Determination of the Water Solubility of PFOS by the Shake Flask Method

WATER SOLUBILITY

TEST SUBSTANCE

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

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

METHOD

Method: OECD 105, OPPTS 830.7840, and 40 CFR 796.1840.
GLP (Y/N): Yes
Year completed: Study completed 1999. Report completed 2000

Remarks: The definitive test consisted of equilibration of an excess amount of test substance with NANOpure® water at 30°C followed by equilibration at 20°C and analyzing subsamples by high performance liquid chromatography with mass spectrometric detection (LCMS).

NANOpure® water is equivalent to ASTM Type II Designation D1193-91.

RESULTS

Value (mg/L) at temperature °C: 519 mg/L at 20 ± 0.5°C.
Description of solubility: Slightly soluble.

Remarks: Triplicate subsamples were removed from the appropriate bottles after one, two and three days of shaking in a water bath maintained at 30 ± 1.0°C and following one day of a 20 ± 0.5°C equilibration period. Analysis of aqueous subsamples after one day had a mean analytical result of 459 mg/L (SD = 8.96, CV = 1.95%). For subsamples collected after two and three days, the mean concentration were 537 mg/L (SD = 27.6, CV = 5.14%) and 501 mg/L (SD = 64.2, CV = 12.8%), respectively.

CONCLUSIONS

The Day 2 and Day 3 mean solubility concentration were within 15% of each other and were averaged to obtain the overall mean solubility concentration. The overall mean solubility concentration of the test substance in NANOpure® water was 519 mg/L (SD = 48.3; CV = 9.31%; N = 6).

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

DATA QUALITY

Reliability: Klimisch ranking 1.

REFERENCES

Study conducted at the request of 3M Company by Wildlife International, Ltd. of Easton, Maryland.

OTHER

Last changed: 5/3/00

DETERMINATION OF THE WATER SOLUBILITY OF PFOS BY THE SHAKE FLASK METHOD

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454C-107

OECD Guideline for the Testing of Chemicals, 105
Water Solubility

AUTHORS:

Raymond L. VanHoven, Ph.D.
Willard B. Nixon, Ph.D.

STUDY INITIATION DATE: February 10, 1999

STUDY COMPLETION DATE: May 3, 1999

AMENDED REPORT DATE: April 26, 2000

Submitted to

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

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

AMENDED

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: Determination of the Water Solubility of PFOS by the Shake Flask Method

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454C-107

3M LAB REQUEST NO.: U2723

STUDY COMPLETION: May 3, 1999

AMENDED REPORT: April 26, 2000

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989 and OECD Principles of Good Laboratory Practice, OCDE/GD (92) 32, Environment Monograph No. 45, Paris 1992, with the following exceptions:

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

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

STUDY DIRECTOR:

Raymond L. VanHoven, Ph.D.
Scientist
Wildlife International Ltd.

4-26-00
DATE

SPONSOR APPPROVAL:

Susan A. Beach
Sponsor

4/27/00
DATE

AMENDED

QUALITY ASSURANCE STATEMENT

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

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Test Substance Preparation	February 15, 1999	February 15, 1999	February 15, 1999
Sample Preparation	February 17 and 18, 1999	February 18, 1999	February 18, 1999
Draft Report and Data	March 16 and 17, 1999	March 17, 1999	March 23, 1999
Final Report	May 3, 1999	May 3, 1999	May 3, 1999
Amended Report	April 26, 2000	April 26, 2000	April 26, 2000

James H. Coleman

James H. Coleman
Quality Assurance Representative

4-26-00

DATE

AMENDED

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: Determination of the Water Solubility of PFOS by the Shake Flask Method

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 454C-107

3M LAB REQUEST NO.: U2723

STUDY DIRECTOR:

Raymond L. VanHoven, Ph.D.
Scientist

4-26-00
DATE

MANAGEMENT:

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

4/26/00
DATE

AMENDED

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SUMMARY

STUDY TITLE:	Determination of the Water Solubility of PFOS by the Shake Flask Method
WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	454C-107
SPONSOR:	3M Corporation
TESTING FACILITY:	Wildlife International Ltd. Easton, Maryland 21601
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International Ltd. Easton, Maryland 21601

TEST SUBSTANCE:	PFOS
TEST DATES:	Experimental Start - February 11, 1999 Experimental Termination - February 20, 1999

TEST SYSTEM:	NANOpure® Water

SUMMARY:	The overall mean solubility concentration of PFOS in NANOpure® water was 519 mg a.i./L (SD = 48.3; CV = 9.31%; N = 6).

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INTRODUCTION

This study was conducted by Wildlife International Ltd. for 3M Corporation at the Wildlife International Ltd. analytical chemistry facility in Easton, Maryland. A test was conducted to determine the water solubility of PFOS (perfluorooctane sulfonic acid, potassium salt). The experimental portion of this study was conducted between February 11, 1999 and February 20, 1999. The original raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 454C-107 in archives located on the Wildlife International Ltd. site.

PURPOSE

The purpose of this study was to determine the water solubility of PFOS at 20 ± 0.5°C by the shake flask method. Determination of water solubility by the shake flask method is applicable to test substances with water solubilities equal to or exceeding 0.01 gram/Liter (g/L).

EXPERIMENTAL DESIGN

The water solubility of PFOS was determined at a temperature of 20 ± 0.5°C. A preliminary test was performed to estimate the water solubility. The test consisted of additions of increasingly large amounts of NANOpure® water to a known weight of test substance at room temperature. The definitive test consisted of equilibration of an excess amount of test substance with NANOpure® water at 30°C followed by equilibration at 20°C and analyzing subsamples by high performance liquid chromatography with mass spectrometric detection (LCMS).

MATERIALS AND METHODS

This study was conducted according to the procedures outlined in the protocol, "Determination of the Water Solubility of PFOS by the Shake Flask Method." The protocol was based on procedures outlined in the OECD Guideline for Testing of Chemicals, 105: *Water Solubility* (1). This study meets data requirements under this guideline and under U.S. EPA Product Properties Test Guidelines, OPPTS, Series:830.7840, *Water Solubility:*

Column Elution Method; Shake Flask Method (2) for shake flask methodology and TSCA Title 40 of the Federal Code of Regulations, Part 796, Section 1840: *Water Solubility* (3).

Test Substance

The test substance was received from 3M Corporation on October 29, 1998 and was assigned Wildlife International Ltd. identification number 4675 upon receipt. The test substance, a white powder, was identified as: FC-95, Lot Number: 217, Expiration Date: 2008 and a reported purity of 98.9%. The test substance was reanalyzed by the Sponsor and the Certificate of Analysis dated March 9, 2000 indicated a purity of 90.49%. The test substance was stored under ambient conditions.

Internal Standard

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

Reagents

All solvents used in the solubility analyses were of ACS reagent grade. Nanopure® water (equivalent to ASTM Type II Designation D1193-91) was used (4).

