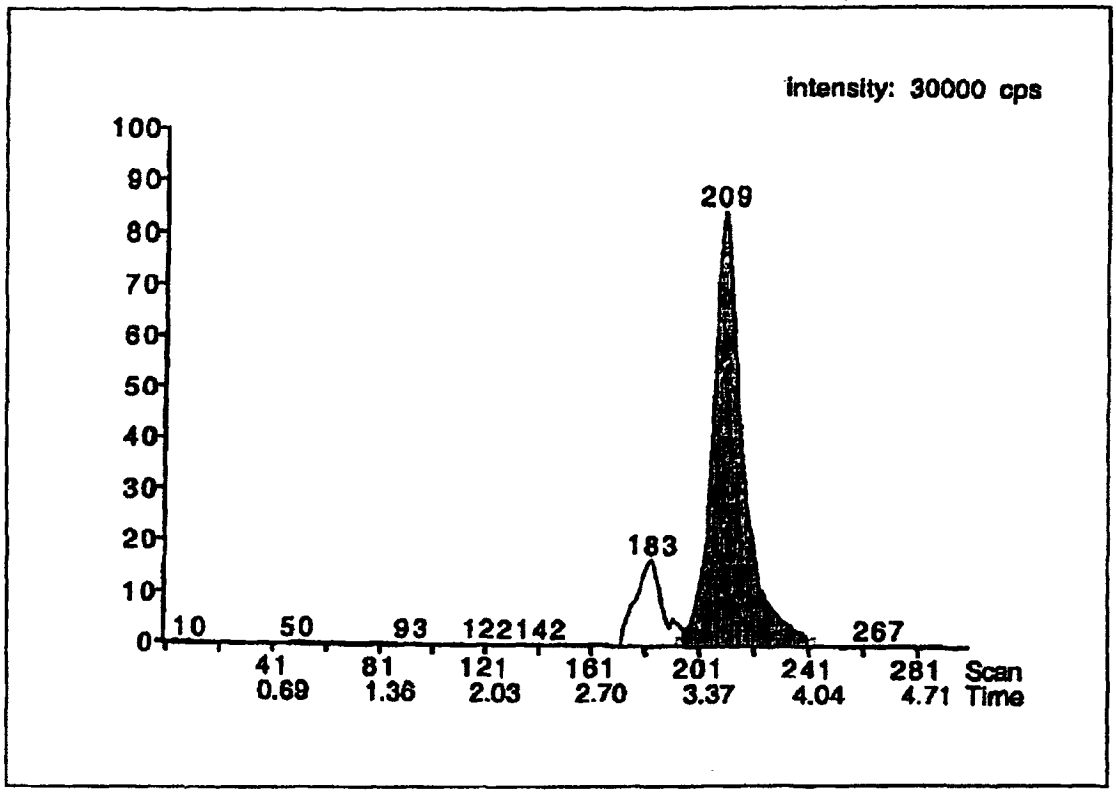


Figure 9. A representative ion chromatogram of a high-level (50.0 µg a.i./L) Perfluorooctanesulfonate, Potassium Salt (PFOS) standard for fish tissue analyses. From high-range (5.00 to 50.0 µg a.i./L) calibration set. (monitored mass = 499 amu →99.1 amu).

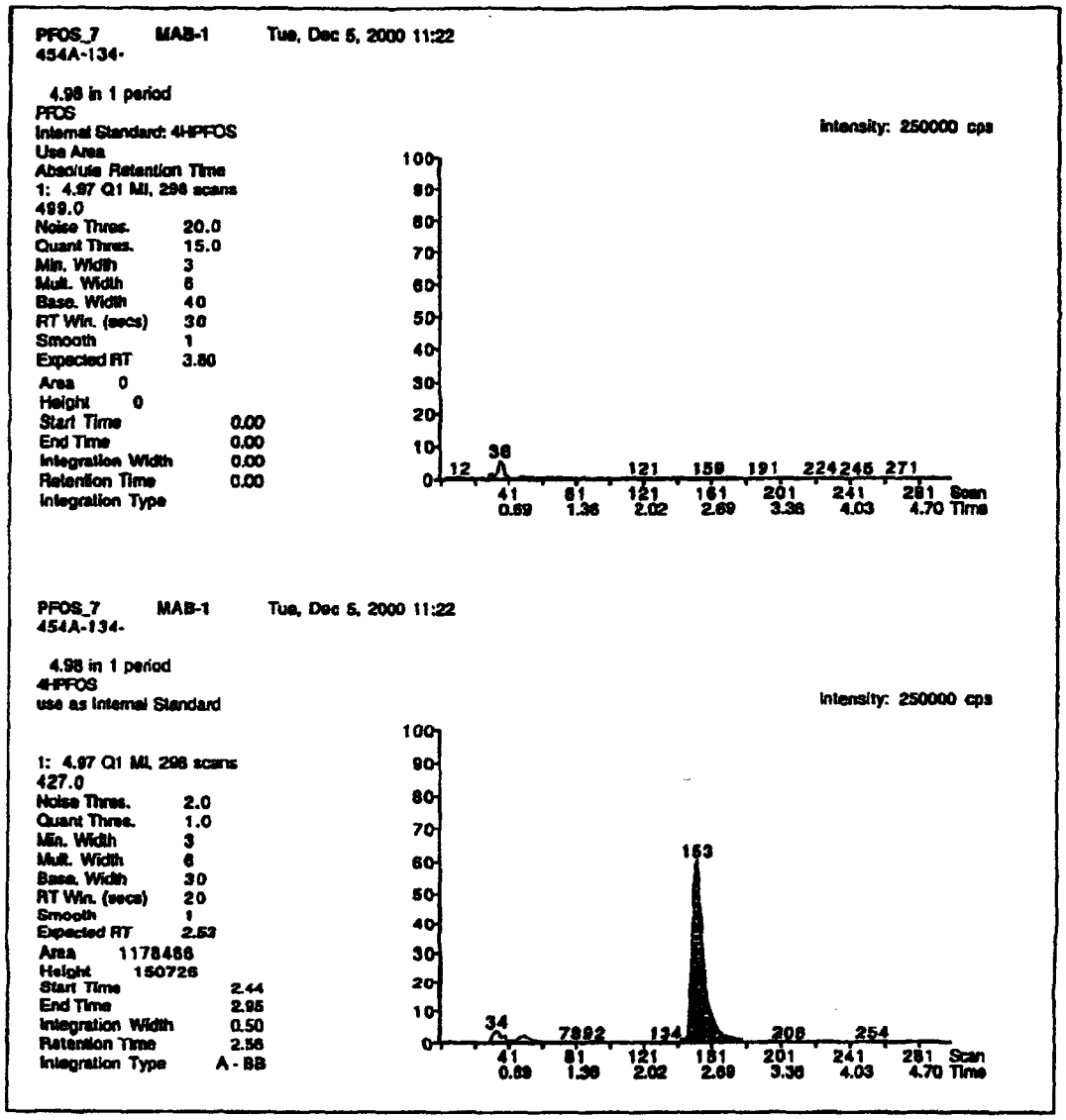


Figure 10. A representative ion chromatogram of a freshwater matrix blank sample (454A – 134 – MAB – 1, dilution = 100x). The arrow indicates the retention time of Perfluorooctanesulfonate, Potassium Salt (PFOS). (monitored masses = 499 amu (PFOS - top) and 427 amu (4HPFOS internal standard - bottom)).

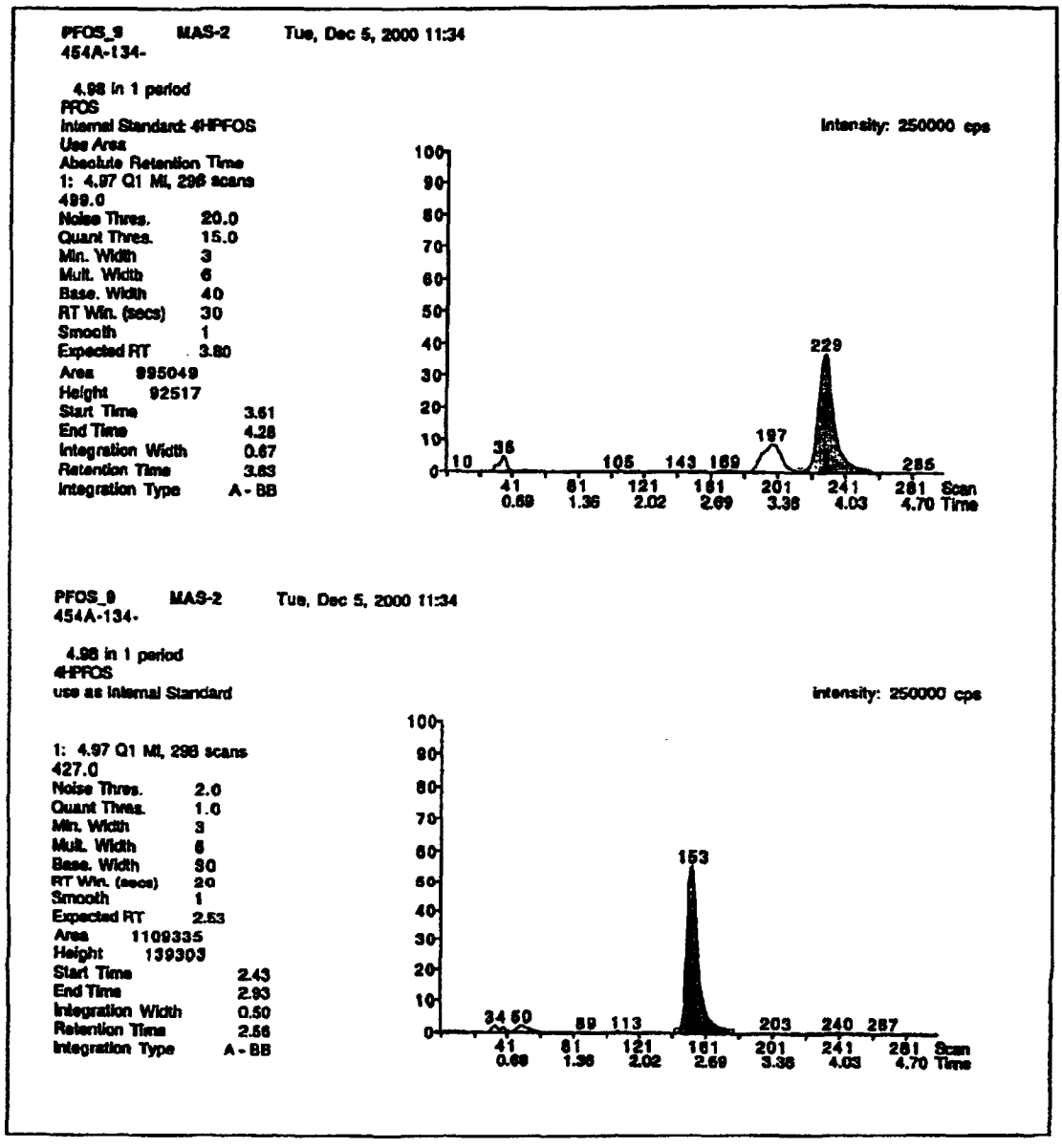


Figure 11. A representative ion chromatogram of a freshwater matrix fortification sample (454A-134-MAS-2, 0.500 mg a.i./L nominal concentration, dilution = 250x). (monitored masses = 499 amu (PFOS - top) and 427 amu (4HPFOS internal standard - bottom)).

