ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS) IN ARTIFICIAL SOIL

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-120

3M Environmental Lab Project No. U2723

AUTHORS:

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STUDY INITIATION DATE: February 5, 2001

STUDY COMPLETION DATE: May 27, 2003

Submitted to:

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

Wildlife International, Ltd.

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

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: Analytical Method Validation for the Determination of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Artificial Soil

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-120

3M ENVIRONMENTAL LAB PROJECT NUMBER: U2723

STUDY COMPLETION: May 27, 2003

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 160, 17 August 1989 and OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17) with the following exceptions:

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. Van Hoven, Ph.D.
Scientist
Wildlife International, Ltd.

5/27/03
DATE

SPONSOR APPROVAL:

Sponsor

5/29/03
DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 160, 17 August 1989 and OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17). 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:
Matrix Fortification	February 7, 2001	February 7, 2001	February 9, 2001
Data and Draft Report	February 14 and 15, 2001	February 15, 2001	February 15, 2001
Final Report	May 27, 2003	May 27, 2003	May 27, 2003

Susan L. Coleman

Susan L. Coleman, B.A.
Senior Quality Assurance Representative

5-27-03

DATE

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: Analytical Method Validation for the Determination of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Artificial Soil

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-120

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STUDY DIRECTOR:

Raymond L. Van Hoven, Ph.D.
Scientist

5/27/03
DATE

MANAGEMENT:

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

5/27/03
DATE

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APPENDICES

SUMMARY

SPONSOR: 3M Corporation

TITLE: Analytical Method Validation for the Determination of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Artificial Soil

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-120

3M ENVIRONMENTAL LAB PROJECT NUMBER: U2723

TEST DATES:
Study Initiation: February 5, 2001
Experimental Start (OECD): February 6, 2001
Experimental Start (EPA): February 7, 2001
Experimental Termination: February 8, 2001

TEST SYSTEM: Non-Target Terrestrial Plant Soil and Invertebrate Testing Soil

FORTIFIED TEST CONCENTRATIONS : wet-weight (dry-weight) basis	Non-Target Terrestrial Plant Soil (mg a.i./Kg)	Invertebrate Testing Soil (mg a.i./Kg)
	2.00 (2.37)	2.00 (2.37)
	10.0 (11.8)	10.0 (11.8)
	100 (118)	100 (118)
	1000 (1180)	1000 (1180)

RESULTS: Non-target terrestrial plant soil samples and invertebrate testing soil samples were each fortified in triplicate with PFOS to reflect nominal concentrations of 2.00, 10.0, 100 and 1000 mg a.i./Kg on a wet-weight basis. The samples were extracted with methanol, vacuum filtered, centrifuged, diluted into the instrumental calibration range with dilution solvent (50% methanol : 50% NANOpure® water) and analyzed by high performance liquid chromatography triple quadruple mass spectrometry (HPLC/MS/MS). Recoveries of PFOS in non-target terrestrial plant soils yielded an overall mean percent recovery of 98.1% (SD = 3.27; CV =3.33%; N = 12). Recoveries of PFOS in invertebrate testing soils yielded an overall mean percent recovery of 96.9% (SD = 3.61; CV = 3.73%; N = 12). Reagent blanks, consisting of solvents, and matrix blank samples, consisting of unfortified non-target terrestrial plant soil and invertebrate testing soil, were devoid of interfering components. The precision of triplicate fortifications in artificial soil matrix taken through the methodology was typically three percent relative, or better.

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for 3M Corporation at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. Validation samples were fortified with Perfluorooctanesulfonate, Potassium Salt (hereafter referred to as PFOS) and analyzed with high performance liquid chromatography mass spectrometry (HPLC/MS/MS) to evaluate the performance of a method for the determination of PFOS in non-target terrestrial plant soils and invertebrate testing soils. Validation samples were prepared and analyzed between February 7, 2001 and February 8, 2001. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454C-120 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to verify the performance of a HPLC/MS/MS methodology for the analyses of PFOS in non-target terrestrial plant soils and invertebrate testing soils. The validated method will be used in support of plant and invertebrate toxicology tests to be conducted with the test substance.

EXPERIMENTAL DESIGN

Non-target terrestrial plant soils and invertebrate testing soils were each fortified at four different concentrations in triplicate and analyzed based on HPLC/MS/MS methodology developed at Wildlife International, Ltd. The fortification levels bracketed the anticipated concentrations of samples from future plant and invertebrate toxicity tests. A reagent blank sample and matrix blank samples in each matrix were analyzed concurrently to evaluate potential analytical method interferences. A calibration curve was generated from analyses of standard solutions of PFOS with each series of samples analyzed.

MATERIALS AND METHODS

This study was conducted according to the procedures outlined in the protocol, "Analytical Method Validation for the Determination of Perfluorooctanesulfonate, Potassium Salt (PFOS) in Artificial Soil" (Appendix 1).

Test Substance

The test substance was received from 3M Corporation on October 29, 1998, assigned Wildlife International, Ltd. identification number 4675, and stored under ambient conditions. The test substance

was described as a white powder. It was identified as FC-95 from lot number 217, with an expiration date of August 31, 2001. Information from the most recent Certificate of Analysis provided by the Sponsor indicated a purity of 86.9% (Appendix 2).

Reagents and Solvents

All solvents used in this study were HPLC grade or equivalent. All reagents were ACS reagent grade or equivalent. NANOpure® water (equivalent to ASTM Type II Designation D1193-91) was used (1).

Test Systems

Invertebrate Testing Soil

The artificial soil used for validating the methodology for invertebrate testing was composed in a ratio of approximately 10 parts sphagnum peat, 20 parts kaolin clay, and 70 parts industrial quartz sand. The dry constituents of the soil were mixed (Soil Mixer #2) and the pH of the dry mixture was adjusted to 5.5 with calcium carbonate powder prior to hydration. After hydration, the moisture content of the soil (30.5%) was determined by weighing soil samples before and after drying at 105°C.

Non-Target Terrestrial Plant Soil

The artificial soil used for validating the methodology for plant testing was composed in a ratio of approximately 4 parts kaolin clay, 50 parts industrial quartz sand and 5 parts peat. Crushed limestone was added to buffer the pH of the soil, and a slow-release fertilizer was added to provide nutrients essential for plant growth. A soil sample representative of that used in this study was sent to Agvise Laboratories, Inc., in Northwood, North Dakota, for analysis of the particle size distribution and organic content of the soil. Reports for the latest soil characterization are stored in the archives at the Wildlife International, Ltd. site in Easton, Maryland. The results of the most recent GLP-compliant analyses performed to measure the concentrations of selected contaminants in soil used for non-target terrestrial plant testing at Wildlife International, Ltd. is presented in Appendix 3. No analytes were measured at levels that were expected to have an impact on the study.

Analytical Validation Procedures

Wildlife International, Ltd. conducted a validation trial using procedures developed at Wildlife International, Ltd. (Appendix 4). Matrix blanks and matrix fortification samples were prepared in non-target terrestrial plant soils and invertebrate testing soils. A reagent blank (containing everything except PFOS and soil) was carried through the entire procedure for each soil matrix. The samples were extracted with methanol. The samples were then vacuum filtered, diluted with methanol, and centrifuged. Dilutions into the calibration range of the HPLC/MS/MS methodology were performed with a solution of 50% methanol (HPLC grade, 99.9+%) and 50% NANOpure® water. Samples were then analyzed by direct injection. Concentrations of PFOS were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with a Perkin-Elmer API 3000LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Chromatographic separations were achieved using a Keystone Betasil C_{18} column (50 mm × 2.0 mm, 3-µm particle size) fitted with a Keystone Javelin C_{18} guard column (20 mm x 2.0 mm). A method flowchart is provided in Appendix 4.1 and the instrument parameters are summarized in Appendix 4.2.

Primary and Secondary Stock Solutions

All primary and secondary stock preparations were adjusted for the purity of the test substance (86.9%). A 10.0 mg a.i./mL primary stock solution of PFOS in methanol was prepared by weighing 1.1508 g of the test substance and bringing to a final volume of 100 milliliters with methanol. Secondary stock solutions (1000, 100, 10.0, 1.00, and 0.100 mg a.i./L) of PFOS in methanol were prepared by serial volumetric dilution from the primary stock.

