39) Analytical method validation for determination of PFBS-K in saltwater and algal media, 454C-117

T-7485

ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF PERFLUOROBUTANE SULFONATE, POTASSIUM SALT (PFBS) IN SALTWATER AND ALGAL MEDIA

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-117

3M Environmental Lab Project No. E00-1429

AUTHORS:

Raymond L. Van Hoven, Ph.D.
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Willard B. Nixon, Ph.D.

STUDY INITIATION DATE: November 7, 2000

STUDY COMPLETION DATE: July13, 2001

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 Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-117

3M ENVIRONMENTAL LAB PROJECT NUMBER: E00-1429

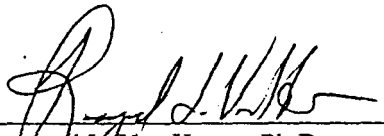
STUDY COMPLETION: July 13, 2001

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 and/or 792 and OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17) with the following exceptions:

The test substance was not characterized in accordance with Good Laboratory Practice Standards.

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.

7-13-01
DATE

SPONSOR APPROVAL:

12/19/01
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 and/or 792, 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 fortifications	November 9, 2000	November 9, 2000	November 13, 2000
Sample preparation	November 13, 2000	November 13, 2000	November 16, 2000
Raw data, draft report	November 28-30, 2000	November 30, 2000	December 1, 2000
Final report	July 13, 2001	July 13, 2001	July 13, 2001

James H. Coleman

James H. Coleman, B.S.
Quality Assurance Representative

7-13-01

DATE

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: Analytical Method Validation for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-117

3M ENVIRONMENTAL LAB PROJECT NUMBER: E00-1429

STUDY DIRECTOR:

Raymond L. Van Hoven, Ph.D. — DATE 7-13-01
Scientist

MANAGEMENT:

Willard B. Nixon, Ph.D. — DATE 7/13/01
Director of Analytical Chemistry

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APPENDICES

SUMMARY

SPONSOR: 3M Corporation

TITLE: Analytical Method Validation for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454C-117

3M ENVIRONMENTAL LAB PROJECT NUMBER: E00-1429

TEST DATES:

Study Initiation:	November 7, 2000
Experimental Start (OECD):	November 9, 2000
Experimental Start (EPA):	November 9, 2000
Experimental Termination:	November 14, 2000

TEST SYSTEM: Freshwater Algal Medium and Filtered Saltwater

FORTIFIED TEST CONCENTRATIONS :

Algal Medium(mg a.i./L)	Filtered Saltwater (mg a.i./L)
100	1.00
1000	10.0
5000	100
10000	1000

RESULTS: Freshwater algal medium samples were fortified with PFBS to reflect nominal concentrations of 100, 1000, 5000 and 10000 mg a.i./L. Filtered saltwater samples were fortified with PFBS to reflect nominal concentrations of 1.00, 10.0, 100 and 1000 mg a.i./L. PFBS stock solutions used for the cited matrix fortification preparations were adjusted for the purity of the potassium salt only. The samples were diluted into the instrumental calibration range with dilution solvent (50% methanol : 50% NANOpure® water) and analyzed by liquid chromatography mass spectrometry (LC/MS). Recoveries of PFBS in freshwater algal medium yielded an overall mean percent recovery of 107% (SD = 3.03; CV =2.83%; N = 12). Recoveries of PFBS in filtered saltwater yielded an overall mean percent recovery of 106% (SD = 3.14%; CV = 2.96%; N = 12). Matrix blank samples, consisting of unfortified freshwater algal medium and filtered saltwater, were devoid of interfering components.

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 Perfluorobutane Sulfonate, Potassium Salt (hereafter referred to as PFBS) and analyzed with liquid chromatography mass spectrometry (LC/MS) to evaluate the performance of a method for the determination of PFBS in filtered saltwater and freshwater algal medium. Validation samples were prepared and analyzed between November 9, 2000 and November 14, 2000. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454C-117 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to verify the performance of a LC/MS methodology for the analyses of PFBS in filtered saltwater and freshwater algal medium. The validated method will be used in support of aquatic toxicology and product chemistry tests to be conducted with the test substance.