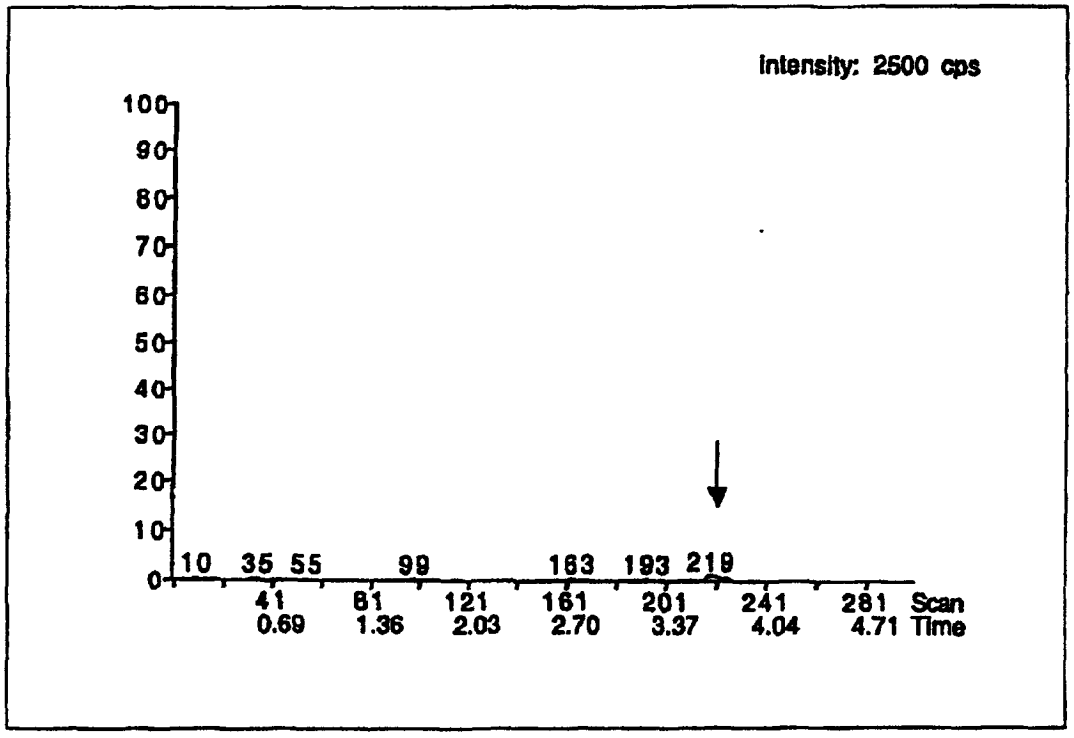


Figure 12. A representative ion chromatogram of an edible fish tissue matrix blank sample (454A-134-E-MAB-1, overall dilution factor = 100 L/Kg). The arrow indicates the retention time of Perfluorooctanesulfonate, Potassium Salt (PFOS). (monitored mass = 499 amu → 99.1 amu).

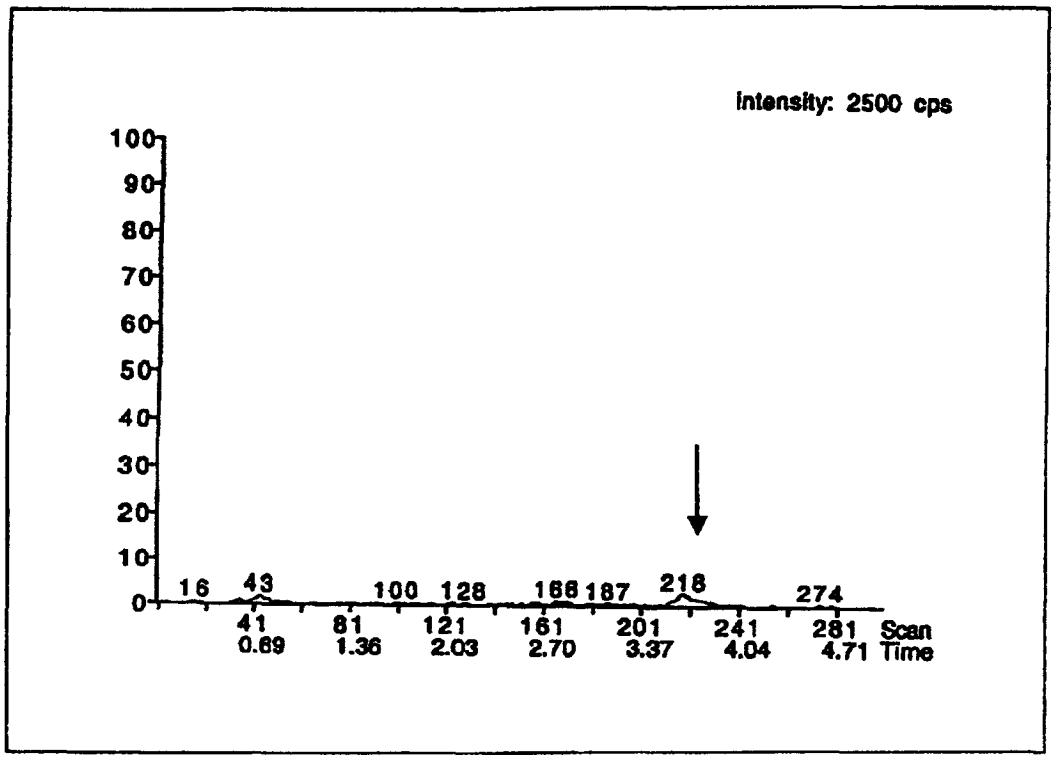


Figure 13. A representative ion chromatogram of a nonedible fish tissue matrix blank sample (454A-134- N-MAB-1, overall dilution factor = 100 L/Kg). The arrow indicates the retention time of Perfluorooctanesulfonate, Potassium Salt (PFOS). (monitored mass = 499 amu → 99.1 amu).

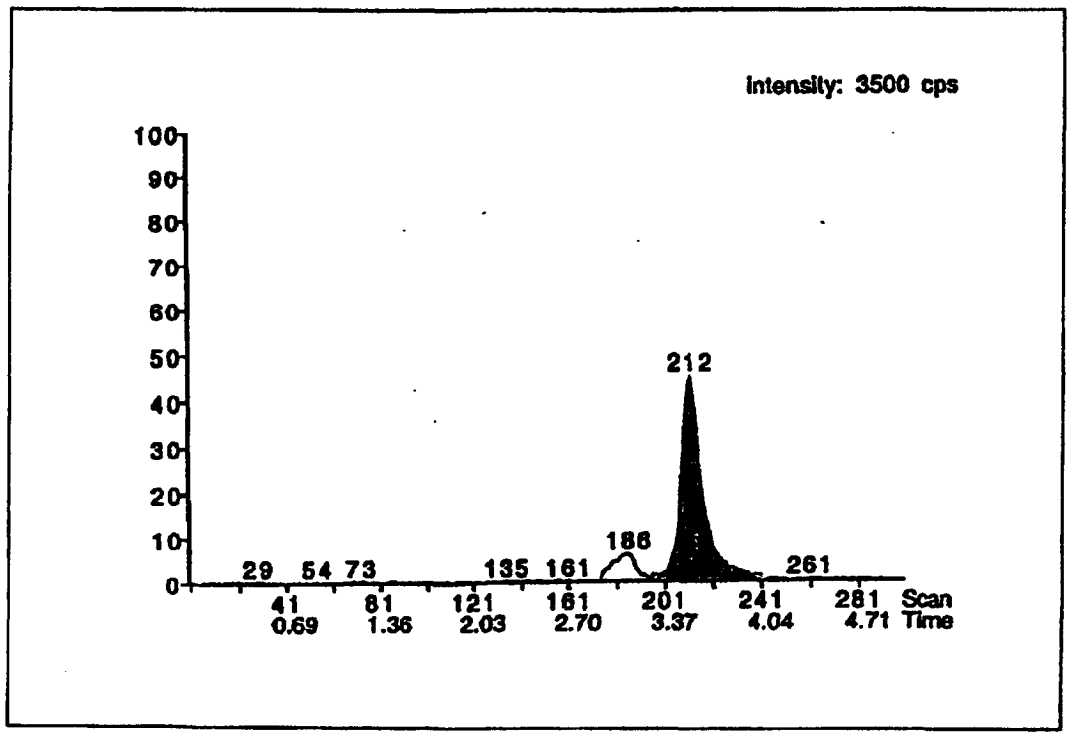


Figure 14. A representative ion chromatogram of an edible fish tissue matrix fortification sample (454A-134-E-MAS-6, 50.0 mg a.i./Kg nominal concentration, overall dilution factor = 20000 L/Kg).
(monitored mass = 499 amu →99.1 amu).