Calibration Standards and Curves

Calibration standards were prepared in 50:50 methanol: NANOpure® water by appropriate dilutions of the 1.00 mg a.i./L stock solution of PFOS in methanol. Calibration standards of PFOS, ranging in concentration from 1.00 to 10.0 µg a.i./L, were analyzed with each sample set. The same and most prominent peak response for PFOS was utilized to monitor PFOS in all calibration and study samples. No attempt was made to quantify PFOS on the basis of individual isomeric components. 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 responses versus the respective concentrations of the calibration standards. A typical calibration curve is presented

in Appendix 4.3. The concentration of PFOS in the samples was determined by substituting the peak area responses into the applicable linear regression equation (Appendix 4.4). Representative ion chromatograms of low and high calibration standards are presented in Appendices 4.5 and 4.6, respectively.

Matrix Blanks and Matrix Fortifications

Selected 10-g aliquots of each artificial soil type were fortified with the appropriate primary or secondary stock solutions of PFOS prepared in methanol using a gas-tight syringe. The fortified soils were allowed to air dry then manually mixed prior to processing. The matrix blanks for each soil type were unfortified soils.

Limit of Quantitation

The method validation levels in both artificial soil types were well above the method capabilities for quantitation of the test substance. Therefore, the method limit of quantitation (LOQ) in each matrix was 1.00 mg a.i./Kg on a wet-weight basis calculated as the product of the lowest calibration standard (0.00100 mg a.i./L) and the overall dilution factor of the matrix blank samples (1000 L/Kg). For the non-target terrestrial plant artificial soil matrix with reported moisture content of 15.5%, the LOQ on a dry-weight basis was calculated as 1.18 mg a.i./Kg. For the invertebrate testing artificial soil matrix with reported moisture content of 30.5%, the LOQ on a dry-weight basis was calculated as 1.44 mg a.i./Kg.

RESULTS

Reagent and Matrix Blank Samples

A reagent blank and three matrix blank samples in each matrix were analyzed to determine possible interferences. No interferences were observed at or above the respective LOQs (Tables 1 and 2). Representative ion chromatograms of non-target terrestrial plant soil and invertebrate testing soil matrix blank samples are presented in Appendices 4.7 and 4.8, respectively.

Method Validation Samples

Non-target terrestrial plant soils were fortified in triplicate at 2.37, 11.8, 118 and 1180 mg a.i./Kg (dry-weight basis) resulting in mean recoveries of 98.3, 101, 97.5, and 96.6%, respectively (Table 1). Representative ion chromatograms of low and high-level fortifications in non-target terrestrial plant soil

are presented in Appendices 4.9 and 4.10, respectively. Invertebrate testing soils were fortified in triplicate at 2.88, 14.4, 144 and 1440 mg a.i./Kg (dry-weight basis) resulting in mean recoveries of 99.7, 100, 93.1 and 95.1%, respectively (Table 2). Representative ion chromatograms of low and high-level fortifications in invertebrate testing soils are presented in Appendices 4.11 and 4.12, respectively.

The precision of the methodology, as demonstrated by the analysis of triplicate samples within each fortification level, was three percent relative, or better, for all four levels of fortification in the invertebrate testing soil matrix. With the exception of one level (1180 mg a.i./Kg on a dry-weight basis = 6.40%), precision in the non-target terrestrial plant matrix was also three percent relative, or better.

CONCLUSIONS

Non-target terrestrial plant and invertebrate testing artificial soils were fortified with PFOS to reflect nominal concentrations of 2.00, 10.0, 100 and 1000 mg a.i./Kg on a wet-weight basis. The samples were extracted with methanol. The samples were then vacuum filtered, diluted with methanol, and centrifuged. Dilutions into the calibration range of the HPLC/MS/MS methodology were performed with a solution of 50% methanol and 50% NANOpure® water. Samples were then analyzed by high performance liquid chromatography mass spectrometry (HPLC/MS/MS). Recoveries of PFOS in non-target terrestrial plant soil yielded an overall mean percent recovery on a wet-weight basis of 98.1% (SD = 3.27%; CV = 3.33%; N = 12). Recoveries of PFOS in invertebrate testing soil yielded an overall mean percent recovery on a wet-weight basis of 96.9% (SD = 3.61%; CV = 3.73%; N = 12). Reagent blanks, consisting of solvents, and matrix blank samples, consisting of unfortified artificial soils, were devoid of interfering components.

REFERENCES

1. **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

Method Validation Recoveries for PFOS in Non-Target Terrestrial Plant Soil

Sample		PFOS Concentration (mg a.i./Kg)				
Number (454C-120-)	Type	Fortified	Measured	Percent Recovered[1]	Mean Measured (mg a.i./Kg)	Mean Percent Recovery
VREB-1	Reagent Blank	0	< 1.18[2]	--	--	--
VMAB-1	Matrix Blank	0	< 1.18	--	--	--
VMAB-2	Matrix Blank	0	< 1.18	--		
VMAB-3	Matrix Blank	0	< 1.18	--		
VMAS-1	Matrix Fortification	2.37	2.33	98.7	2.33	98.3
VMAS-2	Matrix Fortification	2.37	2.30	97.1	± 0.0300	
VMAS-3	Matrix Fortification	2.37	2.36	99.4	1.29%	
VMAS-4	Matrix Fortification	11.8	12.0	101	11.9	101
VMAS-5	Matrix Fortification	11.8	12.0	101	± 0.231	
VMAS-6	Matrix Fortification	11.8	11.6	98.3	1.95%	
VMAS-7	Matrix Fortification	118	115	97.0	115	97.5
VMAS-8	Matrix Fortification	118	113	95.2	± 2.52	
VMAS-9	Matrix Fortification	118	118	99.6	2.18%	
VMAS-10	Matrix Fortification	1180	1205	102	1140	96.6
VMAS-11	Matrix Fortification	1180	1159	97.9	± 73.0	
VMAS-12	Matrix Fortification	1180	1062	89.7	6.40%	
			Overall Mean =	98.1		
			Standard Deviation =	± 3.27		
			CV =	3.33%		
			N =	12		

[1] Percent Recovered values were calculated on a wet-weight basis using MacQuan, version 1.6 software. All other values were converted to dry-weight by dividing the wet-weight value by the percent solids for the matrix soil.

[2] The limit of quantitation (LOQ) was 1.18 mg a.i./Kg on a dry-weight basis calculated as the product of the lowest calibration standard (0.001 mg a.i./L) and the overall dilution factor of the matrix blank samples (1000 L/Kg) divided by the percent solids for the soil (84.5%).

Table 2

Method Validation Recoveries for PFOS in Invertebrate Testing Soil

Sample		PFOS Concentration (mg a.i./kg)				
Number (454C-120-)	Type	Fortified	Measured	Percent Recovered[1]	Mean Measured (mg a.i./Kg)	Mean Percent Recovery
VREB-2	Reagent Blank	0	< 1.44[2]	--	--	--
VMAB-4	Matrix Blank	0	< 1.44	--	--	--
VMAB-5	Matrix Blank	0	< 1.44	--		
VMAB-6	Matrix Blank	0	< 1.44	--		
VMAS-13	Matrix Fortification	2.88	2.82	98.1	2.87	99.7
VMAS-14	Matrix Fortification	2.88	2.82	97.9	± 0.0808	
VMAS-15	Matrix Fortification	2.88	2.96	103	2.82%	
VMAS-16	Matrix Fortification	14.4	14.3	99.3	14.4	100
VMAS-17	Matrix Fortification	14.4	14.5	101	± 0.100	
VMAS-18	Matrix Fortification	14.4	14.4	100	0.694%	
VMAS-19	Matrix Fortification	144	136	94.6	134	93.1
VMAS-20	Matrix Fortification	144	133	92.5	± 2.08	
VMAS-21	Matrix Fortification	144	132	91.4	1.56%	
VMAS-22	Matrix Fortification	1440	1370	95.2	1370	95.1
VMAS-23	Matrix Fortification	1440	1391	96.7	± 25.1	
VMAS-24	Matrix Fortification	1440	1341	93.2	1.83%	
			Overall Mean =	96.9		
			Standard Deviation =	3.61		
			CV =	3.73%		
			N =	12		

[1] Percent Recovered values were calculated on a wet-weight basis using MacQuan, version 1.6 software. All other values were converted to dry-weight by dividing the wet-weight value by the percent solids for the matrix soil.