Preliminary Test Procedure

A preliminary test was conducted to estimate the water solubility of PFOS. The test was performed at room temperature by adding 10.0 mg of the test substance to a 50-mL volumetric flask. Increasing volumes of NANOpure® water were added (10.0, 25.0 and 50.0 mL) to the flask. After each addition, the container was shaken vigorously for approximately 10 minutes and visually examined for undissolved test substance. After the addition of 50-mL of NANOpure® water, no particulates were observed.

Definitive Test Procedure

Approximately 0.500 grams of the test substance was weighed into each of three labelled 16-ounce French square bottles. NANOpure® water, 250 mL equilibrated at 30°C for approximately 30 minutes, was added to

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each bottle. The bottles were sealed with screwcaps and wrapped with parafilm. The bottles were agitated in a shaker-water bath (Lindberg/Blue, Model No. SWB1122A) maintained at 30 ± 1.0°C. After one day, one bottle was removed and placed in a second constant temperature bath (Lindberg/Blue, Model No. SWB1122A, with agitation switched off) maintained at 20 ± 0.5°C for 24 hours with occasional shaking. Triplicate 10-mL subsamples were removed from the bottle, centrifuged at approximately 3000 rpm for approximately 15 minutes in a centrifuge regulated at 20°C. Fifty microliters from each subsample were volumetrically removed and diluted to 500 mL with a solution of 50% methanol and 50% NANOpure® water containing 0.05% v/v formic acid and 0.100 mg/L of the internal standard. The dilutions were placed into autosampler vials for analysis by reverse-phase high performance liquid chromatography (HPLC) using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 100LC Mass Spectrometer equipped with a Perkin-Elmer Turbo IonSpray ion source. The remaining two bottles were treated in a similar manner following initial equilibration periods of 2 and 3 days at 30.0 ± 1.0°C. Instrumental parameters for the analysis of PFOS are summarized in Table 1 and a method flowchart is provided in Figure 1.

Calibration Curve

Calibration standards of PFOS, ranging in concentration from 9.15 to 45.7 µg a.i./L or 9.15 to 68.6 µg a.i./L, were analyzed with each sample set. Each calibration standard contained 0.100 mg/L of the internal standard. A linear regression equation was generated using the peak area responses (ratios of PFOS to the internal standard) versus the respective concentrations of the calibration standards. A typical calibration curve is presented in Figure 2. The concentration of PFOS in the samples was determined by substituting the peak area response ratios into the applicable linear regression equation. Representative ion chromatograms of low and high calibration standards are presented in Figures 3 and 4, respectively.

Reagent Blanks

Reagent blanks were analyzed to assess the presence of potential interferences. No chromatographic interferences were observed in the blanks (prepared from a solution of 50% methanol and 50% NANOpure® water containing 0.05% v/v formic acid and 0.100 mg/L of the internal standard) at or above the limit of quantitation of 9.15 µg a.i./L (Table 2). A representative ion chromatogram of a reagent blank is presented in Figure 5.

Example Calculations

Sample number 454C-107-2A, nominal concentration of 2000 mg/L in NANOpure® water.

Initial Volume: 0.0500 mL
Final Volume: 500 mL
Dilution Factor: 10,000
PFOS Peak Area: 687581
Internal Standard Peak Area: 603048
Peak Area Ratio: 1.140

Calibration curve equation.
Slope: 0.020
Intercept: 0.068
Curve is weighted: (1/x)

Calculated concentration: 539 mg a.i./L
Note: manual calculations may differ.

RESULTS

Water Solubility

Triplicate subsamples were removed from the appropriate bottles after one, two and three days of shaking in a water bath maintained at 30 ± 1.0°C and following one day of a 20 ± 0.5°C equilibration period (Table 3). Analysis of aqueous subsamples after one day had a mean analytical result of 459 mg a.i./L (SD = 8.96, CV = 1.95%). For subsamples collected after two and three days, the mean concentrations were 537 mg a.i./L (SD = 27.6, CV.= 5.14%) and 501 mg a.i./L (SD = 64.2, CV = 12.8%), respectively. A representative ion chromatogram of a Day 2 solubility sample is presented in Figure 6.

CONCLUSIONS

The Day 2 and Day 3 mean solubility concentrations were within 15% of each other and were averaged to obtain the overall mean solubility concentration. The overall mean solubility concentration of PFOS in NANOpure® water was 519 mg a.i./L (SD = 48.3; CV = 9.31%; N = 6).

AMENDED

REFERENCES

1. **Organisation for Economic Cooperation and Development.** 1995. Guideline for Testing of Chemicals, 105: *Water Solubility.*

2. **Product Properties Test Guidelines.** 1996. OPPTS 830.7840. *Water Solubility: Column Elution Method; Shake Flask Method.*

3. **TSCA Title 40 of the Federal Code of Regulations.** 1994. Part 796, Section 1840: *Water Solubility.*

4. **American Society for Testing and Materials.** 1991. Standard Specification for Reagent Water. D1193-91, ASTM Section II Water and Environmental Technology, Vol. 11.01:45-47.

Table 1

Typical LC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 100LC Mass Spectrometer equipped with a Perkin-Elmer Turbo IonSpray ion source. Operated in selective ion monitoring mode (SIM).
ANALYTICAL COLUMN:	Keystone Betasil C_{18} column (100 mm x 2 mm, 3 µm particle size)
OVEN TEMPERATURE:	30°C
STOP TIME:	10 minutes
FLOW RATE:	0.220 mL/minute
MOBILE PHASE:	72% Methanol : 28% NANOpure® Water containing 0.1% Formic Acid
INJECTION VOLUME:	50.0 µL
PFOS RETENTION TIME:	Approximately 7.07 minutes
INTERNAL STANDARD RETENTION TIME:	Approximately 4.85 minutes
PFOS MONITORED MASS:	498.6 amu
INTERNAL STANDARD MONITORED MASS:	426.7 amu

Table 2

Reagent Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured Concentration of PFOS (µg a.i./L)[1]
Number (454C-107-)	Type	
Blank t_{24}	Reagent Blank	< LOQ
Blank t_{48}	Reagent Blank	< LOQ
Blank t_{72}	Reagent Blank	< LOQ

[1] The limit of quantitation (LOQ) of 9.15 µg a.i./L was based upon the product of the lowest calibration standard analyzed (9.15 µg a.i./L) and the dilution factor of the respective blank sample (1.00).