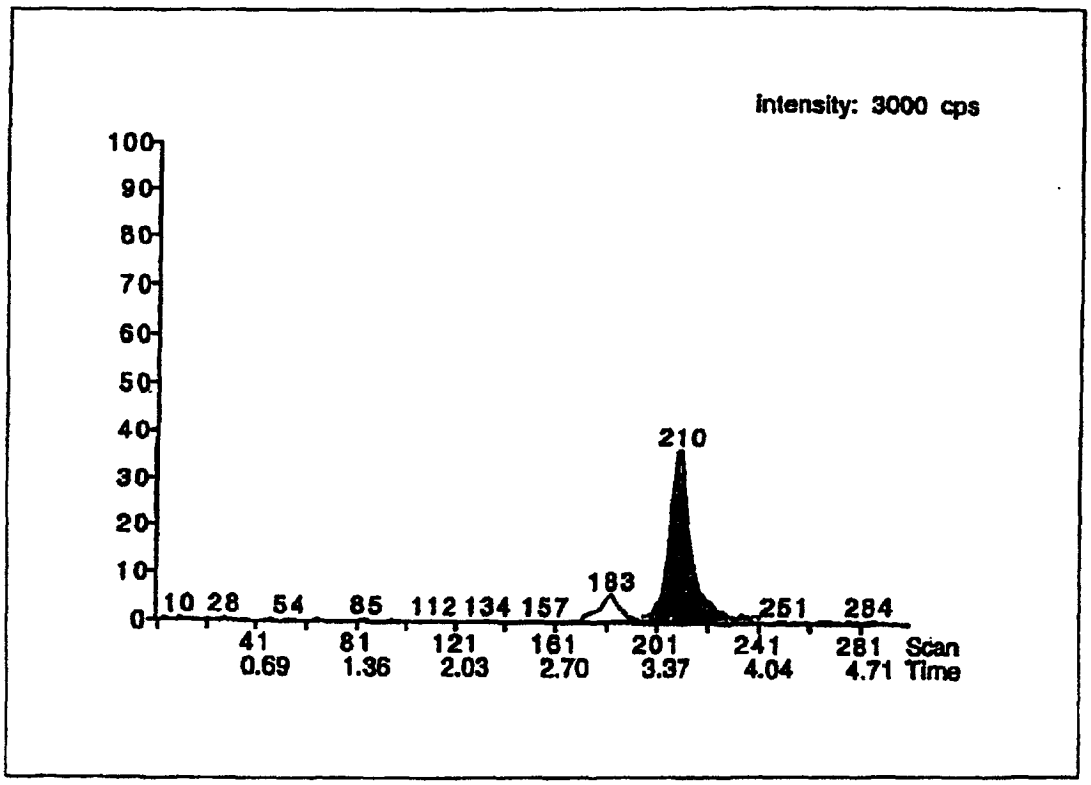


Figure 15. A representative ion chromatogram of a nonedible fish tissue matrix fortification sample (454A-134-NMAS-8, 100 mg a.i./Kg nominal concentration, overall dilution factor = 50000 L/Kg).
(monitored mass = 499 amu→99.1 amu).

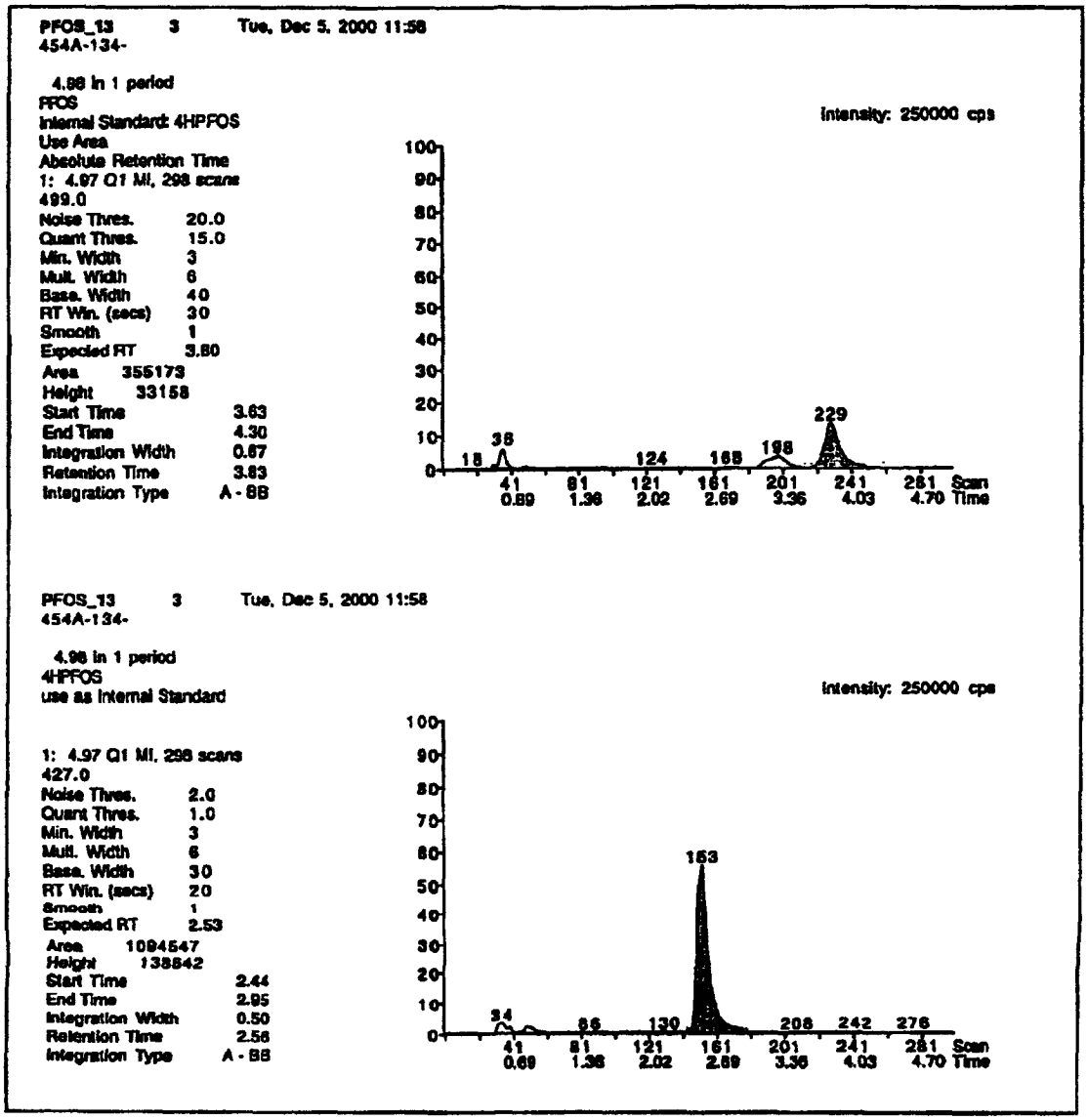


Figure 16. A representative ion chromatogram of a freshwater sample (454A-134-3, dilution = 100x) from the 0.10 mg a.i./L treatment group. (monitored masses = 499 amu (PFOS - top) and 427 amu (4HPFOS internal standard -bottom)).

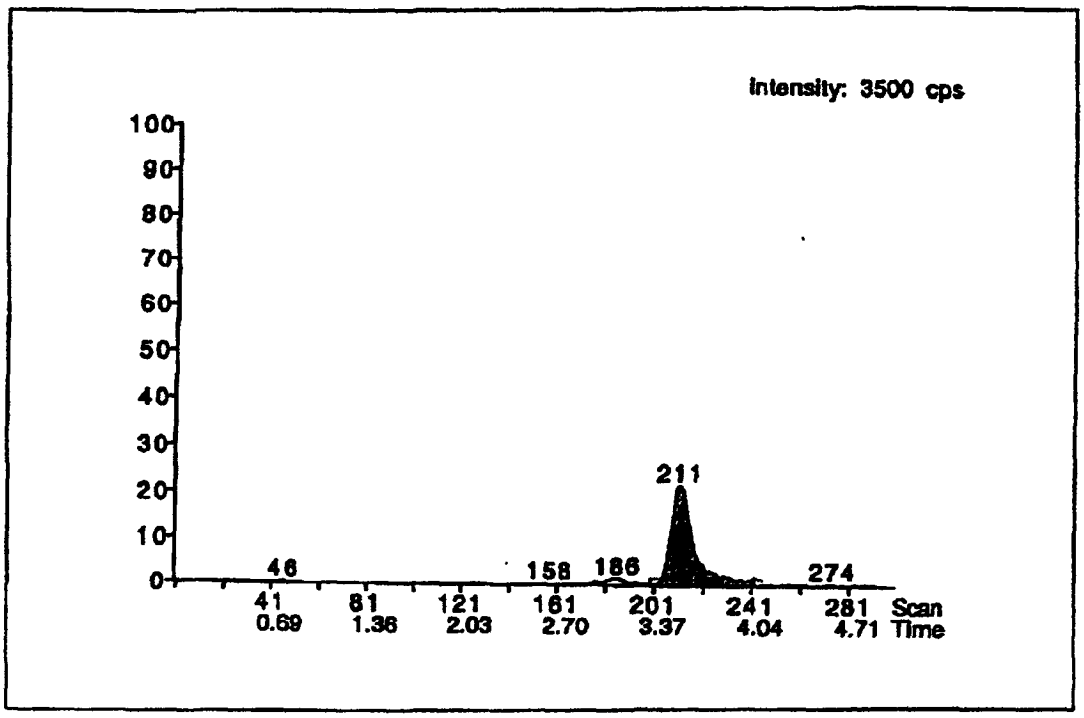


Figure 17. A representative ion chromatogram of an edible fish tissue sample (454A-134-E-30, overall dilution factor = 1420) from the 0.10 mg a.i./L treatment group). (monitored mass = 499 amu →99.1 amu).