EXPERIMENTAL DESIGN

Filtered saltwater and freshwater algal medium were each fortified at four different concentrations in triplicate and analyzed based on LC/MS methodology developed at Wildlife International, Ltd. The fortification levels bracketed the anticipated concentrations of samples from future aquatic toxicity tests. 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 PFBS 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 Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media" (Appendix 1).

Test Substance

The test substance was received from 3M Environmental Technology and Safety Services on March 27, 2000, assigned Wildlife International, Ltd. Identification number 5216, and stored under ambient conditions. The test substance, a white powder, was identified as: Potassium Perfluorobutane Sulfonate,

Lot #2, CAS # 29420-49-3. The test substance was further identified with the 3M Environmental Laboratory test control and reference number Information provided by the Sponsor when the protocol was issued indicated a purity of 97.90% and an expiration date of March 2010. A subsequent revision of the certificate of analysis indicated a purity of 97.3% and an Expiration/Reassessment Date of January 17, 2002 (Appendix 2). Nominal and measured concentration values were not recalculated based on the relatively minor change in purity. The test substance was stored under ambient conditions.

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

Saltwater

Natural seawater collected at Indian River Inlet, Delaware was filtered through a sand filter prior to delivery into a 37,800-L storage tank. The salinity of the seawater was diluted to approximately 20‰ (parts per thousand) with Wildlife International, Ltd. well water in the holding tank to produce the saltwater matrix. The 20 ‰ saltwater was aerated using spray nozzles and filtered (0.45µm) prior to its use in the method validation. Salinity and pH of the filtered saltwater during the four-week period immediately preceding the test are presented in Appendix 3.1. The results of the most recent GLP-compliant analyses performed to measure the concentrations of selected contaminants in well water and saltwater used by Wildlife International, Ltd. are presented in Appendices 3.2 and 3.3, respectively.

Algal Culture Medium

Culture medium used for the method validation was prepared according to Wildlife International, Ltd. Standard Operating Procedures. The concentrations of the components are presented in Appendix 3.4. Stock nutrient solutions were prepared by adding reagent-grade or better chemicals to Wildlife International, Ltd. well water purified by reverse osmosis. Appropriate volumes of the stock nutrient solutions were then diluted with purified well water to prepare the medium. The medium was filter sterilized (0.2 µm) prior to use.

Analytical Validation Procedures

Wildlife International, Ltd. conducted a validation trial using procedures developed at Wildlife International, Ltd. (Appendix 4.1). Validation samples were prepared in filtered saltwater and freshwater algal medium. Dilutions 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 PFBS 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 Prism column (30 mm x 1.5 mm) fitted with a Keystone Prism guard column. A method flow chart is provided in Appendix 4.1 and the instrument parameters are summarized in Appendix 4.2.

Primary Stock Solution, Calibration Standards and Curves

A primary stock solution of PFBS was prepared in methanol. Calibration standards were prepared in 50% methanol: 50% NANOpure® water by appropriate dilutions of this stock solution. Calibration standards of PFBS, ranging in concentration from 0.0100 to 0.0500 mg a.i./L, were analyzed with each sample set. Five calibration standards (different concentrations) were analyzed with the samples. 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 the peak area responses versus the respective concentrations of the calibration standards. A typical calibration curve is presented in Appendix 4.3. The concentration of PFBS 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.

Fortification Stock Solutions

Selected saltwater validation samples were fortified with stock solutions of PFBS prepared in methanol (1.00, 10.0 and 100 mg a.i./L levels). Each stock solution was assigned a unique identification code that was recorded on a stock preparation log sheet. All algal medium validation samples and the high-level (1000 mg a.i./L) saltwater validation samples were fortified directly with the test substance.

Limit of Quantitation

The method validation levels in both the algal medium and saltwater matrices were well above the method capabilities for quantitation of the test substance. Therefore, the method limit of quantitation (LOQ)

in each matrix was calculated as the product of the lowest calibration standard and the dilution factor of the matrix blank samples. In algal medium, the LOQ was (0.0100 mg a.i./L) x (5000) = 50.0 mg a.i./L. In saltwater, the LOQ was (0.0100 mg a.i./L) x (50) = 0.500 mg a.i./L.