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Table 3

Solubility of PFOS in NANOpure® Water

Sample Number (454C-107-)	Day of Test	Concentration of PFOS		Mean SD CV
		Fortified (mg/L)	Measured (mg a.i./L)	
1A	1	2000	464	Mean = 459
				SD = 8.96
1B	1	2000	465	CV = 1.95%
1C	1	2000	449	
2A	2	2000	539	Mean = 537
				SD = 27.6
2B	2	2000	508	CV = 5.14%
2C	2	2000	563	
3A	3	2000	562	Mean = 501
				SD = 64.2
3B	3	2000	434	CV = 12.8%
3C	3	2000	507	
	Totals:	Mean (Days 2 and 3)=	519	
		SD =	48.3	
		CV =	9.31%	
		N =	6	

Note: Results and corrections were generated using MacQuan version 8.3.3 software and manual calculations. Values have been rounded for reporting purposes.

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METHOD OUTLINE FOR THE PROCESSING OF PFOS IN NANOPURE® WATER

Prepare calibration standards in a solution of 50% methanol and 50% NANOpure® water containing 0.05% v/v formic acid and 0.10 mg/L of the internal standard using volumetric flasks and gas-tight syringes.

↓

Prepare solubility samples by weighing 0.500 grams into three 16-ounce French square bottles and adding 250 mL of Nanopure® water to each.

↓

After the appropriate equilibration periods, centrifuge triplicate 10-mL subsamples at approximately 3,000 rpm for approximately 15 minutes in a centrifuge regulated to 20°C. Dilute subsamples to 500 mL with a solution of 50% methanol and 50% NANOpure® water containing 0.05% v/v formic acid and 0.10 mg/L of the internal standard.

↓

Transfer samples and standards to autosampler vials for analysis by LC/MS.

Figure 1. Analytical method flow chart for the analysis of PFOS in Nanopure® water.

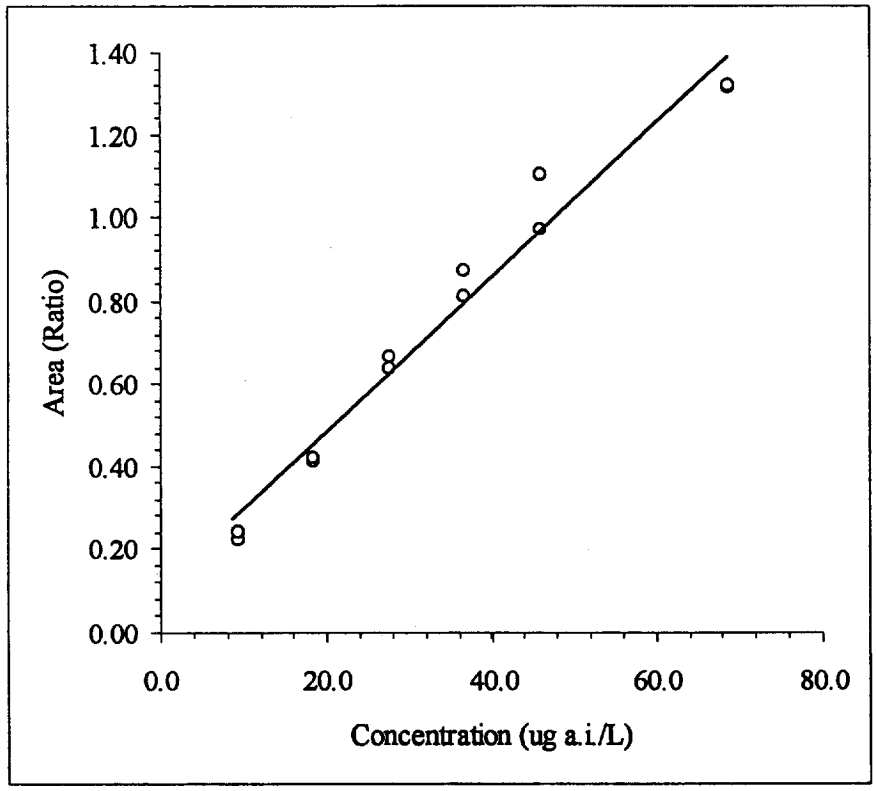

Figure 2. A typical calibration curve for PFOS. Slope = 0.020; Intercept = 0.068; R^2 = 0.990, Weighted (1/x).

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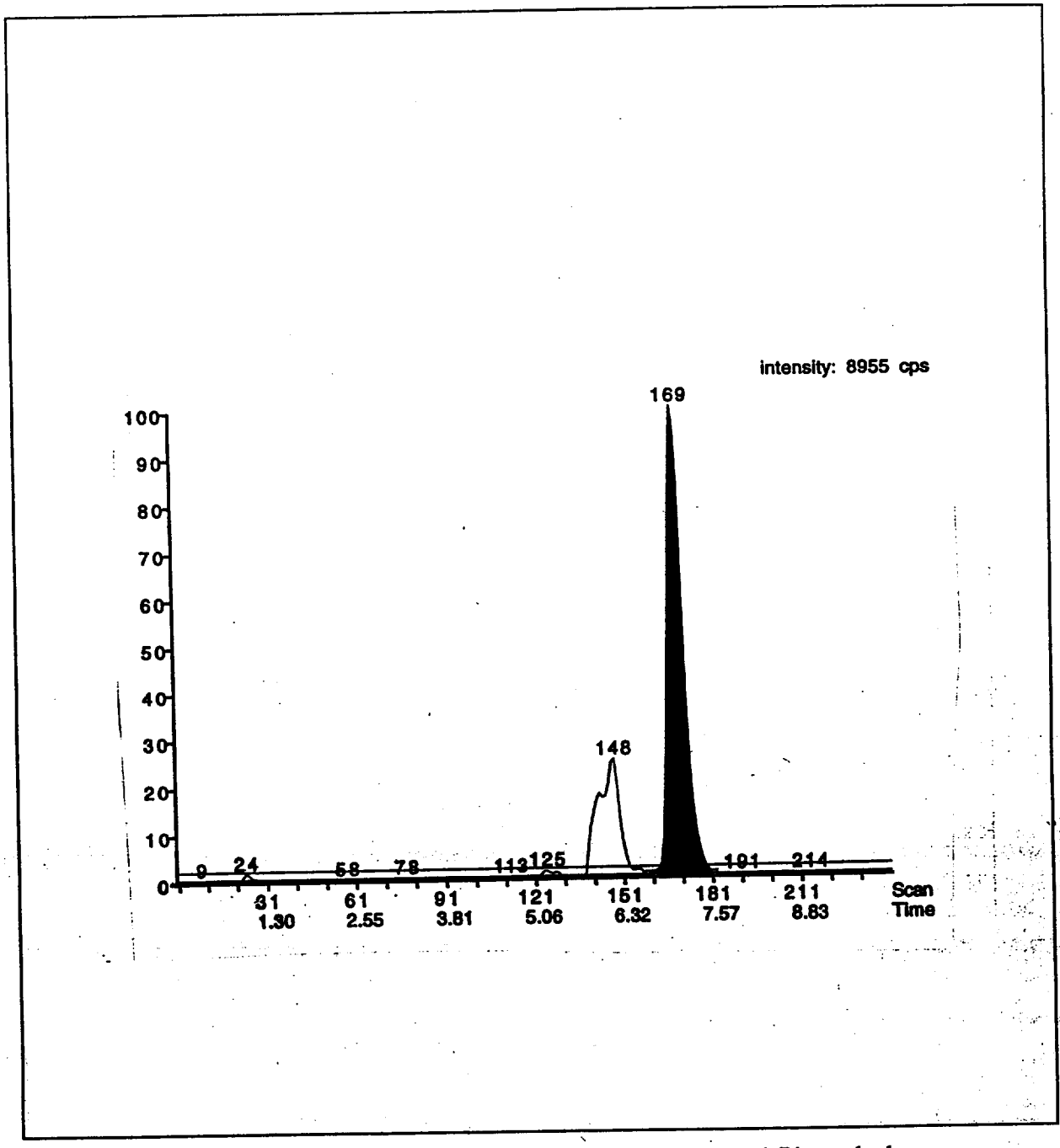

Figure 3. A representative ion chromatogram of a low-level (9.15 µg a.i./L) standard.