[2] The limit of quantitation (LOQ) was 1.44 mg a.i./Kg on a dry-weight basis calculated as the product of the lowest calibration standard (0.001 mg a.i./L) and the overall dilution factor of the matrix blank samples (1000 L/Kg) divided by the percent solids for the soil (69.5%).

Appendix 1

Protocol and Amendments

PROTOCOL

ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS) IN ARTIFICIAL SOIL

Environmental Laboratory Project Number U2723

Submitted to

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

Wildlife International, Ltd.

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

December 7, 2000

Wildlife International, Ltd.

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ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS) IN ARTIFICIAL SOIL

SPONSOR:	3M Corporation Environmental Laboratory 935 Bush Avenue St. Paul, Minnesota 55106
SPONSOR'S REPRESENTATIVE:	Rochelle R. Robideau
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Raymond L. Van Hoven, Ph.D. Scientist Wildlife International, Ltd.
LABORATORY MANAGEMENT:	Willard B. Nixon, Ph.D. Director ~~Manager~~ of Analytical Chemistry Wildlife International, Ltd.

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: February 7, 2001

Experimental Termination Date: March 7, 2001

Project No.: 454C-120

Test Concentrations: Negative Control, 2.00, 10.0, 100, and 1000 mg a.i./kg based on soil moisture content as received

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

PROTOCOL APPROVAL

STUDY DIRECTOR — DATE 02/05/01

LABORATORY MANAGEMENT — DATE 02/05/01

SPONSOR'S REPRESENTATIVE — DATE 12/4/00

Wildlife International, Ltd.

INTRODUCTION

Wildlife International, Ltd. will conduct analytical trials to validate the performance of a liquid chromatography mass spectrometry (LCMS) method for the determination of Perfluorooctanesulfonate, Potassium Salt (hereafter referred to as PFOS) in artificial soil used for earthworm and non-target terrestrial plant tests. The study will be performed at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. The method developed by Wildlife International, Ltd. will be validated by fortifying artificial soil and quantifying the recoveries of PFOS. 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 verify the performance of LCMS methodology for analyses of PFOS in artificial soil used by Wildlife International, Ltd. to conduct non-target terrestrial plant and invertebrate testing.

EXPERIMENTAL DESIGN

Wildlife International Ltd. artificial soils similar to those used in earthworm and non-target terrestrial plant tests will be fortified at four different concentrations and analyzed using LCMS methodology based upon that developed at Wildlife International Ltd. Modifications to the methodology will be made as necessary to achieve quantitation of PFOS in the test matrix. Matrix and/or reagent blanks will be analyzed concurrently to evaluate potential analytical interferences. The levels of fortification will bracket the anticipated concentrations of samples from earthworm and non-target terrestrial plant toxicity tests. Reagent and matrix blanks will be analyzed to evaluate potential analytical interferences. One calibration curve will be prepared and analyzed with each series of matrix fortification samples. The accuracy, precision, and limit of quantitation of the method will be determined.

Wildlife International, Ltd.

MATERIALS AND METHODS

Test Substance

The test substance will be Perfluorooctanesulfonate, Potassium Salt. Information on the characterization of test, control or reference substances is required by Good Laboratory Practice Standards (GLP). The Sponsor is responsible for providing Wildlife International, Ltd. written verification that the test substance has been characterized according to GLPs prior to initiation of the study. The reference material in this study will be the test substance. 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 and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

Reagents and Solvents

All solvents used in the method or procedure will be of reagent grade or better. All reagents will be of ACS grade or better.

Test Soil – Earthworm Soil

The artificial soil used for earthworm tests will be composed of approximately 10% sphagnum peat, 20% kaolin clay and 70% industrial quartz sand. The dry constituents of the soil will be mixed in a PK Twinshell or equivalent mixer. Calcium carbonate powder will be added during the mixing of the dry soil as needed to adjust the pH to 6.0 ± 0.5, prior to hydration. The moisture content of the artificial soil will be determined by weighing samples of the soil before and after drying at approximately 105°C. The moisture content is calculated using the following formula:

$$\% \text{ moisture} = [(\text{net wet weight} - \text{net dry weight}) \div \text{net wet weight}] \times 100$$

The soil will be adjusted to approximately 33% moisture content during the preparation of the test soils.

Wildlife International, Ltd.

Test Soil – Non-Target Terrestrial Plants

Test plants will be grown in plastic pots approximately 16 cm in diameter and 12 cm in depth. An unsterilized artificial sandy-loam or sand soil substrate will be used for testing. The soil will be composed of kaolinite clay, industrial quartz sand and peat mixed in a 4:50:2.5 ratio (w:w:w). Crushed limestone will be added to buffer the pH of the soil, and a slow-release fertilizer will be added to provide nutrients essential for plant growth. Typically, the soil consists of approximately 90 % sand, 2-3 % silt, and 7-10 % clay, with a neutral pH and an organic matter content less than 1.5 %. A sample of soil representative of that used in this study will be sent to Agvise Laboratories, Inc., in Northwood, North Dakota, for analysis of the particle size distribution and organic matter content of the soil. Soil characterization will include, but may not be limited to, the determination of particle size distribution, organic matter content, and pH. Those items relevant to the conduct of the study will be discussed in the final report. The complete report from Agvise Laboratories, Inc. will be filed in the archives located at Wildlife International, Ltd. The results of the characterization will be stored in the archives located at the Wildlife International, Ltd. site, and those items relevant to the conduct of the study will be discussed in the final report.

Analytical Validation Procedures

The analytical method to be used will be LC/MS based upon procedures developed at Wildlife International, Ltd. The method used will be documented in the raw data and summarized in the final report. Prior to the analysis of analytical samples, primary stock solution(s) will be prepared directly from the test substance. Calibration standards will be prepared by appropriate dilution of the primary stock solution(s).

Primary Stock Solution(s), Calibration Standards and Curves

A minimum of five calibration standards (of different concentrations) will be prepared and analyzed along with each analysis set. The calibration standard series will be injected at the beginning and one standard injected, at a minimum, after every five samples. A minimum of two injections of each standard in the calibration standard series will be injected with the analysis set. One calibration curve and regression equation will be prepared from the series of calibration standards.

Wildlife International, Ltd.

Fortification Stock Solutions

The soil will be fortified with test substance in a manner consistent with the method anticipated for the toxicology studies.

Evaluation of Interferences

One reagent blank and three matrix blanks will be analyzed to detect possible interferences. The reagent blank will contain everything except the matrix and the test substance. The matrix blanks will contain everything except the test substance.

Verification Analyses - Method Performance

Matrix fortifications (spiked artificial soil from earthworm and non-target plant studies), prepared at known concentrations of the test substance, will be analyzed to determine the recovery of the analyte and to evaluate method performance. The anticipated verification series will consist of the following analyses:

SUMMARY OF PROPOSED VERIFICATION ANALYSIS SCHEME

Analysis Type	Concentration	Number of Samples	
		Earthworm Soil	Non-Target Plant Soil
Reagent Blank (Solvent)	0 (Control)	1	1
Matrix Blank (Soil)	0 (Control)	3	3
Matrix Fortifications	Level 1 – Low	3	3
	Level 2	3	3
	Level 3	3	3
	Level 4 – High	3	3
Total Number of Analyses		16	16

Matrix fortifications will represent the range of concentrations that are anticipated to be used in subsequent environmental fate and/or effects tests and will be selected in consultation with the Sponsor. Individual samples (identified by project number and a unique sample identification number) will be prepared and analyzed for verification of the analytical methodology.

Wildlife International, Ltd.

If difficulties arise in the validation process (e.g., low recoveries or interferences), the Sponsor will be notified and the need for additional validation and/or method development will be determined through discussions with the Sponsor. Upon completion of the method validation, the Sponsor will review the results and authorize the use of the methodology for analysis of samples, or will authorize further method development trials. Recovery values in the range of 80 to 120% will be used as criteria for method acceptability.