RESULTS

Matrix Blank Samples

Three matrix blank samples in each matrix were analyzed to determine possible interferences. No interferences were observed at or above the LOQ (50.0 and 0.500 mg a.i./L in algal medium and saltwater, respectively) during the sample analyses (Tables 1 and 2). Representative ion chromatograms of freshwater algal medium and filtered saltwater matrix blank samples are presented in Appendices 4.7 and 4.8, respectively.

Method Validation Samples

Freshwater algal medium was fortified in triplicate at 100, 1000, 5000 and 10000 mg a.i./L resulting in mean recoveries of 108, 109, 107 and 105%, respectively (Table 1). Representative ion chromatograms of low and high-level freshwater algal medium matrix fortifications are presented in Appendices 4.9 and 4.10, respectively. Saltwater was fortified in triplicate at 1.00, 10.0, 100 and 1000 mg a.i./L resulting in mean recoveries of 106, 109, 107 and 103%, respectively (Table 2). Representative ion chromatograms of low and high-level saltwater matrix fortifications are presented in Appendices 4.11 and 4.12, respectively.

CONCLUSIONS

Freshwater algal medium samples were fortified with PFBS to reflect nominal concentrations of 100, 1000, 5000 and 10000 mg a.i./L. Filtered saltwater samples were fortified with PFBS to reflect nominal concentrations of 1.00, 10.0, 100 and 1000 mg a.i./L. The samples were diluted into the instrumental calibration range with dilution solvent (50% methanol/50% NANOpure® water) and analyzed by liquid chromatography mass spectrometry (LC/MS). Recoveries of PFBS in freshwater algal medium yielded an overall mean percent recovery of 107% (SD = 3.03%; CV = 2.83%; N = 12). Recoveries of PFBS in saltwater yielded an overall mean percent recovery of 106% (SD = 3.14%; CV = 2.96%; N = 12). Matrix blank samples, consisting of unfortified freshwater algal medium and unfortified saltwater, 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 PFBS in Algal Medium

Sample		PFBS Concentration (mg a.i./L)				
Number (454C-117-)	Type	Fortified	Measured	Percent Recovery	Mean Measured (mg a.i./L)	Mean Percent Recovery
VMAB-1	Matrix Blank	0	<LOQ[1]	–	–	–
VMAB-2	Matrix Blank	0	<LOQ	–		
VMAB-3	Matrix Blank	0	<LOQ	–		
VMAS-1	Matrix Fortification	100	103	103	108	108
VMAS-2	Matrix Fortification	100	111	111		
VMAS-3	Matrix Fortification	100	109	109		
VMAS-4	Matrix Fortification	1000	1110	111	1090	109
VMAS-5	Matrix Fortification	1000	1090	109		
VMAS-6	Matrix Fortification	1000	1060	106		
VMAS-7	Matrix Fortification	5000	5450	109	5340	107
VMAS-8	Matrix Fortification	5000	5290	106		
VMAS-9	Matrix Fortification	5000	5270	105		
VMAS-10	Matrix Fortification	10000	10400	104	10500	105
VMAS-11	Matrix Fortification	10000	10800	108		
VMAS-12	Matrix Fortification	10000	10200	102		
			Overall Mean =	107		
			Standard Deviation =	3.03		
			CV =	2.83		
			N =	12		

[1]The limit of quantitation (LOQ) was 50.0 mg a.i./L based upon the product of the lowest calibration standard (0.0100 mg a.i./L) and the dilution factor of the matrix blank samples (5000).

Table 2

Method Validation Recoveries for PFBS in Saltwater

Sample		PFBS Concentration (mg a.i./L)				
Number (454C-117-)	Type	Fortified	Measured	Percent Recovery	Mean Measured (mg a.i./L)	Mean Percent Recovery
VMAB-4	Matrix Blank	0	<LOQ[1]	–	–	–
VMAB-5	Matrix Blank	0	<LOQ	–		
VMAB-6	Matrix Blank	0	<LOQ	–		
VMAS-25	Matrix Fortification	1.00	1.08	108	1.06	106
VMAS-26	Matrix Fortification	1.00	1.07	107		
VMAS-27	Matrix Fortification	1.00	1.03	103		
VMAS-16	Matrix Fortification	10.0	10.5	105	10.9	109
VMAS-17	Matrix Fortification	10.0	10.8	108		
VMAS-18	Matrix Fortification	10.0	11.3	113		
VMAS-19	Matrix Fortification	100	106	106	107	107
VMAS-20	Matrix Fortification	100	109	109		
VMAS-21	Matrix Fortification	100	107	107		
VMAS-22	Matrix Fortification	1000	1060	106	1030	103
VMAS-23	Matrix Fortification	1000	1020	102		
VMAS-24	Matrix Fortification	1000	1020	102		
			Overall Mean =	106		
			Standard Deviation =	3.14		
			CV =	2.96		
			N =	12		