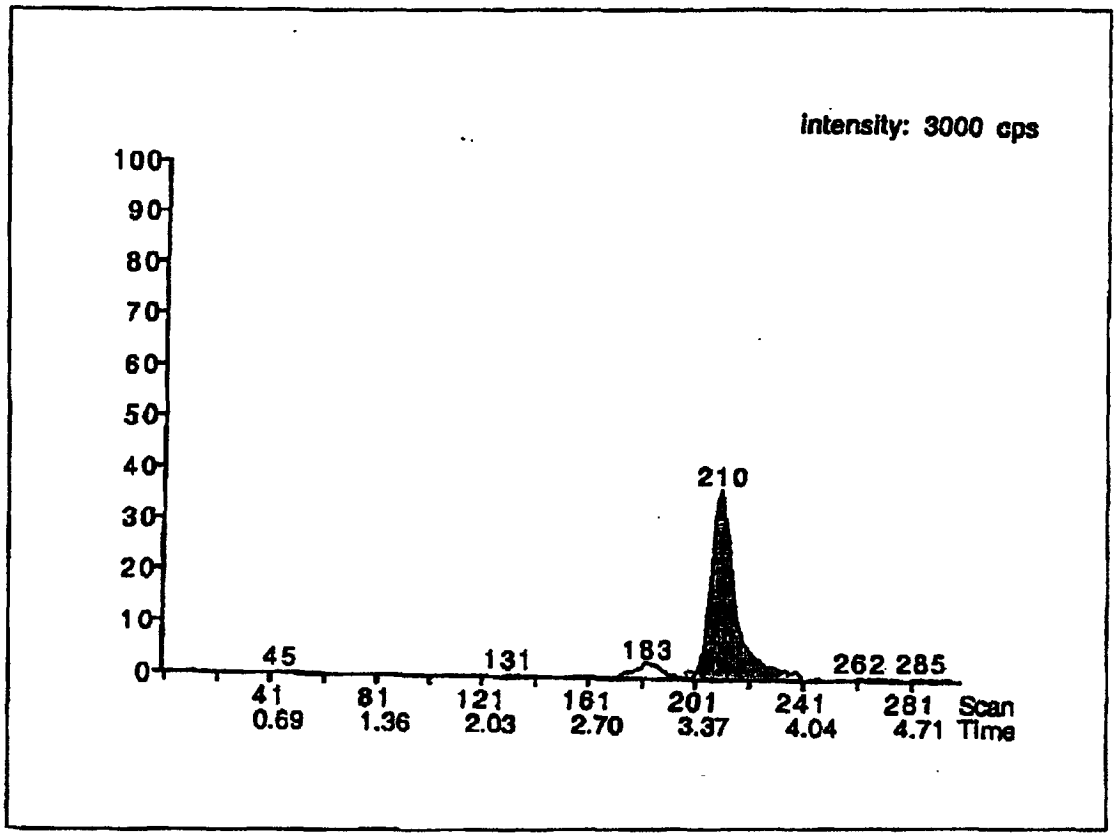


Figure 18. A representative ion chromatogram of a nonedible fish tissue sample (454A-134-N-30, overall dilution factor = 2650) from the 0.10 mg a.i./L treatment group). (monitored mass = 499 amu →99.1 amu).

Appendix 4

Temperature of pH of Water in the Test Chambers
Uptake Phase

Sponsor: 3M Corporation
Test Sustance: PFOS
Test Organism: Bluegill, *Lepomis machrochirus*
Dilution Water: Well Water

Uptake Phase Mean Measured Concentration (mg a.i./L)	Day 0		Day 7		Day 14		Day 21		Day 28	
	Temp[1] (°C)	pH	Temp[1] (°C)	pH	Temp[1] (°C)	pH	Temp[1] (°C)	pH	Temp[1] (°C)	pH
Negative Control	22.0	8.2	21.9	8.0	21.8	8.0	21.8	8.1	21.9	7.9
0.086	21.9	8.2	21.8	8.0	21.8	8.0	21.8	8.1	21.9	7.9
0.87	21.9	8.2	21.8	8.0	21.8	7.9	21.8	8.1	21.8	8.0

[1]Continuous measurements of temperature ranged from 20.0 to 22.0°C.

Uptake Phase Mean Measured Concentration (mg a.i./L)	Day 35		Day 42		Day 49		Day 56	
	Temp[1] (°C)	pH	Temp[1] (°C)	pH	Temp[1] (°C)	pH	Temp[1] (°C)	pH
Negative Control	21.8	8.1	21.8	8.0	21.9	8.0	21.9	8.1
0.086	21.7	8.1	21.8	7.9	21.8	8.0	21.9	8.1
0.87	21.7	8.2	--[2]	--[2]	--	--	--	--

[1] Continuous measurements of temperature ranged from 20.0 to 22.0°C.
[2] Measurements discontinued due to 100% mortality.

Appendix 4 (Continued)

Temperature of pH of Water in the Test Chambers
Depuration Phase

Sponsor: 3M Corporation
Test Sustance: PFOS
Test Organism: Bluegill, *Lepomis machrochirus*
Dilution Water: Well Water

Uptake Phase Mean Measured Concentration (mg a.i./L)	Day 0 Temp[1] (°C)	Day 0 pH	Day 0 Temp[1] (°C)	Day 0 pH	Day 14 Temp[1] (°C)	Day 14 pH	Day 21 Temp[1] (°C)	Day 21 pH	Day 28 Temp[1] (°C)	Day 28 pH
Negative Control	21.9	8.1	21.8	8.2	21.9	8.0	21.9	8.1	21.8	8.1
0.086	21.8	8.1	21.8	8.2	21.9	8.1	21.8	8.1	21.7	8.1

[1]Continuous measurements of temperature ranged from 20.0 to 22.0°C.

Uptake Phase Mean Measured Concentration (mg a.i./L)	Day 35 Temp[1] (°C)	Day 35 pH	Day 42 Temp[1] (°C)	Day 42 pH	Day 49 Temp[1] (°C)	Day 49 pH	Day 56 Temp[1] (°C)	Day 56 pH
Negative Control	21.9	8.1	21.9	8.1	22.0	8.1	21.9	8.1
0.086	21.8	8.1	21.9	8.1	22.0	8.1	21.9	8.1

[1] Continuous measurements of temperature ranged from 20.0 to 22.0°C.

Appendix 5
Dissolved Oxygen (mg/L) of Water in the Test Chambers[1]

Sponsor:	3M Corporation
Test Sustance:	PFOS
Test Organism:	Bluegill, *Lepomis machrochirus*
Dilution Water:	Well Water

Day	Uptake Phase		
	Negative Control	0.086 mg a.i./L	0.87 mg a.i./L
0	8.2	8.2	8.2
1	6.8	6.8	6.8
2	7.4	7.2	6.9
3	7.3	7.1	6.5
4	7.3	7.2	7.3
5	7.3	7.3	7.1
6	7.3	7.3	6.8
7	7.0	7.0	7.0
8	7.6	7.6	7.4
9	7.6	7.4	7.4
10	7.8	7.6	7.2
11	7.7	7.6	7.5
12	7.5	7.3	7.0
13	7.8	7.6	7.1
14	7.3	7.1	6.4
15	7.5	7.2	6.7
16	7.5	7.3	7.4
17	7.9	7.8	8.0
18	8.0	8.0	8.0
19	7.4	7.5	7.5
20	7.8	7.8	7.7
21	7.8	7.8	7.8
22	7.8	7.6	7.7
23	7.8	7.8	7.9
24	NC[2]	NC	NC
25	7.5	7.4	7.4
26	7.5	7.4	7.6
27	7.6	7.6	7.8
28	7.3	7.2	7.4
29	8.0	7.8	8.0
30	7.6	7.4	7.9
31	7.8	7.6	8.0
32	7.5	7.4	7.7
33	7.8	7.9	7.8
34	7.8	7.8	8.2
35	7.5	7.4	8.1

[1] A dissolved oxygen concentration of 52. mg/L represents 60% saturation in freshwater at 22°C.
[2] NC-Data inadvertently not collected.

Appendix 5 (Continued)
Dissolved Oxygen (mg/L) of Water in the Test Chambers[1]

Sponsor:	3M Corporation
Test Sustance:	PFOS
Test Organism:	Bluegill, *Lepomis machrochirus*
Dilution Water:	Well Water

	Uptake Phase		
Day	Negative Control	0.086 mg a.i./L	0.87 mg a.i./L
36	7.8	7.6	--[2]
37	7.7	7.8	--
38	8.2	7.8	--
39	7.8	7.6	--
40	7.8	7.8	--
41	7.8	7.7	--
42	7.4	7.2	--
43	7.7	7.4	--
44	7.3	7.0	--
45	7.8	7.4	--
46	7.8	7.2	--
47	7.6	7.3	--
48	7.9	7.6	--
49	8.0	8.0	--
50	7.5	7.2	--
51	7.6	7.5	--
52	7.5	7.1	--
53	8.0	7.9	--
54	7.4	7.1	--
55	7.8	7.2	--
56	7.8	7.4	--
57	7.7	7.4	--
58	7.4	7.4	--
59	7.6	7.4	--
60	7.6	7.6	--
61	7.6	7.6	--
62	7.6	7.6	--

[1] A dissolved oxygen concentration of 52. mg/L represents 60% saturation in freshwater at 22°C.
[2] NC-Data inadvertently not collected.

Appendix 5 (Continued)
Dissolved Oxygen (mg/L) of Water in the Test Chambers[1]

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Day	Depuration Phase	
	Negative Control	0.086 mg a.i./L
1	7.5	7.6
2	7.6	7.7
3	8.3	8.4
4	8.0	8.0
5	8.2	8.2
6	8.0	8.0
7	8.4	8.4
8	8.3	8.4
9	7.9	8.0
10	8.0	8.0
11	8.0	8.0
12	7.9	8.0
13	8.0	8.0
14	7.9	7.9
15	7.6	8.0
16	8.1	8.2
17	8.0	8.0
18	7.9	8.0
19	7.8	7.8
20	8.2	8.2
21	8.0	8.1
22	8.1	8.2
23	8.4	8.4
24	8.4	8.4
25	8.1	8.2
26	7.5	7.6
27	7.9	7.9
28	7.5	7.6
29	8.2	8.2
30	8.0	8.1
31	8.3	8.3
32	8.4	8.4
33	8.0	8.1
34	8.3	8.4
35	8.5	8.5

[1] A dissolved oxygen concentration of 5.2 mg/L represents 6% saturation in freshwater at 22°C.

Appendix 5 (Continued)
Dissolved Oxygen (mg/L) of Water in the Test Chambers[1]

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Day	Depuration Phase	
	Negative Control	0.086 mg a.i./L
36	8.4	8.5
37	8.2	8.1
38	8.0	8.0
39	8.0	8.0
40	8.1	8.2
41	7.7	7.7
42	7.8	7.8
43	8.2	8.3
44	7.8	7.8
45	8.2	8.3
46	8.1	8.1
47	8.6	8.6
48	8.4	8.4
49	8.5	8.6
50	8.1	8.1
51	8.6	8.6
52	8.1	8.1
53	8.2	8.2
54	8.4	8.4
55	8.2	8.3
56	8.2	8.3

[1] A dissolved oxygen concentration of 5.2 mg/L represents 6% saturation in freshwater at 22°C.