Data Analysis

One calibration curve will be established for each analytical run. A regression equation of the concentration versus peak area for the calibration standards will be generated. The concentration of the samples will be determined by substituting the respective peak area into the regression equation. Statistics to be determined and reported will include the mean and standard deviation for each level of fortification.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by Wildlife International, Ltd. will include:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance and/or analytical standard, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Storage conditions for test substance, analytical standards, and/or samples.
5. Test substance and/or analytical standard use log.
6. Concentration calculations and records of solution preparation.
7. Instrument operating conditions and chromatograms.
8. Statistical calculations.
9. A copy of the final report.
10. Documentation that the steps in the method were followed.

FINAL REPORT

The report will summarize the findings of the verification, the procedural recoveries obtained, and the methods and instrumentation employed. Upon receipt of these findings, the Sponsor will

Wildlife International, Ltd.

review the methods and results and evaluate the results for acceptability prior to initiating any fate and/or effects studies.

The final 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.
3. Objectives and procedures stated in the approved protocol, including any changes in the original protocol or deviations from the protocol.
4. The test substance and/or analytical standard identification, including name, chemical abstract number or code number, strength, purity, composition, date of receipt, lot number, storage conditions, physical characteristics, stability and method of preparation of test concentrations, if provided by the Sponsor.
5. A brief summary of the analytical methodology: A description of the experimental measurements, example calculations, sample preparation (sample weights and dilutions), instrumentation employed, reagents and solvents used, class of water used, and any major modifications to the method.
6. A description of all circumstances that may have affected the quality or integrity of the data.
7. The name of the Study Director, the names of other scientists or professionals, and the names of all supervisory personnel involved in the study.
8. A description of the transformations, calculations, or operations performed on the data.
9. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
10. The location where raw data and final report are to be stored.
11. A statement prepared by the Quality Assurance Unit listing the dates that study inspections were made and the dates of any findings reported to the Study Director/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'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.

Wildlife International, Ltd.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160); and OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17). 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 certification of 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. A copy of the study raw data for all work performed at Wildlife International, Ltd. will be sent to the Sponsor with the final report.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF PERFLUOROOCTANE SULFONATE, POTASSIUM SALT (PFOS) IN ARTIFICIAL SOIL

PROTOCOL NO.: 454/120700/MV-S/SUB454 **AMENDMENT NO.:** 1

SPONSOR: 3M Corporation **PROJECT NUMBER:** 454C-120

SPONSOR STUDY NO.: U2723 **EFFECTIVE DATE:** February 6, 2001

AMENDMENT: Page 5, Test Soil – Non-Target Terrestrial Plants

CHANGE: "The soil will be composed of kaolinite clay, industrial quartz sand and peat mixed in a 4:50:2.5 ratio (w:w:w)."

TO: "The soil will be composed of kaolinite clay, industrial quartz sand and peat mixed in a 4:50:5 ratio (w:w:w)."

REASON: To use a ratio of components in the validation study in line with current standard practice for plant studies.

STUDY DIRECTOR — DATE 02-06-01

LABORATORY MANAGEMENT — DATE 2/6/01

SPONSOR'S REPRESENTATIVE — DATE 2/15/01

Reviewed by QA (SLC) 2-6-01

Project Number 454C-120

Wildlife International, Ltd.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Analytical Method Validation For The Determination Of Perfluorooctane Sulfonate, Potassium Salt (PFOS) In Artificial Soil

PROTOCOL NO.: 454/120700/MV-S/SUB454 **AMENDMENT NO.:** 2

SPONSOR: 3M Corporation **PROJECT NUMBER:** 454C-120

SPONSOR STUDY NO.: U2723 **EFFECTIVE DATE:** May 16, 2003

AMENDMENT: Page 2, Sponsor's Representative

Change: Rochelle R. Robideau

To: Susan A. Beach

REASON:

Reassignment of Sponsor's Representative responsibilities at the request of the Sponsor.

AMENDMENT: Page 8, Changing of Protocol

Change: "Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative."

To: "Planned changes to the protocol will be in the form of written amendments signed by the Study Director and approved by the Sponsor's Representative."

REASON:

To expedite finalization of the study.

STUDY DIRECTOR — DATE 5/19/03

LABORATORY MANAGEMENT — DATE 5/19/03

Reviewed by QA
JHC 5-19-03

Appendix 2

Certificate of Analysis

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-018A

3M Product: PFOS, Lot 217

Reference #: SD-018

Purity: 86.9%

Test Name	Specifications	Result
Purity[1]		86.9%
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		 1. 0.005 wt./wt.% 2. 0.001 wt./wt.% 3. 1.439 wt./wt.% 4. 6.849 wt./wt.% 5. <0.001 wt./wt.% 6. 0.005 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.93 wt./wt.%
Total % Impurity (LC/MS)		8.41 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.33 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable[3]
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		 1. <0.015 wt./wt.% 2. 0.59 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.76 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		 1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. 0.10 wt./wt.% 4. 0.28 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	 1. 12.48 wt./wt.% 2. 0.244 wt./wt.% 3. 1.74 wt./wt.% 4. 8.84 wt./wt.% 5. 54.1 wt./wt.%

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

[1]Purity = 100% - (sum of metal impurities, 1.45% +LC/MS impurities, 8.41%+Inorganic Fluoride, 0.59%+NMR impurities, 1.93%+organic acid impurities, 0.38%+POAA, 0.33%)

Total impurity from all tests = 13.09%
Purity = 100% - 13.09% = 86.9%

[2]Potassium is expected in this salt form and is therefore not considered an impurity.

[3]Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

[4]Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

[5]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3^-K^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801 www.centrelab.com
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.22
C5	1.33
C6	4.72
C7	1.14
Total	**8.41**

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average result from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average result from the C6 and C8 standard curves.

Prepared By: _____ 10/11/01
Charles Simons Date
Scientist, Centre Analytical Laboratories

Reviewed By: John M Flaherty 10/11/01
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

Appendix 3

Analyses of Pesticides, Organics and Metals
in Wildlife International, Ltd. Greenhouse Soil[1]

Pesticides And Organics			
Component	Measured Concentration	Component	Measured Concentration
α-chlordane	<0.01 mg/kg	Azinphos-methyl	Not Determined
γ-chlordane	<0.01 mg/kg	Chlorfenvinphos	<0.01 mg/kg
P,P'-DDE	<0.01 mg/kg	Chlorpyriphos	<0.01 mg/kg
O,P'-DDT	<0.01 mg/kg	Chlorpyriphos-methyl	<0.01 mg/kg
Aldrin	<0.01 mg/kg	Diazinon	<0.01 mg/kg
P,P'-DDT	<0.01 mg/kg	Dichlorvos	<0.01 mg/kg
Dieldrin	<0.01 mg/kg	Fenitrothion	<0.01 mg/kg
β-endosulfan	<0.01 mg/kg	Malathion	<0.01 mg/kg
Endosulfansulphate	<0.01 mg/kg	Mevinphos (Cis+Trans)	Not Determined
Endrin	<0.01 mg/kg	Parathion	<0.01 mg/kg
Endrin-keton	<0.01 mg/kg	Parathion-methyl	<0.01 mg/kg
α-HCH	<0.01 mg/kg	Pirimiphos-methyl	<0.01 mg/kg
β-HCH	<0.01 mg/kg	Sulfotep	<0.01 mg/kg
Lindane (γ-HCH)	<0.01 mg/kg	PCB 28	<0.01 mg/kg
δ-HCH	<0.01 mg/kg	PCB 52	<0.01 mg/kg
Heptachlor	<0.01 mg/kg	PCB 101	<0.01 mg/kg
β-Heptachlorepoxide	<0.01 mg/kg	PCB 118	<0.01 mg/kg
Hexachlorbenzene	<0.05 mg/kg	PCB 138	<0.01 mg/kg
Methoxychlor	<0.05 mg/kg	PCB 153	<0.01 mg/kg
P,P'-tDE	<0.01 mg/kg	PCB 180	<0.01 mg/kg
Metals			
Magnesium	3,000 mg/kg	Nickel	<2.0 mg/kg
Sodium	60 mg/kg	Copper	<2.0 mg/kg
Calcium	4,700 mg/kg	Zinc	6.1 mg/kg
Iron	6,100 mg/kg	Molybdenum	<1.2 mg/kg
Potassium	220 mg/kg	Silver	<0.40 mg/kg
Aluminum	5,200 mg/kg	Cadmium	<2.0 mg/kg
Manganese	51 mg/kg	Arsenic	3.8 mg/kg
Beryllium	<0.04 mg/kg	Mercury	12.0 µg/kg
Chromium	6.0 mg/kg	Selenium	0.11 mg/kg
Cobalt	0.50 mg/kg		

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

Appendix 4

Analytical Method Validation Data

Appendix 4.1

Analytical Method Flowchart for the Analysis of PFOS in Artificial Soil

Weigh approximately 10.0 grams of soil per sample into a tared weigh boat. Record weights. Transfer to 8-oz French-square bottles. For matrix fortification samples, fortify each sample with the appropriate volume of PFOS stock solution in methanol with a gas-tight syringe. Allow to air dry. The matrix blank is unfortified soil matrix.