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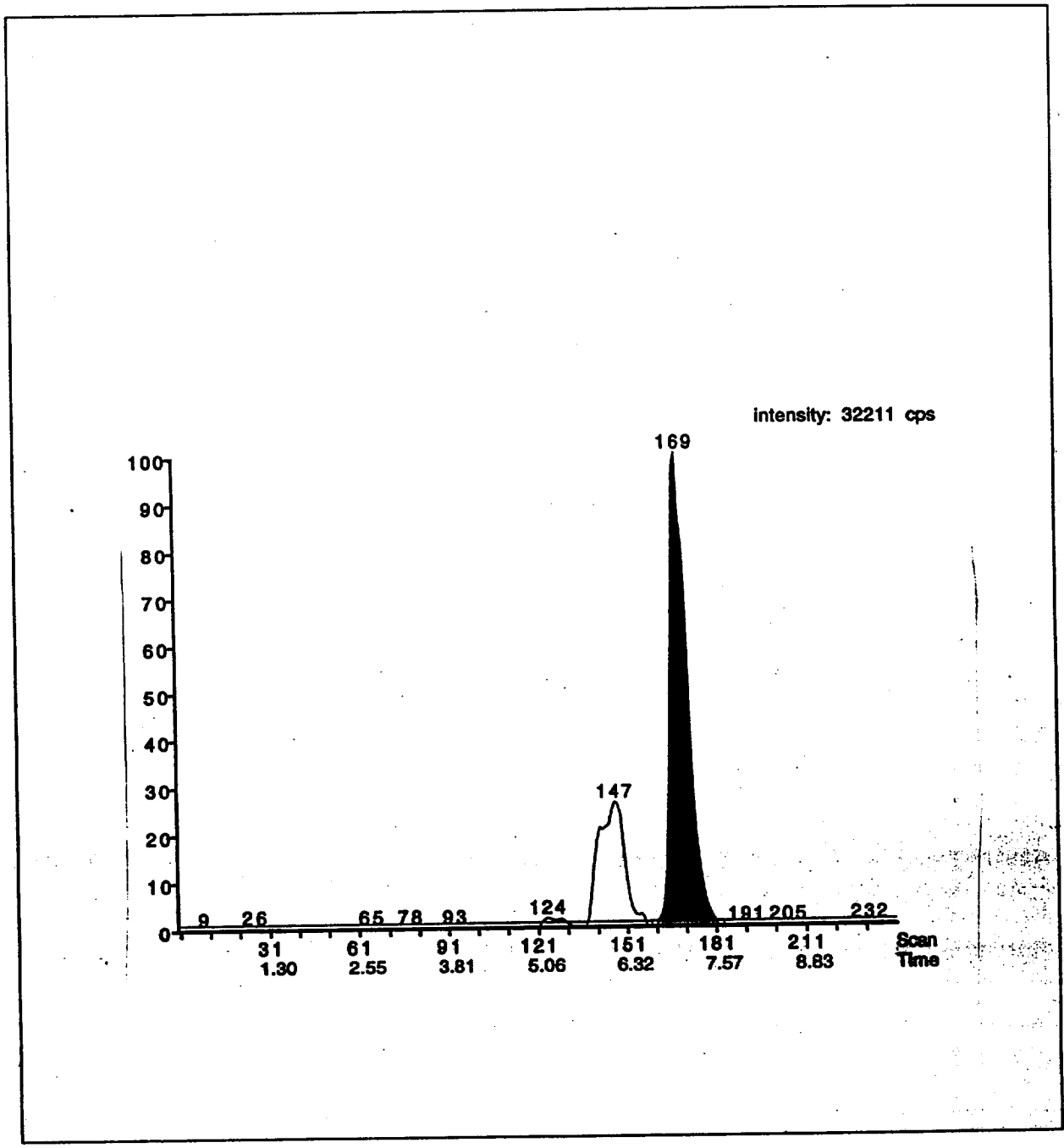

Figure 4. A representative ion chromatogram of a high-level (45.7 µg a.i./L) standard.

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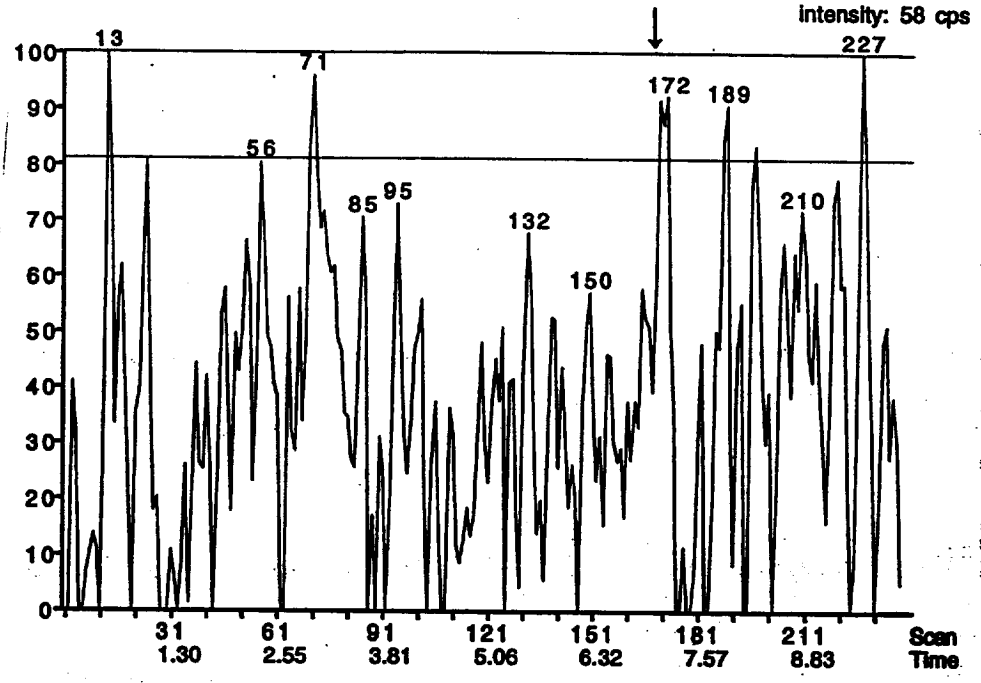

Figure 5. A representative ion chromatogram of a reagent blank sample (454C-107-Blank-t_{48}). The arrow indicates the retention time of PFOS.