Appendix 6

Hardness, Alkalinity, Conductivity and TOC of Water in the Negative Control

Uptake Phase

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Parameter	Day								
	0	7	14	21	28	35	42	49	56
Hardness (mg/L as $CaCO^3$)	132	112	126	130	136	132	104	134	130
Alkalinity (mg/L as $CaCO^3$)	183	174	179	183	181	183	183	184	181
Conductivity (µmhos/cm)	310	325	330	330	325	325	310	320	330
TOC (mg C/L)	<1	<1	<1	<1	<1	<1	<1	<1	<1

Depuration Phase

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Parameter	Day								
	1	7	14	21	28	35	42	49	56
Hardness (mg/L as $CaCO^3$)	134	110	126	124	130	132	132	128	126
Alkalinity (mg/L as $CaCO^3$)	179	178	178	180	183	180	175	179	178
Conductivity (µmhos/cm)	330	330	325	320	325	325	330	320	325
TOC (mg C/L)	<1	<1	<1	<1	<1	<1	<1	<1	<1

Appendix 7
Cumulative Mortality and Treatment-Related Effects[1]
Negative Control – Uptake Phase

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Day	Observations	Cumulative Number Dead	Number Remaining	Number Sampled
0	AN	0	90	5
1	AN	0	85	5
2	AN	0	80	0
3	AN	0	80	5
4	AN	0	75	0
5	AN	0	75	0
6	AN	0	75	0
7	AN	0	75	5
8	AN	0	70	0
9	AN	0	70	0
10	AN	0	70	0
11	AN	0	70	0
12	AN	0	70	0
13	AN	0	70	0
14	AN	0	70	5
15	AN	0	65	0
16	AN	0	65	0
17	AN	0	65	0
18	AN	0	65	0
19	AN	0	65	0
20	AN	0	65	0
21	AN	0	65	5
22	AN	0	60	0
23	AN	0	60	0
24	AN	0	60	0
25	AN	0	60	0
26	AN	0	60	0
27	AN	0	60	0
28	AN	0	60	5
29	AN	0	55	0
30	AN	0	55	0
31	AN	0	55	0
32	AN	0	55	0
33	AN	0	55	0
34	AN	0	55	0
35	AN	0	55	5

[1] Observed Effects: AN = Appears Normal

Appendix 7 (Continued)
Cumulative Mortality and Treatment-Related Effects[1]
Negative Control – Uptake Phase

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Day	Observations	Cumulative Number Dead	Number Remaining	Number Sampled
36	AN	0	50	0
37	AN	0	50	0
38	AN	0	50	0
39	AN	0	50	0
40	AN	0	50	0
41	AN	0	50	0
42	AN	0	50	5
43	AN	0	45	0
44	AN	0	45	0
45	AN	0	45	0
46	AN	0	45	0
47	AN	0	45	0
48	AN	0	45	0
49	AN	0	45	5
50	AN	0	40	0
51	AN	0	40	0
52	AN	0	40	0
53	AN	0	40	0
54	AN	0	40	0
55	AN	0	40	0
56	AN	0	40	5
57	AN	0	35	0
58	AN	0	35	0
59	AN	0	35	0
60	AN	0	35	0
61	AN	0	35	0
62	AN	0	35	10

[1] Observed Effects: AN = Appears Normal

Appendix 7 (Continued)
Cumulative Mortality and Treatment-Related Effects[1]
Negative Control – Uptake Phase

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Day	Observations	Cumulative Number Dead	Number Remaining	Number Sampled
1	AN	0	25	0
2	AN	0	25	0
3	AN	0	25	0
4	AN	0	25	0
5	AN	0	25	0
6	AN	0	25	0
7	AN	0	25	0
8	AN	0	25	0
9	AN	0	25	0
10	AN	0	25	0
11	AN	0	25	0
12	--*	0	25	0
13	AN	0	25	0
14	AN	0	25	5
15	AN	0	20	0
16	AN	0	20	0
17	AN	0	20	0
18	AN	0	20	0
19	AN	0	20	0
20	AN	0	20	0
21	AN	0	20	0
22	AN	0	20	0
23	AN	0	20	0
24	AN	0	20	0
25	AN	0	20	0
26	AN	0	20	0
27	AN	0	20	0
28	AN	0	20	4
29	AN	0	16	0
30	AN	0	16	0
31	AN	0	16	0
32	AN	0	16	0
33	AN	0	16	0
34	AN	0	16	0
35	AN	0	16	0

[1] Observed Effects: AN = Appears Normal
*Biological observations not recorded on this day.

Appendix 7 (Continued)
Cumulative Mortality and Treatment-Related Effects[1]
Negative Control – Uptake Phase

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Day	Observations	Cumulative Number Dead	Number Remaining	Number Sampled
36	AN	0	16	0
37	AN	0	16	0
38	AN	0	16	0
39	AN	0	16	0
40	AN	0	16	0
41	AN	0	16	0
42	AN	0	16	4
43	AN	0	12	0
44	AN	0	12	0
45	AN	0	12	0
46	AN	0	12	0
47	AN	0	12	0
48	AN	0	12	0
49	AN	0	12	0
50	AN	0	12	0
51	AN	0	12	0
52	AN	0	12	0
53	AN	0	12	0
54	AN	0	12	0
55	AN	0	12	0
56	AN	0	12	12

[1] Observed Effects: AN = Appears Normal

Appendix 7 (Continued)
Cumulative Mortality and Treatment-Related Effects[1]
0.086 mg a.i./L – Uptake Phase

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Day	Observations	Cumulative Number Dead	Number Remaining	Number Sampled
0	AN	0	90	5
1	AN	0	85	5
2	AN	0	80	0
3	AN	0	80	5
4	AN	0	75	0
5	AN	0	75	0
6	AN	0	75	0
7	AN	0	75	5
8	AN	0	70	0
9	AN	0	70	0
10	AN	0	70	0
11	AN	0	70	0
12	AN	0	70	0
13	AN	0	70	0
14	AN	0	70	5
15	AN	0	65	0
16	AN	0	65	0
17	AN	0	65	0
18	AN	0	65	0
19	AN	0	65	0
20	AN	0	65	0
21	AN	0	65	5
22	AN	0	60	0
23	AN	0	60	0
24	AN	0	60	0
25	AN	0	60	0
26	AN	0	60	0
27	AN	0	60	0
28	AN	0	60	5
29	AN	0	55	0
30	AN	0	55	0
31	AN	0	55	0
32	AN	0	55	0
33	AN	0	55	0
34	AN	0	55	0
35	AN	0	55	5

[1] Observed Effects: AN = Appears Normal

Appendix 7 (Continued)
Cumulative Mortality and Treatment-Related Effects[1]
0.086 mg a.i./L – Uptake Phase

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Day	Observations	Cumulative Number Dead	Number Remaining	Number Sampled
36	AN	0	50	0
37	AN	0	50	0
38	AN	0	50	0
39	AN	0	50	0
40	AN	0	50	0
41	AN	0	50	0
42	AN	0	50	5
43	AN	0	45	0
44	AN	0	45	0
45	AN	0	45	0
46	AN	0	45	0
47	AN	0	45	0
48	AN	0	45	0
49	AN, 1X	1	45	5
50	AN	1	39	0
51	AN	1	39	0
52	AN	1	39	0
53	AN	1	39	0
54	AN	1	39	0
55	AN	1	39	0
56	AN	1	39	5
57	AN	1	34	0
58	AN	1	34	0
59	AN, 1X	2	34	0
60	AN	2	33	0
61	AN	2	33	0
62	AN	2	33	10

[1] Observed Effects: AN = Appears Normal. X = Dead.

Appendix 7 (Continued)
Cumulative Mortality and Treatment-Related Effects[1]
0.086 mg a.i./L – Depuration Phase

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Day	Observations	Cumulative Number Dead	Number Remaining	Number Sampled
1	AN	2	23	0
2	AN	2	23	0
3	AN	2	23	0
4	AN	2	23	0
5	AN	2	23	0
6	AN	2	23	0
7	AN	2	23	0
8	AN	2	23	0
9	AN	2	23	0
10	AN	2	23	0
11	AN	2	23	0
12	–*	2	23	0
13	AN	2	23	0
14	AN	2	23	5
15	AN	2	18	0
16	AN	2	18	0
17	AN	2	18	0
18	AN	2	18	0
19	AN	2	18	0
20	AN	2	18	0
21	AN	2	18	0
22	AN	2	18	0
23	AN	2	18	0
24	AN	2	18	
25	AN	2	18	0
26	AN	2	18	0
27	AN	2	18	0
28	AN	2	18	4
29	AN	2	14	0
30	AN	2	14	0
31	AN	2	14	0
32	AN	2	14	0
33	AN	2	14	0
34	AN	2	14	0
35	AN	2	14	0

[1] Observed Effects: AN = Appears Normal
*Biological observations not recorded on this day.