↓

For each soil sample, measure 100 milliliters of methanol with a graduated cylinder and transfer into the French-square bottle. Add 100 milliliters of methanol into an empty French-square bottle and process as for the soil samples. The additional sample is the reagent blank.

↓

Cap bottles and place on a shaker table. Allow the samples to shake for a minimum of 30 minutes at approximately 250 rpm.

↓

Vacuum filter with qualitative filter paper and rinse retained soil three times with methanol into filtrate.

↓

Transfer the filtrate into a 200-mL volumetric flask and bring to volume with methanol. Mix well with several repeat inversions.

↓

Transfer approximately twenty milliliters of each sample into a separate glass centrifuge tube or scintillation vial and cap. Centrifuge samples for approximately five minutes at 2000 rpm.

↓

Dilute samples into the calibration range of the PFOS LC/MS methodology: Partially fill Class A volumetric flasks with dilution solvent (50% methanol: 50% NANOpure® water). Add appropriate volume of sample with a gas-tight syringe and bring to volume with dilution solvent. Process matrix blank samples using the same dilution and aliquot volumes as for the lowest fortification level.

↓

Ampulate samples and submit for HPLC/MS/MS analysis.

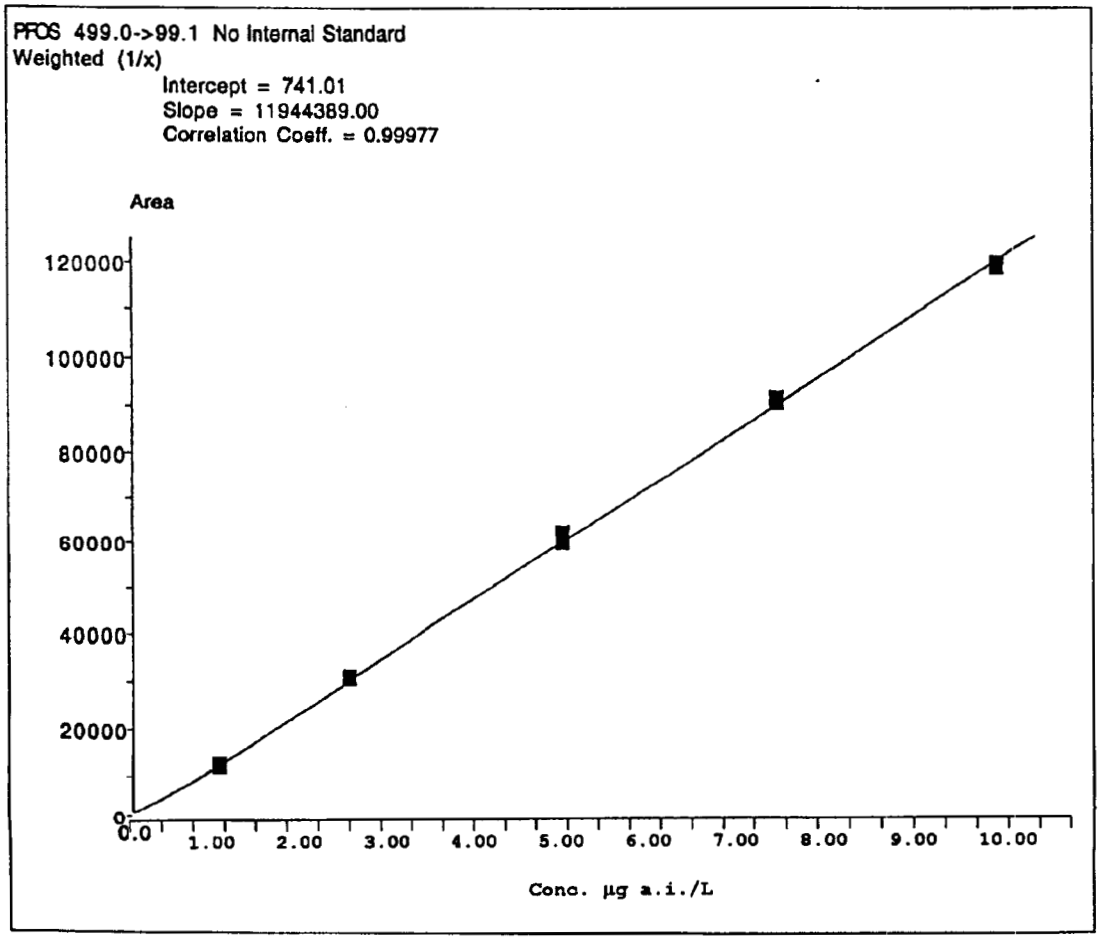
Appendix 4.2

Typical HPLC/MS/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 3000 Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in multiple ion reaction monitoring (MRM) mode.
ANALYTICAL COLUMN:	Keystone Betasil C_{18} column (50 mm × 2 mm I.D., 3-µm particle size)
GUARD COLUMN:	Keystone Javelin C_{18} column (20 mm × 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 4.0 minutes
PFOS MONITORED MASS:	499.0 amu → 99.1 amu

Appendix 4.3

A Typical Calibration Curve for PFOS



Appendix 4.4

Example Calculations for a Representative Sample

Sample number 454C-120-VMAS-1, nominal concentration of 2.00 mg a.i./Kg on a wet-weight basis in plant soil

Peak Area = 24317
Y-intercept = 741.01
Slope = 11944389
Primary Dilution:
Initial Weight (W_i) = 10.0 g
Original Final Volume (V_1)= 200 mL
Secondary Dilution:
Initial Volume (V_i) = 0.200 mL
Final Volume (V_f) = 10.0 mL
Overall Dilution Factor ($V_1/W_i \times V_f/V_i$) = 1000 mL/g = 1000 L/Kg

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

$$= \frac{(24317 - 741.01)}{11944389}$$

$$= 0.0019738 \text{ mg a.i./L}$$

PFOS in sample (mg a.i./Kg) = PFOS at instrument (mg a.i./L) × overall dilution factor (L/Kg)

$$\text{PFOS in sample (mg a.i./Kg)} = \frac{0.0019738 \text{ mg a.i.}}{\text{L}} \times \frac{1000\text{L}}{\text{Kg}}$$