[1]The limit of quantitation (LOQ) was 0.500 mg a.i./L based upon the product of the lowest calibration standard (0.0100 mg a.i./L) and the dilution factor of the matrix blank samples (50).

Appendix 1

Protocol and Amendments

PROTOCOL

ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF
PERFLUOROBUTANE SULFONATE, POTASSIUM SALT (PFBS)
IN SALTWATER AND ALGAL MEDIA

3M Lab Project No. E00-1429

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

July 17, 2000

Wildlife International, Ltd.

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ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF
[PERFLUOROBUTANE SULFONATE, POTASSIUM SALT (PFBS)]
IN SALTWATER AND ALGAL MEDIA

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

SPONSOR'S REPRESENTATIVE: [ ]

TESTING FACILITY: Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601

STUDY DIRECTOR: Raymond L. VanHoven, Ph.D.
Scientist

LABORATORY MANAGEMENT: Willard B. Nixon, Ph.D.
Manager of Analytical Chemistry

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: _____ Experimental Termination Date: _____

Project No.: 454C-117 Test Concentrations: _____

Test Substance No.: 5216 Reference Substance No.: N/A

PROTOCOL APPROVAL

_____ 11/07/00
STUDY DIRECTOR DATE

_____ 11/7/00
LABORATORY MANAGEMENT DATE

[ ] 7/20/00
SPONSOR'S REPRESENTATIVE DATE

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 Perfluoro Butane Sulfonate, Potassium Salt (PFBS) in filtered saltwater and algal media. The study will be performed at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. The method will be verified by fortifying each water matrix with the test substance and determining recoveries of PFBS. 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 PFBS in filtered salt water and algal media. The method to be validated will be used in support of aquatic toxicology and product chemistry tests to be conducted with the test substance.

EXPERIMENTAL DESIGN

Wildlife International, Ltd. filtered saltwater and algal media will each 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 and documented as necessary to achieve quantitation of PFBS in each of the test matrices. 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 aquatic toxicity tests. One or more calibration curves will be generated from analyses of standard solutions of PFBS to be analyzed with each series of matrix fortification samples.

Wildlife International, Ltd.

MATERIALS AND METHODS

Test and Reference Substances

The test substance will be PFBS. Information on the characterization of test and reference substances is required by Good Laboratory Practice Standards (GLP). The Sponsor is responsible for providing Wildlife International, Ltd. written verification that the test and reference substances have been characterized according to GLPs prior to initiation of the study. If written verification of GLP test and/or reference substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report.

Reagents and Solvents

All solvents used in the method or procedure will be HPLC grade or equivalent. All reagents will be ACS reagent grade or higher quality. Nanopure water (equivalent to ASTM Type II Designation D1193-91) will be used (1). The solvents and reagents are not expected to contain contaminants capable of interfering with the purpose of this study.

Algal Culture Medium

Culture medium to be used for the method validation will be prepared according to Wildlife International, Ltd. Standard Operating Procedures. The concentrations of the components in the medium are presented in Table 1 (2). Stock nutrient solutions will be prepared by adding reagent-grade or better chemicals to Wildlife International, Ltd. well water purified by reverse-osmosis. Appropriate volumes of the stock nutrient solutions will then be diluted with purified well water to prepare the medium. The medium will be filter sterilized (0.2 µm) or autoclaved prior to use. The pH of the medium should be 7.5 ± 0.1.

Saltwater

Natural seawater collected at Indian River Inlet, Delaware will be filtered through a sand filter prior to delivery to a 37,800-L holding tank. The salinity of the seawater will be diluted to approximately 20 ‰ (parts per thousand) with Wildlife International, Ltd. well water in the holding tank to produce the saltwater matrix. The 20 ‰ saltwater will be aerated using spray nozzles and filtered (0.45 µm) prior to its use in the method validation.