Appendix 7 (Continued)
Cumulative Mortality and Treatment-Related Effects[1]
0.086 mg a.i./L – Depuration Phase

Sponsor: 3M Corporation
Test Substance: PFOS
Test Organism: Bluegill, *Lepomis macrochirus*
Dilution Water: Well Water

Day	Observations	Cumulative Number Dead	Number Remaining	Number Sampled
36	AN	2	14	0
37	AN	2	14	0
38	AN	2	14	0
39	AN	2	14	0
40	AN	2	14	0
41	AN	2	14	0
42	AN	2	14	0
43	AN	2	14	4
44	AN	2	10	0
45	AN	2	10	0
46	AN	2	10	0
47	AN	2	10	0
48	AN	2	10	0
49	AN	2	10	0
50	AN	2	10	0
51	AN	2	10	0
52	AN	2	10	0
53	AN	2	10	0
54	AN	2	10	0
55	AN	2	10	0
56	AN	2	10	10

[1] Observed Effects: AN = Appears Normal

Appendix 7 (Continued)
Cumulative Mortality and Treatment-Related Effects[1]
0.087mg a.i./L – Uptake Phase

Sponsor:	3M Corporation
Test Substance:	PFOS
Test Organism:	Bluegill, *Lepomis macrochirus*
Dilution Water:	Well Water

Day	Observations	Cumulative Number Dead	Number Remaining	Number Sampled
0	AN	0	90	5
1	AN	0	85	5
2	AN	0	80	0
3	AN	0	80	5
4	AN	0	75	0
5	AN	0	75	0
6	AN	0	75	0
7	AN	0	75	5
8	AN	0	70	0
9	AN, 1X	1	70	0
10	AN	1	69	0
11	AN	1	69	0
12	AN, 1R	1	69	0
13	AN, 1X	2	69	0
14	AN, 6R, 14X	16	68	5
15	AN, 8X	24	49	0
16	AN, 6X	30	41	0
17	AN	30	35	0
18	AN	30	35	0
19	AN	30	35	0
20	AN	30	35	0
21	AN, 3R, 5X	35	35	5
22	AN, 1R, 4X	39	25	0
23	AN, 1R, 2X	41	21	0
24	AN, 1X	42	19	0
25	AN, 5R, 1X	43	18	0
26	AN, 6X	49	17	0
27	AN, 1R	49	11	0
28	AN, 3X	52	11	5
29	AN, 1C, 1X	53	3	0
30	1C, 1X	54	2	0
31	1C	54	1	0
32	1C	54	1	0
33	1C	54	1	0
34	1C	54	1	0
35	1X	55	1	0

[1] Observed Effects: AN = Appears Normal, C = Lethargic, R= Lying on Bottom, X = Dead.

Appendix 8

Changes to Protocol

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

1. Amendment: The proposed experimental start and termination dates were added to the protocol.
2. Amendment: The frequency of light intensity measurements was added to the protocol.
3. Amendment: The frequency of TOC measurements was added to the protocol.
4. Amendment: The methodology for TOC measurements was added to the protocol.
5. Amendment: Storage stability QC samples were added to the protocol.
6. Amendment: The protocol was clarified to indicate when fish would be sampled for lipid analysis on Day 0.
7. Amendment: The internal reference standard was added to the protocol.
8. Deviation: Dissolved oxygen was not measured on Day 24 of the uptake phase.
9. Deviation: Test temperature was out of range for approximately 2 hours.
10. Deviation: The 0.87 mg a.i./L test concentration adversely affected the test organisms.
11. Deviation: Biological observations were not recorded on Day 12 of the depuration phase.
12. Deviation: Four fish were collected from each treatment on Days 28 and 42 of the depuration phase.
13. Deviation: The depuration phase was terminated on Day 56.
14 Deviation: Fish in the 0.87 mg a.i./L treatment group were not collected for lipid analysis at the end of the uptake or depuration phases.

Appendix 9

Protocol, Amendments and Deviations

PROTOCOL

**PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS):
A FLOW-THROUGH BIOCONCENTRATION TEST WITH
THE BLUEGILL (*Lepomis macrochirus*)**

**U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.1730
and
OECD Guideline 305**

Environmental Laboratory Request Number U2723

Submitted to

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

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8601

October 18, 2000

Wildlife International, Ltd.

-2-

PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS):
A FLOW-THROUGH BIOCONCENTRATION TEST WITH
THE BLUEGILL (*Lepomis macrochirus*)

SPONSOR:	3M Corporation Environmental Laboratory P.O. Box 33331 St. Paul, Minnesota 55133
SPONSOR'S REPRESENTATIVE:	Ms. Susan A. Beach
TESTING FACILITY:	Wildlife International Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Kurt R. Drottar
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.: 454A-134

Test Concentrations: Negative Control, 0.1 and 1.0 mg a.i./L

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

PROTOCOL APPROVAL

STUDY DIRECTOR — DATE 10/31/00

LABORATORY MANAGEMENT — DATE 10/31/00

SPONSOR'S REPRESENTATIVE — DATE 10/24/00

Wildlife International, Ltd.

INTRODUCTION

Wildlife International, Ltd. will conduct a bioconcentration test with the bluegill sunfish (*Lepomis macrochirus*) for the Sponsor at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures in U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.1730 (1); *ASTM Standard E1022-84 Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs* (2); and OECD Guideline for Testing of Chemicals 305, *Bioaccumulation: Flow-Through Fish Test* (3). 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.

OBJECTIVE

The objective of this study is to obtain laboratory data characterizing the bioconcentration potential of Perfluorooctanesulfonate (hereafter referred to as PFOS) in the bluegill, *Lepomis macrochirus*.

EXPERIMENTAL DESIGN

Bluegill will be exposed to two test concentrations and a negative control. Each group will consist of one test chamber with up to approximately 450 grams of fish biomass in each chamber. The test will be divided into an uptake and a depuration phase. During the uptake phase, fish in the treatment groups will be exposed to sublethal concentrations of PFOS, while fish in the control group will be exposed to dilution water. The duration of the uptake phase (3 hours to 28 days) and the depuration phase (6 hours to 60 days) may vary according to the time required to reach steady-state. During both phases of the study, test organisms and water samples are collected and analyzed for PFOS content. These values will be used to determine the uptake rate constant (k_1), the depuration rate constant (k_2), the kinetic bioconcentration factor (BCFK) and the steady-state bioconcentration factor (BCF) for whole body tissues, edible tissue (skin on) and nonedible tissue.

MATERIALS AND METHODS

Test Substance

The test substance will be Perfluorobutanesulfonate, Potassium Salt, hereafter referred to as PFOS. Information on the characterization of test, control or reference substances is required by Good

Wildlife International, Ltd.

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 initiation of 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 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 organisms in water. This route of administration was selected because it represents the most likely route of exposure to aquatic organisms. A primary stock solution of PFOS will be prepared by transferring the PFOS to a volumetric flask and diluting to an appropriate volume with dilution water.

Test Organism

The test organism used in this study will be the bluegill, *Lepomis macrochirus*. Bluegill are one of the recommended fish species for use in bioconcentration tests (1,2,3). Bluegills will be obtained as juveniles from a commercial supplier, gradually acclimated to Wildlife International, Ltd. well water, and held for a period of at least 14 days prior to the test. If the fish are held at water temperatures that differ from the test temperature, they will be brought to test temperature at a rate not exceeding 3°C per 72 hours during holding. If mortality exceeds 3% during the 48-hour period immediately preceding the test, the entire batch of fish will be rejected or held for an additional 14-day period to ensure that they are healthy.

Fish will be handled as little as possible, but when handling is necessary, it will be done carefully, gently, and quickly. To control bias, fish will be removed from holding tanks with nets and impartially distributed to the test chambers. No other forms of bias are expected to affect the results of the study.

Wildlife International, Ltd.

The minimum acceptable size of individual test organisms will be determined by the quantity of tissue required for measurement of residues. Loading (the total wet weight of fish per liter of test solution) will not exceed 1.0 gram of fish per liter of solution that passes through a test chamber in 24 hours.

Bluegill will be fed flake food or another commercial feed, once daily. Excess feed will be siphoned from the tanks approximately 30 minutes after feeding. Feeding and sampling schedules will be coordinated so that fish will be sampled at least four hours after feeding. Specifications for acceptable levels of contaminants in fish diets have not been established. However, there are no known levels of contaminants reasonably expected to be present in the diet that are considered to interfere with the purpose or conduct of the test.

Dilution Water

Water used for the holding and testing of bluegill will be obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The water will be passed through a sand filter and pumped into a 37,800-L storage tank where the water will be aerated with spray nozzles. Prior to use the water will be filtered to 0.45 μm in order to remove fine particles. Water used for holding and testing is characterized as moderately hard. Typical values for hardness, alkalinity, pH and specific conductance are approximately:

Hardness, mg/L as $CaCO_3$	130
Alkalinity, mg/L as $CaCO_3$	170
pH	8.2
Specific Conductance, µmhos/cm	320

Hardness, alkalinity, pH and specific conductance will be measured weekly to monitor the consistency of the well water. Means and ranges of the measured parameters for the four-week period preceding the test will be provided in the final report. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and results of the most recent GLP analyses will be summarized in the final report.

Diluter System and Test Conditions

A continuous-flow diluter will be used to provide two concentrations of PFOS and a control. The flow of dilution water to control and treatment test chambers will be regulated by rotameters. A

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peristaltic pump will be used to inject the test substance working stock solution into a mixing chamber where dilution to the test concentration occurs.

Flow rates will be adjusted so that each test chamber receives at least six volume additions of water every 24 hours. Delivery of the test substance working stock solution will be initiated at least 48 hours prior to the test. Duplicate water samples from each test chamber will be collected at least twice to verify the establishment of equilibrium concentrations of PFOS in the test chambers before introduction of the test organisms (i.e., measured concentrations should remain within 70 to 120% of nominal concentrations). The general operation of the diluter will be checked visually at least two times per day during the test.

Stainless steel aquaria filled with approximately 80 L of test water will be used as test chambers. Test chambers will be placed in a water bath to maintain a test temperature of 22±1°C. The test chambers and water bath will be enclosed in a plexiglass hood in order to minimize potential cross-contamination. Ambient light during testing will be provided by fluorescent tubes that emit wavelengths similar to natural sunlight (e.g. Colortone® 50 or equivalent). A photoperiod of 16 hours of light and 8 hours of darkness will be controlled with an automatic timer. A 30-minute transition period of low light intensity will be provided when lights go on or off to avoid sudden changes in light intensity. Test chambers will be identified by the project number and test concentration.

PROCEDURES

The test will be divided into an uptake and a depuration phase. Organisms in the control group will be exposed to well water in the uptake and depuration phases. Organisms in the treatment groups will be exposed to the test substance in the uptake phase and well water in the depuration phase.

Selection of Test Concentrations

The test concentrations selected will not stress, irritate, or otherwise adversely affect the organisms. The test concentrations will be selected in conjunction with the Sponsor and will be based on acute or chronic toxicity data. The two exposure concentrations should differ by a factor of 10.

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Chemical/Physical Measurements

Temperature will be monitored and recorded continuously during the entire test in one control replicate using a Fulscope ER/C Recorder (1900 J Series Model A), or equivalent. Recorder measurements will be verified prior to the test and at approximately weekly intervals during the test using a liquid-in-glass thermometer. Temperature also will be measured in every 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 will be measured daily in every test chamber during the test using a Yellow Spring Instruments Model 51B dissolved oxygen meter, or equivalent. In the event that dissolved oxygen levels fall below 60% saturation, appropriate actions will be taken after consultation with the Sponsor.