$$= 1.97 \text{ mg a.i./Kg}$$

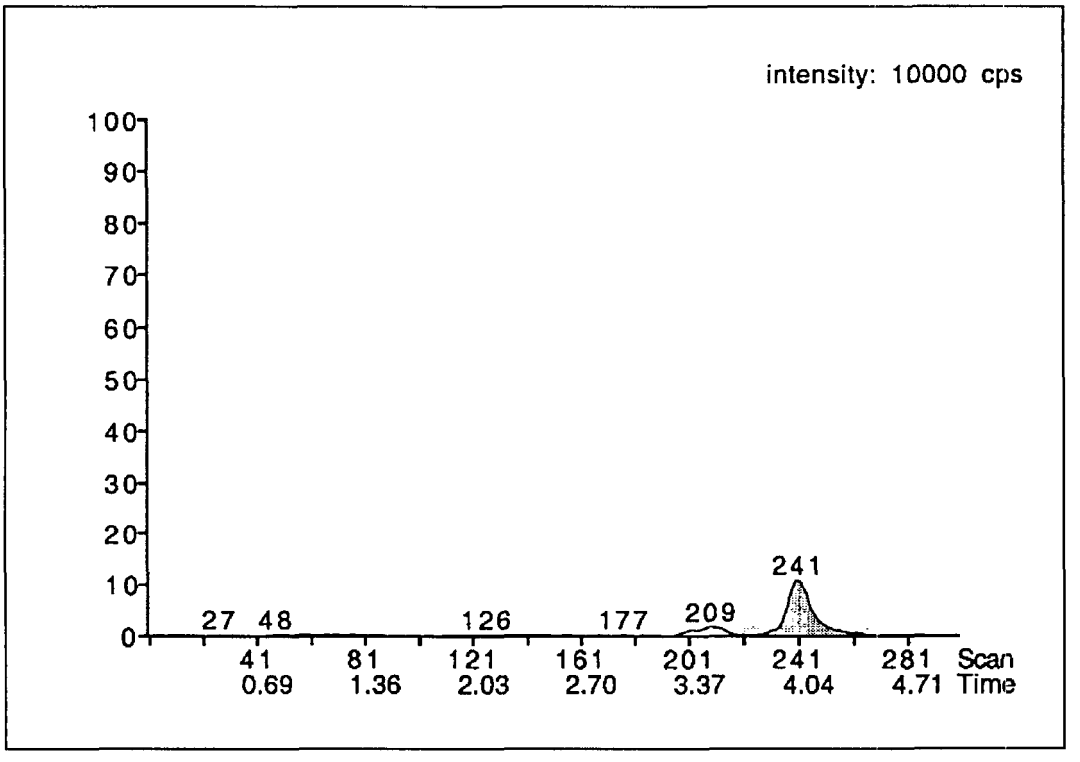
$$\text{Percent of Nominal Concentration} = \frac{\text{PFBS (mg a.i./L) in sample}}{\text{PFBS (mg a.i./L) nominal}} \times 100$$

$$= \frac{1.97}{2.00} \times 100 = 98.7\%$$

Instrument Software: MacQuan, version 1.6.

Appendix 4.5

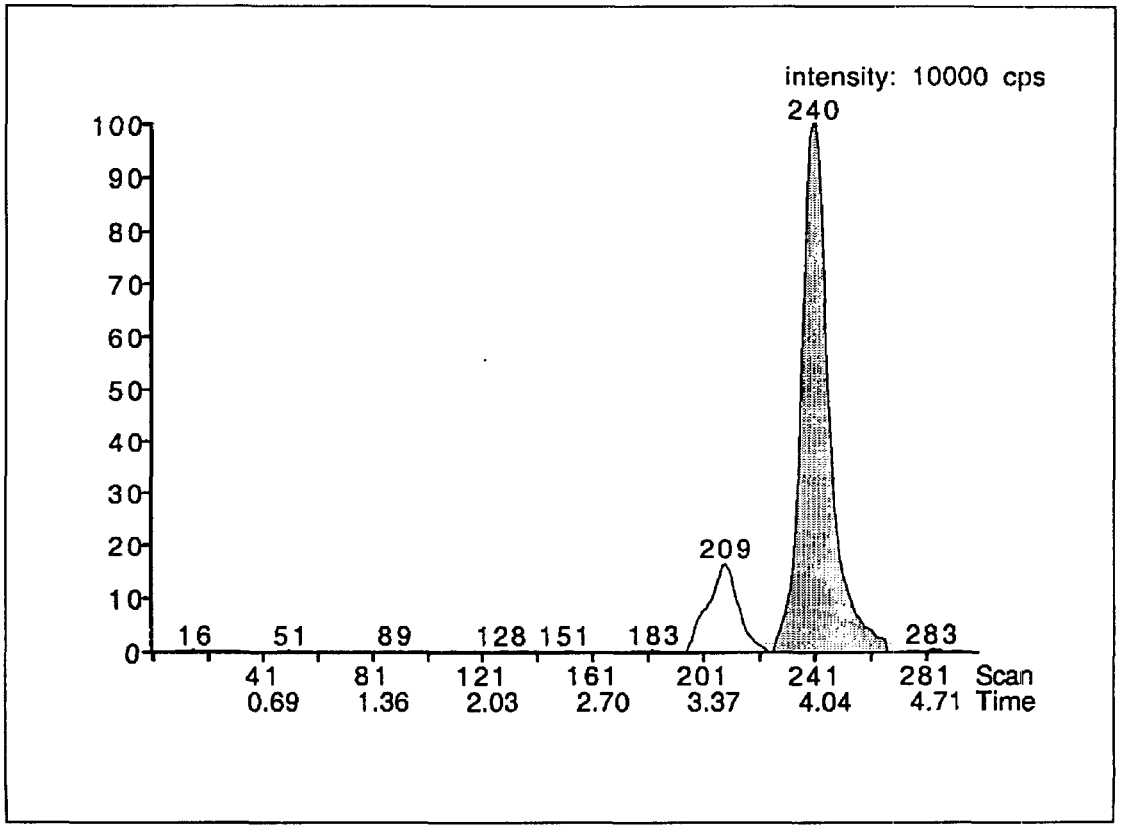
Ion Chromatogram of a Low-level PFOS Standard



Nominal concentration: 0.00100 mg a.i./L

Appendix 4.6

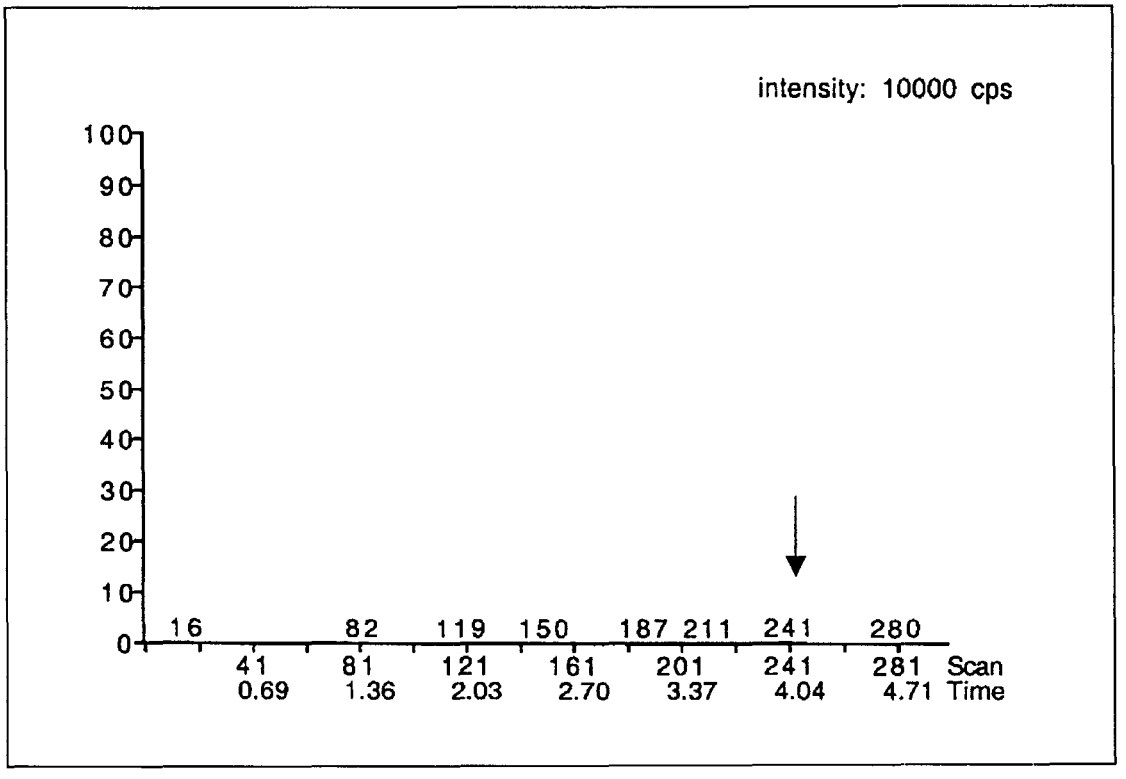
Ion Chromatogram of a High-level PFOS Standard