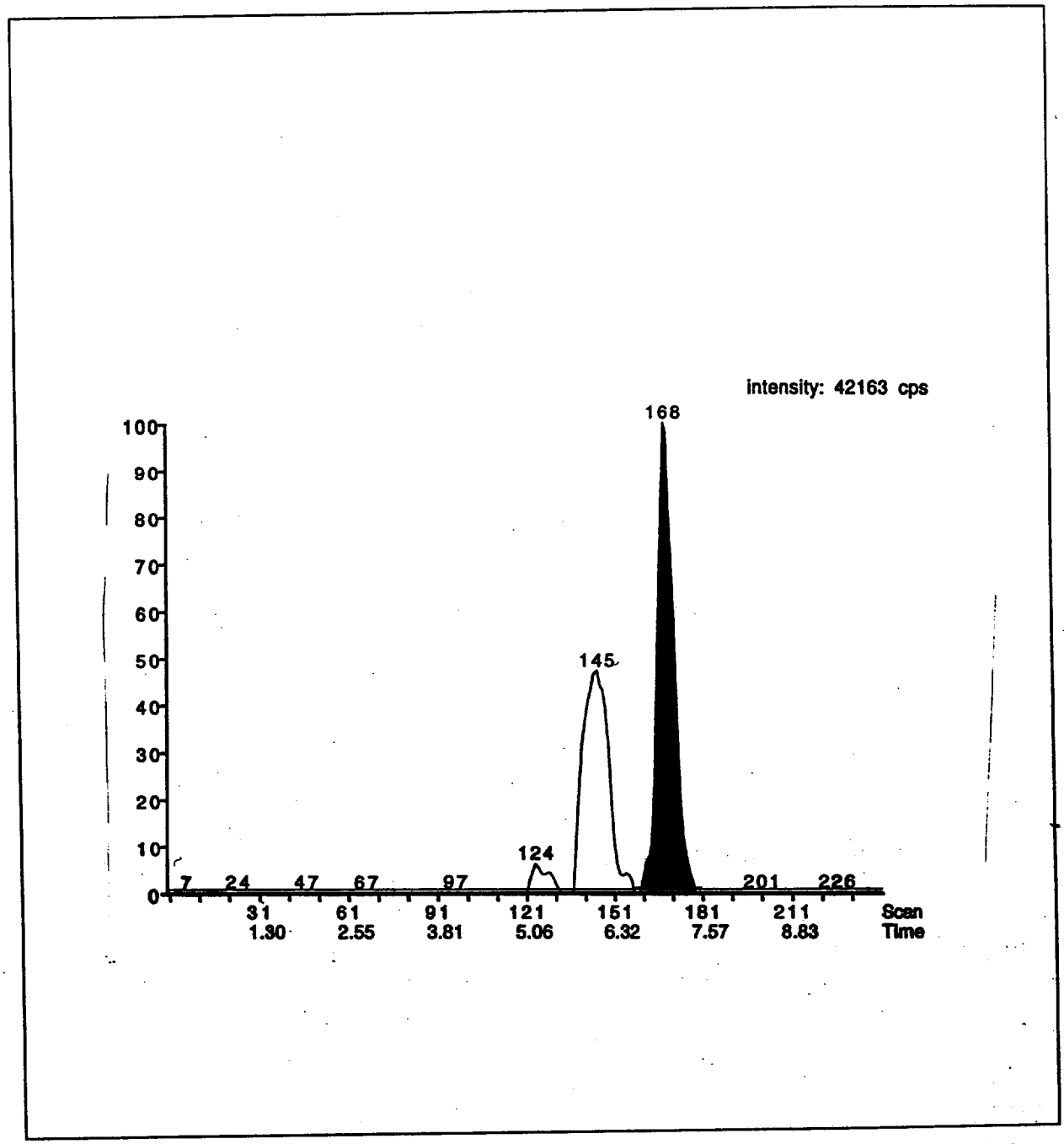

Figure 6. A representative ion chromatogram of a Day 2 solubility sample, 454C-107-2A (nominal concentration of 2000 mg/L).

APPENDIX I

Protocol

PROTOCOL

DETERMINATION OF THE WATER SOLUBILITY OF PFOS
BY THE SHAKE FLASK METHOD

OECD Guideline for the Testing of Chemicals, 105
Water Solubility

3M Lab Request No. U2723

Submitted to

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

WILDLIFE INTERNATIONAL LTD.
8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

December 2, 1998

PROTOCOL NO.: 454/120298/830.7840/SUB454 3M LAB REQUEST NO. U2723

WILDLIFE INTERNATIONAL LTD.

DETERMINATION OF THE WATER SOLUBILITY OF PFOS
BY THE SHAKE FLASK METHOD

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:	~~Ronda Jacobs~~ Raymond L. VanHoven, Ph.D. ~~Senior Chemist~~ Scientist
LABORATORY MANAGEMENT:	Willard B. Nixon, Ph.D. Manager of Chemistry

FOR LABORATORY USE ONLY

Proposed Dates:			
Experimental Start Date:	2/10/99	Experimental Termination Date:	3/10/99
Project No.:	454C-107	Int/Date:	RJL 2/10/99
Test Substance No.:	41675	Receipt Date:	10/29/98

PROTOCOL APPROVAL

Raymond L. VanHoven — STUDY DIRECTOR — DATE 2/10/99

Willard B. Nixon — LABORATORY MANAGEMENT — DATE 2/10/99

Susan A. Beach — SPONSOR'S REPRESENTATIVE — DATE 12/7/98

PROTOCOL NO.: 454/120298/830.7840/SUB454 3M LAB REQUEST NO. U2723

INTRODUCTION

Wildlife International Ltd. will experimentally determine the water solubility of PFOS (perfluorooctane sulfonic acid, potassium salt). The study will be conducted at the Wildlife International Ltd. analytical chemistry facility in Easton, Maryland. The study will be performed following the guidance presented in the OECD Guideline for Testing of Chemicals, 105: *Water Solubility* (1) for shake flask methodology. This study is intended to meet data requirements under this guideline and under EPA Product Properties Test Guidelines, OPPTS 830.7840, *Water Solubility: Column Elution Method; Shake Flask Method* (2) and TSCA Title 40 of the Federal Code of Regulations, Part 796, Section 1840: *Water Solubility* (3). The method of analysis for quantitation provided by the Sponsor will be verified at Wildlife International Ltd. Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report.