Wildlife International, Ltd.

Salinity and pH will be measured weekly to monitor the consistency of the saltwater. Means and ranges of the measured parameters for the four-week period preceding the test will be provided in the final report.

Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and seawater. Results of the most recent GLP-compliant analyses will be summarized in the final report. Specifications for acceptable levels of contaminants in culture medium, well water, and seawater have not been established. However, there are no known levels of contaminants reasonably expected to be present in these well matrices that are considered to interfere with the purpose or conduct of the test.

Analytical Validation Procedures

Wildlife International, Ltd. will conduct validation trials using procedures based on LC/MS methodology developed at Wildlife International, Ltd. The method may be modified to yield a method capable of quantitating PFBS at the desired concentrations in filtered salt water and algal media. The method used, including any modifications, will be documented in the raw data and summarized in the final report.

Reference Stock Solution(s), Calibration Standards and Curves

A primary stock solution(s) will be prepared from PFBS reference substance, if available, or directly from the test substance in an appropriate solvent. Calibration standards will be prepared by appropriate dilution of this primary stock solution(s). The concentration range of the calibration standards will bracket the instrumental concentrations of the study samples. A minimum of five concentrations of calibration standards will be prepared and analyzed along with each analysis set of validation samples. The calibration standard series will be injected at the beginning and end of the analytical run with, in addition, a minimum of one mid-level standard injected following every five samples. One or more calibration curves will be derived from regression analysis of the instrumental responses of the standards.

Fortification Stock Solution(s)

Saltwater and algal media will each be fortified with a stock solution(s) of PFBS. The fortification levels used will bracket the levels expected in anticipated aquatic toxicology studies

Wildlife International, Ltd.

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with PFBS. Each stock solution will be assigned a unique identification code which will be recorded on a stock preparation log sheet.

Evaluation of Interferences

Matrix and reagent blanks, if applicable, will be analyzed to assess the presence of potential interferences. Matrix blanks will consist of saltwater and algal media without addition of test substance. Reagent blanks, if applicable, will consist of reagents processed through the method.

Validation Analyses - Method Performance

Matrix fortifications (fortified saltwater and algal media) prepared at known concentrations of PFBS, will be analyzed to determine recoveries and to evaluate method performance. The anticipated validation series will consist of the following analyses:

SUMMARY OF PROPOSED VALIDATION ANALYSIS SCHEME

Analysis Type	Concentration	Number of Samples	
		Algal Medium	Saltwater
Matrix Blank	0 (Control)	3	3
Matrix Fortifications	Level 1 - Low[1]	3	3
	Level 2	3	3
	Level 3	3	3
	Level 4 - High	3	3
Total Number of Analyses		30	

[1] Matrix fortifications will be prepared at four concentrations, the lowest and highest of which will bracket the anticipated treatment range for environmental effects studies.

Matrix fortifications will span the range of concentrations that are anticipated to be used in subsequent environmental effects tests. Individual fortification samples (identified by project number and a unique sample identification number) will be prepared and analyzed.

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

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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 for each analytical run will be derived from the instrumental responses of PFBS standards of known concentration using regression analysis. The concentrations of PFBS in fortified samples will be determined by substituting the respective instrumental response into the regression equation

If the fortification range and calibration curve standard concentrations are significantly higher than method capabilities for quantitation, the limit of quantitation (LOQ) will be defined as the concentration equivalent to the lowest standard accounting for dilutions and other manipulations in the method for matrix blanks. If the fortification range is near the method capability for accurate quantitation of the analyte, the LOQ will be determined from the responses of matrix blank samples. The mean (Sb) and standard deviation (σ) of responses will be determined for the replicate control sample and converted to ppm equivalents using a standard curve and method dilution factors. The LOQ will be expressed as the concentration equivalent of Sb + 10σ. The limit of detection (LOD), defined as the lowest concentration of analyte which can be injected and produce a measurable response above background, will be expressed as the amount equivalent to Sb +3σ.

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 test and reference substances, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Storage conditions for test and reference substances, and samples.
5. Test and reference substance use logs.
6. Concentration calculations and records of solution preparation.