Nominal concentration: 0.0100 mg a.i./L

Appendix 4.7

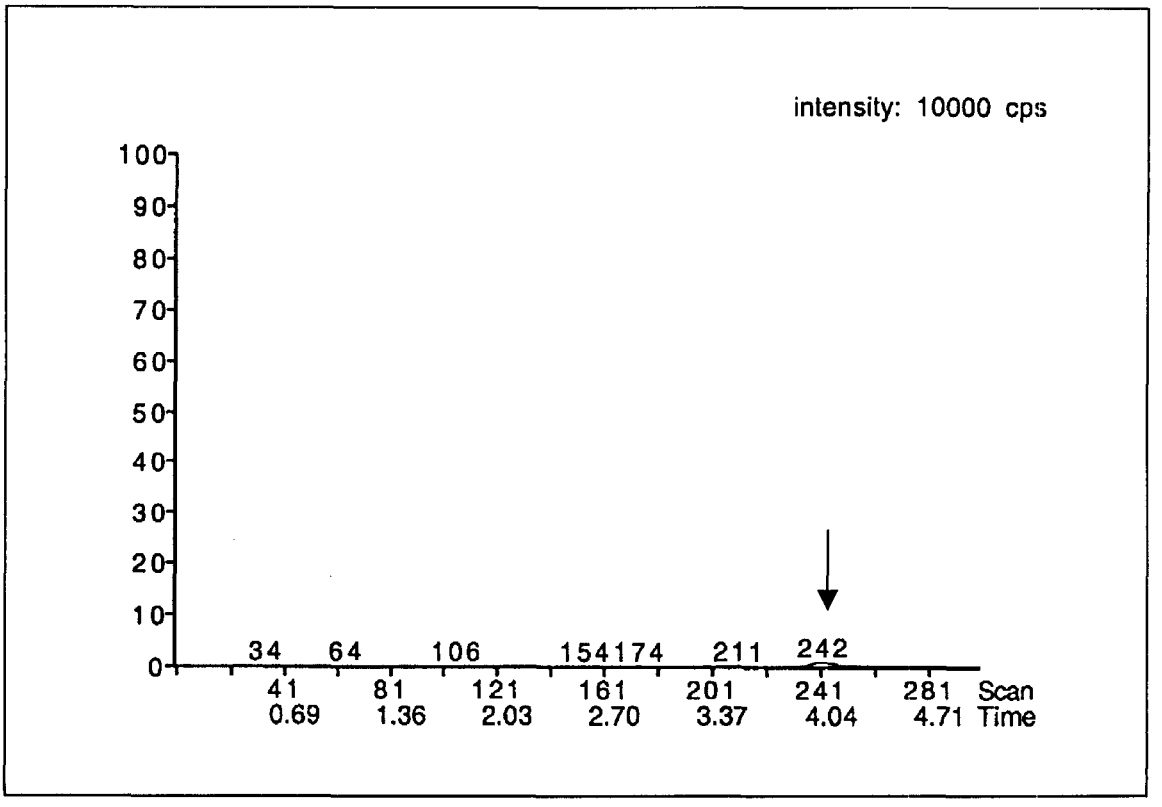
Ion Chromatogram of Non-Target Terrestrial Plant Soil Matrix Blank



Sample number 454C-120-VMAB-1. The arrow indicates the approximate retention time of PFOS.

Appendix 4.8

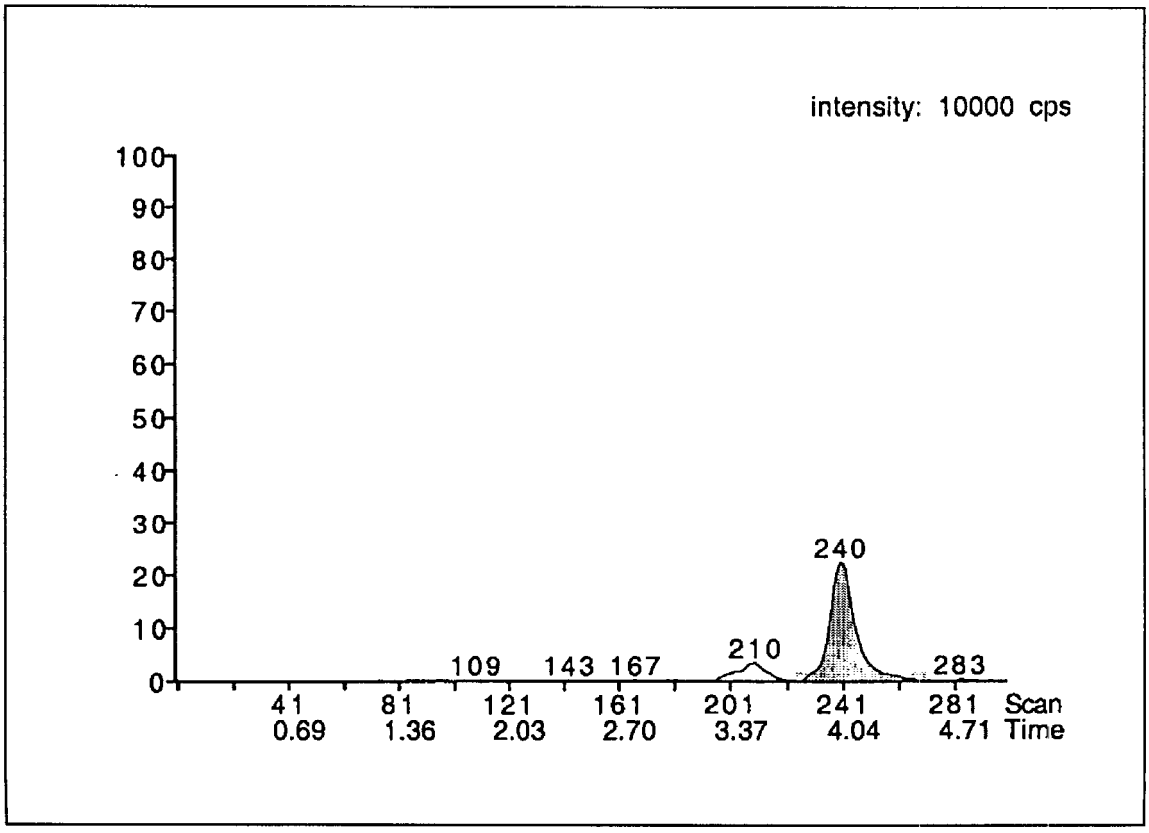
Ion Chromatogram of an Invertebrate Testing Soil Matrix Blank



Sample number 454C-120-VMAB-4. The arrow indicates the approximate retention time of PFOS.

Appendix 4.9

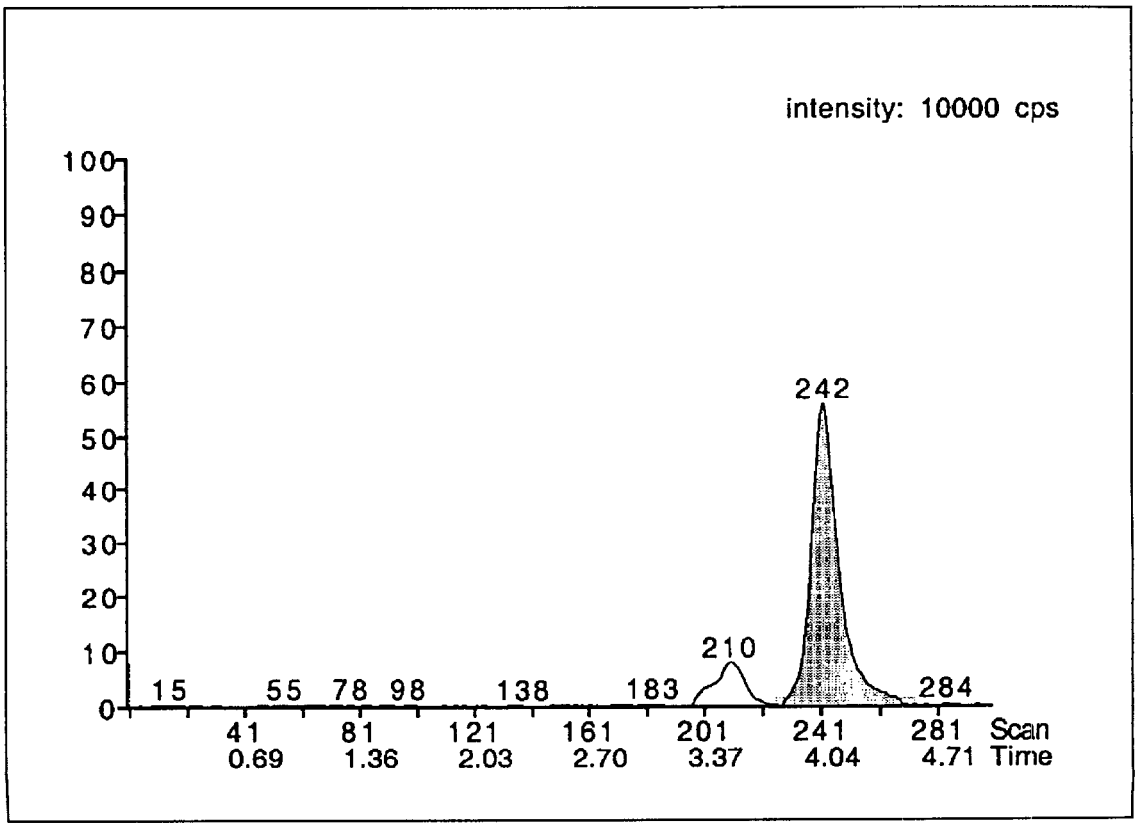
Ion Chromatogram of a Low-level Non-Target Terrestrial Plant Soil Fortification Sample