PURPOSE

The purpose of this study is to determine the water solubility of PFOS at 20 ± 0.5°C by the shake flask method. Determination of water solubility by the shake flask method is applicable to test substances with water solubilities equal to or exceeding 0.01 gram/Liter (g/L). The methodology may not be applicable to surface active or volatile test substances.

EXPERIMENTAL DESIGN

The water solubility of PFOS will be determined at a temperature of 20 ± 0.5°C. A preliminary test will be performed to estimate the water solubility at room temperature. The preliminary test consists of additions of increasingly large amounts of water to a known weight of test substance until solubilization is effected or the solubility is estimated to be less than 0.01 g/L. For substances with an estimated water solubility greater than 0.01 g/L, a definitive test will be performed. The definitive test consists of equilibration of an excess amount of test substance with water at an elevated temperature, 30°C, followed by equilibration at 20°C.

MATERIALS AND METHODS

Test Substance

The test substance will be the highest purity available. The test substance should be at least 99% pure by weight. The Sponsor will be asked to provide the following information concerning the test substance, if available:

WILDLIFE INTERNATIONAL LTD.

Chemical Name	Lot or Batch Number
Empirical Formula and/or Molecular Weight	Chemical Purity:
Dissociation Constant in Water	Vapor Pressure
Expiration Date	Storage Conditions
Method of Analysis	

The attached form IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR (Appendix I), along with a Material Safety Data Sheet, will be used to provide information necessary for the handling and testing of the test substance. The Sponsor agrees to accept any unused test substance and/or test substance containers at the end of the study.

Reagents

Water that meets ASTM Type II standards (ASTM D 1193-91) will be used (4). Other solvents may be needed for the analytical method or preparation of stock solutions and analytical standards. All solvents will be ACS reagent grade or better, and will be determined to be free of contaminants that interfere with the quantitation of the test substance.

Preliminary Test Procedure

A preliminary test will be conducted to estimate the solubility of the test substance at room temperature. Approximately 10 mg of the test substance will be placed in a plastic tube. Increasing volumes of water will be added stepwise as shown below:

Total volume of water added (mL)	0.1	0.5	1	2	10
Approximate solubility (g/L)	>100	20	10	5	1

After each addition of water to yield the total volume indicated, the container will be capped and shaken vigorously for approximately 10 minutes and visually examined for undissolved test substance. If the test substance is not dissolved after addition of 10 mL of water, the contents of the container will be transferred to a larger container and water added for a final volume of 100 mL. Following capping and shaking, the container will be visually checked for undissolved material, i.e., undissolved material would indicate a water solubility <0.1 g/L. Preliminary testing of the solubility at lower concentrations (<0.1 g/L) may be performed by transferring the contents of the 100-mL vessel to a 1-L vessel or reinitiating the test with a lesser amount of test substance, e.g., 1 mg. Amounts and volumes noted above are only guides for conduct of the preliminary study; larger amounts of test substance with proportionally larger volumes of water may be used.

Definitive Test Procedure

The quantity of test substance necessary to saturate the desired volume of water is estimated from the preliminary test. The volume of water used will depend on the analytical method and solubility range. Greater than (or equal to) five times the quantity of test substance required to achieve water solubility will be transferred to a minimum of three test vessels (plastic and/or other suitable container material). The desired volume of water will be added to each vessel and the vessels sealed. The closed vessels will be agitated (shaken or stirred) at 30 ± 1.0°C in a water bath. After 1 day, one of the vessels will be removed and re-equilibrated for 24 hours at 20 ± 0.5°C with occasional shaking or stirring. The contents of the vessel will then be centrifuged in a centrifuge regulated at 20°C. The concentration of the test substance in a minimum of two aliquots of the aqueous phase will be determined. The remaining two vessels are treated similarly following initial equilibration periods of 2 and 3 days at 30 ± 1.0°C. The test will be terminated if the concentrations of the test substance in the aqueous phase of two consecutive vessels agree within 15%. If greater variation is observed and/or at the request of the Sponsor, the test may be reinitiated with longer equilibration periods.

Method

The analytical method for quantitation of the test substance will be based on procedures provided by the Sponsor. The method will be appended to this protocol in Appendix II and referenced in the report. The method may be verified as part of the preliminary test(s) and may be modified to measure the observed test substance concentrations. Major modifications will be approved by the Sponsor and Study Director, and documented in the raw data.

Calculations

The concentration of the test substance in each vessel will be expressed in g/L, mg/L or µg/L in water as appropriate. The average solubility for test vessels with solubilities within 15% of each other will be averaged and reported.

Sample Handling and Safety

The Sponsor will identify any special handling or safety precautions to be used with the above referenced test substance. All normal precautions with respect to handling and storage will be taken.

Sample and Test Substance Retention

Upon completion of testing, portions of the test substance used as part of this study will be disposed of in accordance with federal, state and local regulations. Any unused portion of the test substance will be returned to the Sponsor.

RECORDS TO BE MAINTAINED

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

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and completion of the study.
4. Dates of experimental start and termination.
5. Storage conditions of the test substance.
6. Test substance use log.
7. Concentration calculations and records of solution preparation.
8. Instrument operating conditions and chromatograms, if applicable.
9. Statistical calculations.
10. Test conditions.
11. A copy of the final report.

FINAL REPORT

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

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Purpose and procedure, as stated in the approved protocol, including all amendments and deviations to the protocol.
5. The test substance identification, including name or code number, purity, empirical formula, molecular formula, lot or batch number, method of analysis, and any other information provided by the Sponsor.
6. Description of the test method or reference to the method used along with any modifications made.

WILDLIFE INTERNATIONAL LTD.

7. The individual concentrations of each sample.
8. The means and standard deviations of solubility determinations at each interval for each temperature tested.
9. Description of any problems experienced and how they were resolved.
10. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and findings reported to the Study Director and Management.

CHANGING OF PROTOCOL

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

GOOD LABORATORY PRACTICES

This study will be conducted according to the Good Laboratory Practices described in OECD (ISBN 92-84-12367-9) and EPA (40 CFR Part 792). 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. Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report

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REFERENCES

1 **Organisation for Economic Cooperation and Development.** 1995. Guideline for Testing of Chemicals, 105: *Water Solubility.*

2 **Product Properties Test Guidelines.** 1996. OPPTS 830.7840. *Water Solubility: Column Elution Method; Shake Flask Method.*

3 **TSCA Title 40 of the Federal Code of Regulations.** 1994. Part 796, Section 1840. *Water Solubility.*

4 **American Society for Testing and Materials.** 1991. Standard Specification for Reagent Water. D1193-91, ASTM Section II Water and Environmental Technology, Vol. 11.01: 45-47.

WILDLIFE INTERNATIONAL LTD.

APPENDIX I
IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

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

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

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

Test Substance Sample Code or Batch Number: Lot 217

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

II. Test Substance Characterization

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

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International Ltd.