Measurements of pH will be made in every test chamber at the beginning and end of the test and at weekly intervals during the test using a Fisher Accumet Model 915 pH meter, or equivalent. Hardness, alkalinity, and conductivity will be measured in the control treatment at the beginning and end of the test and at weekly intervals during the test. Hardness and alkalinity measurements will be made by titration using procedures based on methods in *Standard Methods for the Examination of Water and Wastewater* (4). Conductivity will be measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter, or equivalent.

Biological Measurements

Observations of behavior and mortality will be made daily. For a test to be considered valid, mortality and abnormal behavior should not be apparent in more than 10% of the fish in the treatment or control groups.

Duration of Uptake Phase

The statistically optimum duration of the uptake phase (u) is near $u = 1.6/k2$, but not more than $3.0/k2$, which is equivalent to 95 percent of steady state. The uptake phase will continue until the concentration of PFOS residues in body tissues reaches steady-state. The criterion for attainment of steady-state is that three consecutive PFOS residue concentrations in fish are not statistically different from each other.

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Duration of Depuration Phase

Two times α is usually a sufficient time for about 95 percent removal of the body burden ($t_{95} = 0.69/k_2$), however, the depuration phase will continue until the concentration of PFOS residue in the organisms attains any one of the following three conditions:

1. Residues reach less than 10% of steady-state; or
2. Residues fall below the LOQ; or
3. Sixty (60) days of depuration have elapsed.

Sample Intervals for Water and Fish Collection

Fish and water will be sampled no less than five times during the uptake phase and four times during the depuration phase of the study at the same intervals. In addition, control and treated water will be sampled no less than twice prior to addition of fish to the chambers to confirm the concentrations desired. In the absence of information on the bioaccumulation potential of the test substance and/or reliable physico-chemical data from which bioaccumulation potential may be estimated, the recommended sampling schedule is as follows.

Phase	Sample Interval	Water[1]	Fish[2]	Action
Pre-Uptake[3]	$-n_1$	X	-	Start
	$-n_2$	X	-	
	0	X	-	Add fish
Uptake	~ 4 hours	X	X	
	1 day	X	X	
	3 day	X	X	
	7	X	X	
	14	X	X	
	21	X	X	
	28	X	X	Transfer
Depuration	1	X	X	
	3	X	X	
	7	X	X	
	10	X	X	
	14[4]	X	X	

[1] At least five replicates of water samples will be collected at each interval listed for the analysis of PFOS. Additional samples will be drawn as necessary for the determination of pH, hardness, alkalinity, specific conductance, and total organic carbon (TOC).
[2] Sufficient fish will be collected to allow the preparation of four replicate samples from each treatment group and duplicate samples from the control group for the analysis of PFOS. Additional samples will be drawn as necessary for the analysis of lipid content.
[3] Pre-Uptake water sampling ($-n_1$, $-n_2$) for confirmation of treatment levels prior to addition of fish.
[4] Depuration phase sampling will continue until the concentration of PFOS residue in the organisms attains any one of the conditions outlined in the duration of depuration phase on page 8.

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The number and frequency of sample intervals may be changed based on physico-chemical properties of the test substance from which predictions of bioaccumulation rate can be derived and/or at the direction of the Sponsor.

At each sampling interval, two water samples will be collected from the control and three water samples will be collected from the PFOS treatment groups. The PFOS concentration will be determined for a minimum of one replicate of a control and two replicates at each treatment level for each interval in which samples are analyzed (as specified by the Study Director). The remaining replicates of samples drawn for PFOS analysis will be held in reserve and may be analyzed at the direction of the Study Director or Chemistry Principal Investigator. The concentration of PFOS in the water at each sample interval will be presented as the average of the replicate measurements, as applicable.

Additional samples will be drawn as necessary for the determination of pH, hardness, alkalinity, specific conductance, and total organic carbon (TOC).

Fish Sampling

To assess the PFOS content in fish tissues, sufficient fish will be collected to allow the preparation of four replicate samples from each treatment group and duplicate samples from the control group. One additional sample from each group will be collected and held in reserve and may be analyzed at the direction of the Study Director or Chemistry Principal Investigator. The concentration of PFOS in fish at each sample interval will be presented as the average of the replicate measurements.

Fish sampled for determination of PFOS content will be impartially removed from the test chambers, rinsed with dilution water, blotted dry, and sacrificed. The weight (wet weight, blotted dry) and total length of each fish will be determined within approximately 15 minutes of collection, if possible. Each fish will be separated into edible and nonedible tissue and the wet weight of each tissue fraction (i.e., edible and nonedible) will be recorded. Fish will be stored frozen if not analyzed immediately.

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Additional samples will be drawn as necessary for the analysis of lipid content..

Analytical Method

Water and tissue samples will be analyzed for PFOS using liquid chromatography-mass spectrometry. Water samples will be analyzed according to the method entitled "Analytial Method Validation for the Determination of Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS) in Freshwater, Saltwater and Algal Media" (Wildlife International, Ltd Project No. 454C-109). Tissue samples will be analyzed according to the method entitled "Analytical Method Validation for the Determination of Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS) in Fish Tissues" (Project No. 454C-119). Any modifications to the above methodologies will be documented in the raw data and described in the final report.

Tissue Lipid Content

At a *minimum*, fish will be sampled from the control aquarium at Day 0 (upon transfer of fish to aquaria), at an interval where the PFOS concentration in fish has reached steady state (or following 28 days of exposure), and at the end of the depuration period. Fish will be sampled to generate four samples from the control and two treated aquaria at each of the intervals noted. The lipid content of the tissue homogenates will be determined by chloroform/methanol extraction.

Data Analysis

Results of tissue analyses will be presented on a wet weight basis. The steady-state bioconcentration factor for the test substance (BCF), kinetic bioconcentration factor (BCFK) uptake rate (k_1), and depuration rate (k_2) will be expressed for edible tissue, nonedible tissue and whole fish (5).

RECORDS TO BE MAINTAINED

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

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Test organism holding and acclimation records.

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5. Methods used to prepare stock solutions and dilutions of the test substance.
6. Daily observations.
7. Water chemistry calculations (e.g., hardness and alkalinity).
8. Organism weight and length measurements.
9. The methods used to analyze test substance concentrations and the results of analytical measurements.
10. Statistical calculations.
11. A copy of the final report.

FINAL REPORT

A 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:

1. Name and address of the facility performing the study.
2. Dates on 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 stated in the approved protocol, including any changes in the original protocol.
5. Statistical methods employed for analyzing the data.
6. The test, control and reference substances identified by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor.
7. Stability and, when relevant to the conduct of the study, the solubility of the test, control and reference substances under the conditions of administration, if provided by the Sponsor or contracted to Wildlife International, Ltd.
8. A description of the methods used.
9. A description of the test system used. Where applicable, the final report shall include the number of animals used, body weight range, source of supply, species, age, and procedure used for identification.
10. A description of the dosage, dosage regimen, route of administration, and duration.
11. A description of all circumstances that may have affected the quality or integrity of the data.

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12. The name of the Study Director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
13. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.
14. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
15. The location where all specimens, raw data, and the 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 shall be in the form of amendment by the Study Director. The amendment should clearly identify the part of the final report that is being added to or corrected and the reasons for the correction or addition. Amendments shall 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); 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.

- 13 -

REFERENCES

1. U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (*Public Draft*), OPPTS Number 850.1730: *Fish BCF.*

2. ASTM Standard E1022-84. 1988. *Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs.* American Society for Testing and Materials.

3. OECD Guideline for Testing of Chemicals 305. 1996. *Bioconcentration: Flow-Through Fish Test.*

4. APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

5. G. E. Blau and G. L. Agin. 1978. BIOFAC. The Dow Company. Midland, MI.

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WILDLIFE INTERNATIONAL LTD.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL (*Lepomis macrochirus*)

PROTOCOL NO.: 454/101800/BLU-BIO/SUB454 **AMENDMENT NO.:** 1

SPONSOR: 3M Corporation **PROJECT NO.:** 454A-134

EFFECTIVE DATE: November 7, 2000

ENVIRONMENTAL LABORATORY REQUEST NO.: U2723

AMENDMENT: Page 2

Add: Experimental Start Date: 12/5/00
Experimental Termination Date: 1/16/01

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

AMENDMENT: Diluter System and Test Conditions, Page 6

Add: Light intensity will be measured at test initiation with a SPER Scientific Ltd. light meter or equivalent.

REASON: To specify the frequency of light intensity measurement.

AMENDMENT: Chemical/Physical Measurements, Page 7

Change: Hardness, alkalinity, and conductivity will be measured in the control treatment at the beginning and end of the test and at weekly intervals during the test.

To: Hardness, alkalinity, conductivity and total organic carbon (TOC) will be measured in the control treatment at the beginning and end of the test and at weekly intervals during the test.

REASON: To specify the frequency of TOC measurements.

AMENDMENT: Chemical/Physical Measurements, Page 7

Add: TOC will be measured using a Shimadzu Model TOC-5000 TOC analyzer.

REASON: To add the methodology for TOC measurements.

Reviewed by QA
GHC 11-7-00

AMENDMENT: Analytical Method, Page 10

Add: Tissue stability samples will be prepared at test initiation to establish test substance stability in fish tissues stored frozen during the study.

REASON: To add storage stability QC samples.

AMENDMENT: Tissue Lipid Content, Page 10

Change: At a minimum, fish will be sampled from the control aquaria at Day 0. . .

To: At a minimum, fish will be sampled on Day 0. . .

REASON: Fish sampled on Day 0 for lipid analysis will be collected prior to distribution to the test chambers.

STUDY DIRECTOR	11/13/00 DATE
LABORATORY MANAGEMENT	11/13/00 DATE
SPONSOR'S REPRESENTATIVE	11/30/00 DATE

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PROJECT NO.: 454A-134
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AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL (*Lepomis macrochirus*)

PROTOCOL NO.: 454/101800/BLU-BIO/SUB454 **AMENDMENT NO.:** 2

SPONSOR: 3M Corporation **PROJECT NO.:** 454A-134

EFFECTIVE DATE: November 28, 2000

ENVIRONMENTAL LABORATORY REQUEST NO.: U2723

AMENDMENT: Page 2

Add: Reference Substance No.: 4526

REASON: To add the internal reference standard.