Sample number 454C-120-VMAS-1, 2.00 mg a.i./Kg wet-weight nominal concentration, overall dilution factor = 1000 L/Kg.

Appendix 4.10

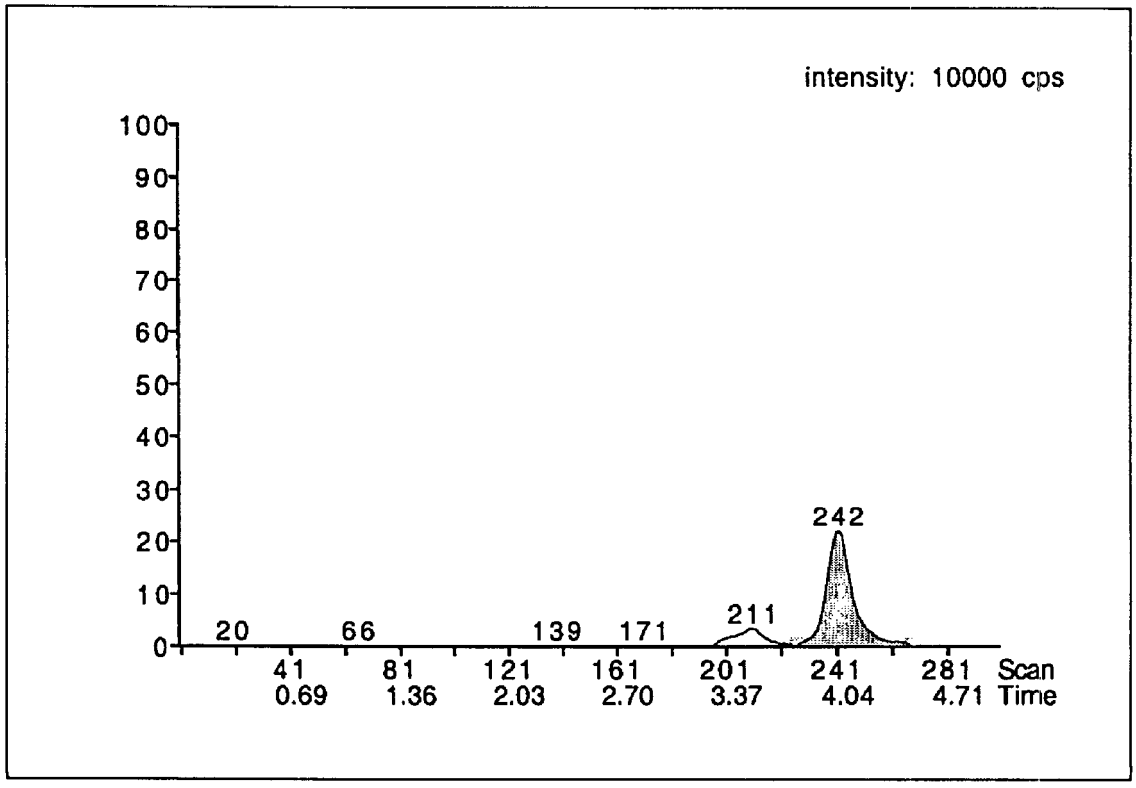
Ion Chromatogram of a High-level Non-Target Terrestrial Plant Soil Fortification Sample



Sample number 454C-120-VMAS-10, 1000 mg a.i./Kg wet-weight nominal concentration, overall dilution factor = 200000 L/Kg.

Appendix 4.11

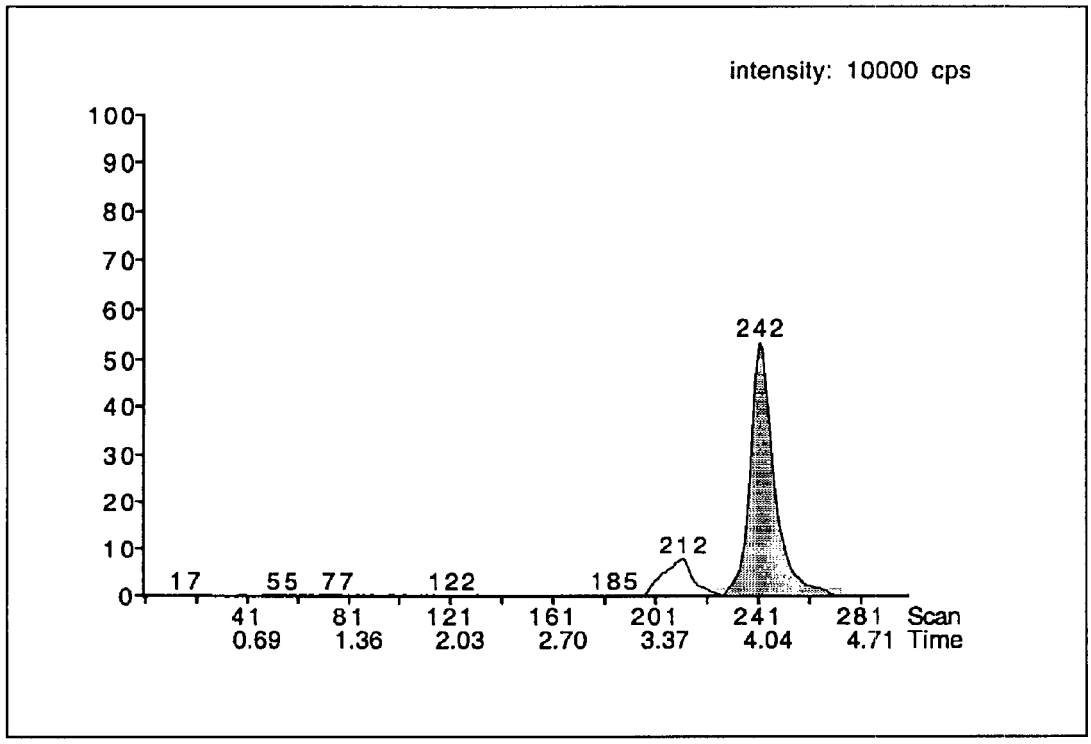
Ion Chromatogram of a Low-level Invertebrate Testing Soil Fortification Sample



Sample number 454C-120-VMAS-13, 2.00 mg a.i./Kg wet-weight nominal concentration, overall dilution factor = 1000 L/Kg.

Appendix 4.12

Ion Chromatogram of a High-level Invertebrate Testing Soil Fortification Sample



Sample number 454C-120-VMAS-22, 1000 mg a.i./Kg wet-weight nominal concentration, overall dilution factor = 200000 L/Kg.

Appendix 5

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., Director, Analytical Chemistry
2. Raymond L. Van Hoven, Ph.D., Scientist
3. Jon MacGregor, B.S., Scientist
4. Frank J. Lezotte, B.S., Chemist