Ambient room temperature

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

Other pertinent stability information:

IV. Test Concentrations:

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

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

V. Toxicity Information:

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

Aquatic:

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

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

Please see MSDS

WILDLIFE INTERNATIONAL LTD.

APPENDIX II

ANALYTICAL METHOD

Analytical Method for the Determination of PFOS in Water

PFOS may be determined in water using high performance liquid chromatography with mass spectrometric detection (LCMS). Sample preparation for the fortification samples will involve dilution (using the same matrix water) into the concentration range of instrumental response. Matrix blanks will be directly injected. The following presents approximate chromatographic conditions for LCMS quantitation of PFOS. Chromatographic conditions may be changed to provide conditions more optimized for retention and/or chromatographic separation of PFOS.

Instrument: Hewlett-Packard Model 1090A HPLC
Column: Keystone Betacil C-18, 100 mm x 2.0 mm, 3μm
Mobile Phase: Methanol: Ammonium Acetate Buffered Water 65:35 (v:v), isocratic.
Flow Rate: 250 μL/minute
Column Temperature: 40C
Injection Volume: 100 μL
Ion Source: Turbo IonSpray
Mass Spectrometer: Perkin-Elmer API100
Run Time: 6.0 minutes

APPENDIX II

Certificate of Analysis

FC-95, Lot 217

March 9, 2000

Richard M. Payfer

This sample was analyzed using LC/MS, ^{1}H-NMR, ^{19}F-NMR, and elemental analyses techniques. The results of these tests show the sample to contain the following weight percent composition:

$C_2F_5SO_3^-K^+$	0.04 %
$C_3F_7SO_3^-K^+$	0.83 %
$C_4F_9SO_3^-K^+$	1.38 %
$C_5F_{11}SO_3^-K^+$	1.30 %
$C_6F_{13}SO_3^-K^+$	3.71 %
$C_7F_{15}SO_3^-K^+$	1.19 %
$C_8F_{17}SO_3^-K^+$	90.49 %
$C_9F_{19}SO_3^-K^+$	0.49 %
$C_{10}F_{21}SO_3^-K^+$	0.13 %
$C_{11}F_{23}SO_3^-K^+$	0.04 %
CF_3-$CO_2^-K^+$	0.05 %
$SF_5C_4F_{16}SO_3^-K^+$	0.35 %

Additionally, the isomer distribution of the sample was determined using ^{19}F-NMR techniques and found to contain the following mole percent composition:

$CF_3(CF_2)_x$-$SO_3^-K^+$ (Normal chain, where x is mainly 7)	70.5%
$CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-$SO_3^-K^+$ (Internal monomethyl branch, where x+y is mainly 5, and $x \neq 0$, $y \neq 0$)	17.1%
$(CF_3)_2CF$-$(CF_2)_x$-$SO_3^-K^+$ (Isopropyl branch, where x is mainly 5)	10.3%
C_2F_{5x+1}-$CF(CF_3)$-$SO_3^-K^+$ (Alpha branch, where x is mainly 6)	1.6%
$(CF_3)_3C$-$(CF_2)_x$-$SO_3^-K^+$ (t-butyl branch, where x is mainly 4)	0.2%
CF_3-$(CF_2)_x$-$C(CF_3)_2$-$(CF_2)_y$-$SO_3^-K^+$ (Internal gem-dimethyl branch, where x+y is mainly 4, and $x \neq 0$)	0.2%

AMENDED

APPENDIX III

Personnel Involved in the Study

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

1. Willard B. Nixon, Ph.D., Manager, Analytical Chemistry
2. Raymond VanHoven, Ph.D., Scientist
3. Norman J. Glassbrook, M.S., Senior Chemist
4. Keri Leach, B.S., Technician

APPENDIX IV

Report Amendment

1. Original Report: Pages 1-4 and 6

   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 VI.

   Reason: To reflect the issuing of an amended report.

2. Original Report: Page 9

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

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

3. Original Report: Entire report

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

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

AMENDMENT SIGNATURES:

Raymond J. VanHoven, Ph.D.
Study Director — DATE 4-26-00

Willard B. Nixon, Ph.D.
Manager, Analytical Chemistry — DATE 4/26/00

REVIEWED BY:

James H. Coleman
Quality Assurance — DATE 4-26-00

AMENDED

PROTOCOL

DETERMINATION OF THE WATER SOLUBILITY OF PFOS BY THE SHAKE FLASK METHOD

OECD Guideline for the Testing of Chemicals, 105
Water Solubility

3M Lab Request No. U2723

Submitted to

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

WILDLIFE INTERNATIONAL LTD.
8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

December 2, 1998

PROTOCOL NO.: 454/120298/830.7840/SUB454

3M LAB REQUEST NO. U2723

DETERMINATION OF THE WATER SOLUBILITY OF PFOS
BY THE SHAKE FLASK METHOD

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:	~~Ronda Jacobs~~ Raymond L. VanHoven, Ph.D. ~~Senior Chemist~~ Scientist
LABORATORY MANAGEMENT:	Willard B. Nixon, Ph.D. Manager of Chemistry

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date:	2/10/99	Experimental Termination Date:	3/10/99
Project No.:	454C-107	Int/Date:	RLB 2/10/99
Test Substance No.:	46675	Receipt Date:	10/29/98

PROTOCOL APPROVAL

Raymond L VanHoven — STUDY DIRECTOR — 2/10/99 — DATE

Willard B. Nixon — LABORATORY MANAGEMENT — 2/10/99 — DATE

Susan A. Beach — SPONSOR'S REPRESENTATIVE — 12/7/98 — DATE

INTRODUCTION

Wildlife International Ltd. will experimentally determine the water solubility of PFOS (perfluorooctane sulfonic acid, potassium salt). The study will be conducted at the Wildlife International Ltd. analytical chemistry facility in Easton, Maryland. The study will be performed following the guidance presented in the OECD Guideline for Testing of Chemicals, 105: *Water Solubility* (1) for shake flask methodology. This study is intended to meet data requirements under this guideline and under EPA Product Properties Test Guidelines, OPPTS 830.7840, *Water Solubility: Column Elution Method; Shake Flask Method* (2) and TSCA Title 40 of the Federal Code of Regulations, Part 796, Section 1840: *Water Solubility* (3). The method of analysis for quantitation provided by the Sponsor will be verified at Wildlife International Ltd. Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report.

PURPOSE

The purpose of this study is to determine the water solubility of PFOS at 20 ± 0.5 °C by the shake flask method. Determination of water solubility by the shake flask method is applicable to test substances with water solubilities equal to or exceeding 0.01 gram/Liter (g/L). The methodology may not be applicable to surface active or volatile test substances.