STUDY DIRECTOR	DATE 11/30/00
LABORATORY MANAGEMENT	DATE 11/30/00
SPONSOR'S REPRESENTATIVE	DATE 12/6/00

Reviewed by QA
AHC 11-29-00

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DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL (*Lepomis macrochirus*)

PROTOCOL NO: 454/101800/BLU-BIO/SUB454 **DEVIATION NO.:** 1

SPONSOR: 3M Corporation **PROJECT NO.:** 454A-134

DATE OF DEVIATION: December 29, 2000

DEVIATION: The protocol states that dissolved oxygen will be measured daily in every test chamber. Dissolved oxygen was not measured on Day 24 of the uptake phase of the test.

REASON: Biologist oversight. All dissolved oxygen measurements during the test were >60% of saturation. Consequently, it is the best judgement of the Study Director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 5/23/01

LABORATORY MANAGEMENT — DATE 5/23/01

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DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL (*Lepomis macrochirus*)

PROTOCOL NO: 454/101800/BLU-BIO/SUB454 **DEVIATION NO.:** 2

SPONSOR: 3M Corporation **PROJECT NO.:** 454A-134

DATE OF DEVIATION: February 24, 2001

DEVIATION: The protocol states that the test chambers will be placed in a water bath to maintain a test temperature of 22 ± 1°C. On Day 19 of depuration, the continuous temperature recorder reached 20°C. The temperature was out of range for approximately 2 hours.

REASON: Unknown. Based on the short duration of the temperature deviation, it is the best judgement of the Study Director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 5/23/01

LABORATORY MANAGEMENT — DATE 5/23/01

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DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL (*Lepomis macrochirus*)

PROTOCOL NO: 454/101800/BLU-BIO/SUB454 **DEVIATION NO.:** 3

SPONSOR: 3M Corporation **PROJECT NO.:** 454A-134

DATE OF DEVIATION: December 14, 2000

DEVIATION: The protocol states that the test concentrations selected will not stress, irritate, or otherwise affect the organisms. The 0.87 mg a.i./L treatment group was actually chronically toxic to bluegill.

REASON: Information on the chronic toxicity of PFOS to bluegill was not known when the test concentrations were selected. This deviation adversely affected the results of the study because adequate bioconcentration data was not obtained from the 0.87 mg a.i./L treatment group.

STUDY DIRECTOR — DATE 6/14/01

LABORATORY MANAGEMENT — DATE 6/14/01

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WILDLIFE INTERNATIONAL LTD.

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL (*Lepomis macrochirus*)

PROTOCOL NO: 454/101800/BLU-BIO/SUB454 **DEVIATION NO.:** 4

SPONSOR: 3M Corporation **PROJECT NO.:** 454A-134

DATE OF DEVIATION: February 17, 2001

DEVIATION: The protocol states that observations of behavior and mortality will be made daily. Biological observations were not recorded on Day 12 of depuration.

REASON: Biologist oversight. Biological observations on Day 11 and 13 of depuration were the same. Consequently, it is the best judgement of the study director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 6/14/01

LABORATORY MANAGEMENT — DATE 6/14/01

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DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL (*Lepomis macrochirus*)

PROTOCOL NO: 454/101800/BLU-BIO/SUB454 **DEVIATION NO.:** 5

SPONSOR: 3M Corporation **PROJECT NO.:** 454A-134

DATES OF DEVIATION: March 5 and 19, 2001

DEVIATION: The protocol states that sufficient fish will be collected to allow the preparation of four replicate samples from each treatment group and duplicate samples from the control group. One additional sample from each group will be collected and held in reserve and may be analyzed at the discretion of the Study Director or Chemistry Principal Investigator. On Days 28 and 42 of depuration, reserve samples were not collected.

REASON: Reserve samples were not collected to conserve the number of fish remaining in the study. It is the best judgement of the study director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 6/14/01

LABORATORY MANAGEMENT — DATE 6/14/01

PROJECT NO.: 454A-134
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WILDLIFE INTERNATIONAL LTD.

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL (*Lepomis macrochirus*)

PROTOCOL NO: 454/101800/BLU-BIO/SUB454 **DEVIATION NO.:** 6

SPONSOR: 3M Corporation **PROJECT NO.:** 454A-134

DATE OF DEVIATION: April 2, 2001

DEVIATION: The protocol states that the depuration phase will continue until the concentration of PFOS residue in the organisms attains any one of the following three conditions: 1) Residues reach less than 10% of steady-state, or 2) Residues fall below the LOQ, or 3) Sixty (60) days of depuration have elapsed. The residues did not meet 1 or 2 above and depuration was terminated on Day 56 of depuration.

REASON: The Sponsor requested that sampling occur every two weeks during depuration. It is the best judgement of the study director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 6/14/01

LABORATORY MANAGEMENT — DATE 6/14/01

PROJECT NO.: 454A-134
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DEVIATION TO STUDY PROTOCOL

STUDY TITLE: PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE BLUEGILL (*Lepomis macrochirus*)

PROTOCOL NO: 454/101800/BLU-BIO/SUB454 DEVIATION NO.: 7

SPONSOR: 3M Corporation PROJECT NO.: 454A-134

DATES OF DEVIATION: February 5 and April 2, 2001

DEVIATION: The protocol states that fish will be samples for lipid analysis at steady-state and at the end of the depuration phase. No fish were sampled from the 0.87 mg a.i./L treatment group.

REASON: There were no fish remaining in the 0.87 mg a.i./L treatment group at the above times. It is the best judgement of the study director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 6/14/01

LABORATORY MANAGEMENT — DATE 6/14/01

Appendix 10

Personnel Involved in the Study

The following key Wildlife International Ltd. personnel were involved in the conduct or management of this study:

1. Mark Jaber, Director of Research
2. Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants
3. Willard B. Nixon, Ph.D., Director, Analytical Chemistry
4. Kurt R. Drottar, Senior Aquatic Biologist
5. Cary A. Sutherland, Laboratory Supervisor
6. Raymond L. Van Hoven, Ph.D., Scientist
7. Susan T. Plantania, Biologist

AMENDED

Appendix 11

Calculations Of The Kinetic Concentration Factor (BCFK),
The Uptake Rate Constant (k_1), The Depuration Rate Constant (k_2),
The Half-Life For Clearance, And The Time To Reach 90% Of Steady State.

1. The depuration rate constant (k_2) was determined from data collected during the depuration phase and was defined as the slope of the regression line where x equals days of depuration and y equals the natural log of the tissue concentration. Results of all calculations were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

depuration rate constant (k_2) values

Edible Fish Tissue	Non-Edible Fish Tissue	Whole Fish Tissue
0.0080	0.0060	0.0062

2. The half-life for clearance in tissue during the depuration phase was determined by the following equation:

$$t_{1/2} = (1/k_2)\ln 2$$

Half-lives for Clearance (days)

Edible Fish Tissue	Non-Edible Fish Tissue	Whole Fish Tissue
86.4	115.5	111.7

3. The uptake rate constant (k_1) was determined from the following equation:

$$k_1 = \frac{C_f k_2}{C_w(1-e^{-k_2 t})}$$

where C_f is the concentration of fish tissue from the uptake/depuration curve near the uptake midpoint (mean concentrations from day 35 were used in calculations);

where C_w is the mean measured concentration in water (0.086 mg a.i./L);

and where t = 35 days.

AMENDED

Appendix 11
(continued)

uptake rate constant (k_1) values

Edible Fish Tissue	Non-Edible Fish Tissue	Whole Fish Tissue
9.022	24.08	17.35

4. It is assumed that the two compartment, two-parameter model applies. This means that the time to reach 90% steady state in the uptake phase is about equal to the time it takes to reach 90% of depuration in the depuration phase. Therefore, k_2, the depuration rate constant, was used in the following equation to determine the time to reach 90% steady state for uptake:

$$0.9 = 1 - e^{-k_2 t}$$

or

$$t_{90} = 2.3 / k_2$$

Time to reach 90% of steady-state (days)

Edible Fish Tissue	Non-Edible Fish Tissue	Whole Fish Tissue
287	383	371

5. BCFK values (k_1 / k_2) are provided below:

BCFK values

Edible Fish Tissue	Non-Edible Fish Tissue	Whole Fish Tissue
1124	4013	2796

AMENDED

Appendix 12

Report Amendment

1. Original Report: Pages 1-8

   Amendment: The pages were changed to include the amended report date, revised page numbers, and new signatures and dates due to the addition of the report amendment as Appendix 12.

   Reason: To reflect the issuing of an amended report.

2. Original Report: Page 2 and 4

   Amendment: The Study Director was changed to Henry Krueger and Management was changed to Mark Jaber.

   Reason: Reassignment of responsibilities due to the departure of the original Study Director.

3. Original Report: Data Analysis Section, Page 15

   Amendment: This section was modified to clarify that the tests for normality and homogeneity met the assumptions of the tests. Additionally, the section was modified to explain why the computer program BIOFAC was not used to estimate the kinetic concentration factor (BCFK), the uptake rate constant (k_1), the depuration rate constant (k_2), the half-life for clearance, and the time to reach 90% of steady state. However, after further review, the BIOFAC program may not be the best way to estimate these parameters.

   Reason: After consultation with the Sponsor it was believed that the BIOFAC model underestimates the uptake and depuration rate constants, and overestimates the kinetic concentration factor, the estimated time to reach 90% steady state, and the half-life for clearance. Therefore, the data have been reanalyzed using the equations and graphical methods outlined in the draft OPPTS 850.1730 Guidance Document.

4. Original Report: Pages 17-18 and 31-33

   Amendment: The results and conclusion sections, as well as the Table 6 and Figures 1-3 have been changed to reflect the recalculated numbers.

   Reason: To report the results of the test based on recalculated numbers.

AMENDED

Appendix 12

-Continued-

Report Amendment

5. Original Report: Addition of Appendix 11.

Amendment: Appendix 11 was added to detail how calculations in the amended report were performed.

Reason: To provide adequate explanation of how the new calculations were performed.

AMENDMENT SIGNATURES:

Henry O. Krueger, Ph.D. — DATE: 6 June 02
Director of Aquatic Toxicology and Non-Target Plants

Mark Jaber — DATE: 6/6/02
Director of Research

REVIEWED BY:

Marshall T Hynson — DATE: 6/6/2002
Quality Assurance

AMENDED