EXPERIMENTAL DESIGN

The water solubility of PFOS will be determined at a temperature of 20 ± 0.5 °C. A preliminary test will be performed to estimate the water solubility at room temperature. The preliminary test consists of additions of increasingly large amounts of water to a known weight of test substance until solubilization is effected or the solubility is estimated to be less than 0.01 g/L. For substances with an estimated water solubility greater than 0.01 g/L, a definitive test will be performed. The definitive test consists of equilibration of an excess amount of test substance with water at an elevated temperature, 30 °C, followed by equilibration at 20 °C.

MATERIALS AND METHODS

Test Substance

The test substance will be the highest purity available. The test substance should be at least 99% pure by weight. The Sponsor will be asked to provide the following information concerning the test substance, if available:

Chemical Name	Lot or Batch Number
Empirical Formula and/or Molecular Weight	Chemical Purity:
Dissociation Constant in Water	Vapor Pressure
Expiration Date	Storage Conditions
Method of Analysis	

The attached form **IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR** (Appendix I), along with a Material Safety Data Sheet, will be used to provide information necessary for the handling and testing of the test substance. The Sponsor agrees to accept any unused test substance and/or test substance containers at the end of the study.

Reagents

Water that meets ASTM Type II standards (ASTM D 1193-91) will be used (4). Other solvents may be needed for the analytical method or preparation of stock solutions and analytical standards. All solvents will be ACS reagent grade or better, and will be determined to be free of contaminants that interfere with the quantitation of the test substance.

Preliminary Test Procedure

A preliminary test will be conducted to estimate the solubility of the test substance at room temperature. Approximately 10 mg of the test substance will be placed in a plastic tube. Increasing volumes of water will be added stepwise as shown below:

Total volume of water added (mL)	0.1	0.5	1	2	10
Approximate solubility (g/L)	>100	20	10	5	1

After each addition of water to yield the total volume indicated, the container will be capped and shaken vigorously for approximately 10 minutes and visually examined for undissolved test substance. If the test substance is not dissolved after addition of 10 mL of water, the contents of the container will be transferred to a larger container and water added for a final volume of 100 mL. Following capping and shaking, the container will be visually checked for undissolved material, i.e., undissolved material would indicate a water solubility <0.1 g/L. Preliminary testing of the solubility at lower concentrations (<0.1 g/L) may be performed by transferring the contents of the 100-mL vessel to a 1-L vessel or reinitiating the test with a lesser amount of test substance, e.g., 1 mg. Amounts and volumes noted above are only guides for conduct of the preliminary study; larger amounts of test substance with proportionally larger volumes of water may be used.

Definitive Test Procedure

The quantity of test substance necessary to saturate the desired volume of water is estimated from the preliminary test. The volume of water used will depend on the analytical method and solubility range. Greater than (or equal to) five times the quantity of test substance required to achieve water solubility will be transferred to a minimum of three test vessels (plastic and/or other suitable container material). The desired volume of water will be added to each vessel and the vessels sealed. The closed vessels will be agitated (shaken or stirred) at 30 ± 1.0°C in a water bath. After 1 day, one of the vessels will be removed and re-equilibrated for 24 hours at 20 ± 0.5°C with occasional shaking or stirring. The contents of the vessel will then be centrifuged in a centrifuge regulated at 20°C. The concentration of the test substance in a minimum of two aliquots of the aqueous phase will be determined. The remaining two vessels are treated similarly following initial equilibration periods of 2 and 3 days at 30 ± 1.0°C. The test will be terminated if the concentrations of the test substance in the aqueous phase of two consecutive vessels agree within 15%. If greater variation is observed and/or at the request of the Sponsor, the test may be reinitiated with longer equilibration periods.

Method

The analytical method for quantitation of the test substance will be based on procedures provided by the Sponsor. The method will be appended to this protocol in Appendix II and referenced in the report. The method may be verified as part of the preliminary test(s) and may be modified to measure the observed test substance concentrations. Major modifications will be approved by the Sponsor and Study Director, and documented in the raw data.

Calculations

The concentration of the test substance in each vessel will be expressed in g/L, mg/L or µg/Lin water as appropriate. The average solubility for test vessels with solubilities within 15% of each other will be averaged and reported.

Sample Handling and Safety

The Sponsor will identify any special handling or safety precautions to be used with the above referenced test substance. All normal precautions with respect to handling and storage will be taken.

Sample and Test Substance Retention

Upon completion of testing, portions of the test substance used as part of this study will be disposed of in accordance with federal, state and local regulations. Any unused portion of the test substance will be returned to the Sponsor.

RECORDS TO BE MAINTAINED

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

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and completion of the study.
4. Dates of experimental start and termination.
5. Storage conditions of the test substance.
6. Test substance use log.
7. Concentration calculations and records of solution preparation.
8. Instrument operating conditions and chromatograms, if applicable.
9. Statistical calculations.
10. Test conditions.
11. A copy of the final report.

FINAL REPORT

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

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Purpose and procedure, as stated in the approved protocol, including all amendments and deviations to the protocol.
5. The test substance identification, including name or code number, purity, empirical formula, molecular formula, lot or batch number, method of analysis, and any other information provided by the Sponsor.
6. Description of the test method or reference to the method used along with any modifications made.

7. The individual concentrations of each sample.
8. The means and standard deviations of solubility determinations at each interval for each temperature tested.
9. Description of any problems experienced and how they were resolved.
10. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and findings reported to the Study Director and Management.

CHANGING OF PROTOCOL

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

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4 **American Society for Testing and Materials.** 1991. Standard Specification for Reagent Water. D1193-91, ASTM Section II Water and Environmental Technology, Vol. 11.01: 45-47.

APPENDIX I
IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

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

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

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

Test Substance Sample Code or Batch Number: Lot 217

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

II. Test Substance Characterization

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

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International Ltd.

Ambient room temperature

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

Other pertinent stability information: ____

IV. Test Concentrations:

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

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

V. Toxicity Information:

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

Aquatic:

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

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

Please see MSDS

APPENDIX II

ANALYTICAL METHOD

Analytical Method for the Determination of PFOS in Water

PFOS may be determined in water using high performance liquid chromatography with mass spectrometric detection (LCMS). Sample preparation for the fortification samples will involve dilution (using the same matrix water) into the concentration range of instrumental response. Matrix blanks will be directly injected. The following presents approximate chromatographic conditions for LCMS quantitation of PFOS. Chromatographic conditions may be changed to provide conditions more optimized for retention and/or chromatographic separation of PFOS.

Instrument: Hewlett-Packard Model 1090A HPLC
Column: Keystone Betacil C-18, 100 mm x 2.0 mm, 3μm
Mobile Phase: Methanol: Ammonium Acetate Buffered Water 65:35 (v:v), isocratic.
Flow Rate: 250 μL/minute
Column Temperature: 40C
Injection Volume: 100 μL
Ion Source: Turbo IonSpray
Mass Spectrometer: Perkin-Elmer API100
Run Time: 6.0 minutes