Wildlife International, Ltd.

7. Instrument operating conditions and chromatograms.
8. Statistical calculations.
9. A copy of the final report.

FINAL REPORT

The report will summarize the findings of the validation, the fortification recoveries obtained, and method(s) and instrumentation employed. Upon receipt of these findings, the Sponsor will review the methods and results, and evaluate the results for acceptability.

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. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures stated in the approved protocol, including any changes in the original protocol or deviations from the protocol.
5. Identification of test and reference substances, 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.
6. A brief summary of the analytical methodology to include, where applicable, a description of the experimental measurements, example calculations, sample preparation (sample weights and/or dilutions), instrumentation employed, reagents used, purity of reagents and solvents, and any major modifications to the method.
7. A description of any circumstances that may have affected the quality or integrity of the data.
8. The name of the Study Director, the names of other scientists or professionals, and the names of all supervisory personnel involved in the study.
9. A description of the transformations, calculations, or operations performed on the data.
10. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
11. The location where raw data and final report are to be stored.

Wildlife International, Ltd.

12. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.

CHANGING OF PROTOCOL

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

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or 792) and OECD Principles of Good Laboratory Practices (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 compliance with Good Laboratory Practices for procedures performed by other laboratories.

Wildlife International, Ltd.

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

2 **American Society for Testing and Materials.** 1990. Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae. E 1218-90. ASTM Section II Water and Environmental Technology, Vol. 11.05.

3 **American Society for Testing and Materials.** 1991. Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G-3. E 1415-91. ASTM Section II Water and Environmental Technology, Vol. 11.05.

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TABLE 1

FRESHWATER ALGAL MEDIUM CONSTITUENTS

Component	Nominal Concentration
$MgCl_2 \cdot 6H_2O$	12.16 mg/L
$CaCl_2 \cdot 2H_2O$	4.40 mg/L
H_3BO_3	0.1856 mg/L
$MnCl_2 \cdot 4H_2O$	0.416 mg/L
$ZnCl_2$	3.28 µg/L
$FeCl_3 \cdot 6H_2O$	0.1598 mg/L
$CoCl_2 \cdot 6H_2O$	1.428 µg/L
$Na_2MoO_4 \cdot 2H_2O$	7.26 µg/L
$CuCl_2 \cdot 2H_2O$	0.012 µg/L
$Na_2EDTA \cdot 2H_2O$	0.300 mg/L
$NaNO_3$	25.50 mg/L
$MgSO_4 \cdot 7H_2O$	14.70 mg/L
K_2HPO_4	1.044 mg/L
$NaHCO_3$	15.00 mg/L

The pH of the medium will be adjusted, as necessary, to 7.5 ± 0.1 using 0.1 N NaOH or 10% HCl.

Project Number: 454C-117

Wildlife International, Ltd. Page 1 of 1

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Analytical Method Verification for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media

PROTOCOL NO.: 454/071700/MVAL/SUB454 AMENDMENT NO.: 1

SPONSOR: 3M Corporation PROJECT NUMBER: 454C-117

3M LAB PROJECT NO.: E00-1429 EFFECTIVE DATE: November 9, 2000

AMENDMENT: Page 2, Proposed Dates and Test Concentrations

Add: Experimental Start Date: 11/09/00
Experimental Termination Date: 12/31/00
Test Concentrations: (Algal Medium) 0.0, 100, 1000, 5000, and 10000 mg a.i./L

REASON: The above information was required to complete the algal medium portion of the protocol.

STUDY DIRECTOR 11-09-00 DATE

LABORATORY MANAGEMENT 11/9/00 DATE

11/30/00 DATE

Reviewed by QA
JHC 11-8-00

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Analytical Method Verification for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media

PROTOCOL NO.: 454/071700/MVAL/SUB454 AMENDMENT NO.: 2

SPONSOR: 3M Corporation PROJECT NUMBER: 454C-117

3M LAB PROJECT NO.: E00-1429 EFFECTIVE DATE: November 9, 2000

AMENDMENT: Page 5, Reference Stock Solution(s), Calibration Standards and Curves

Change: "The calibration standard series will be injected at the beginning and end of the analytical run with, in addition, a minimum of one mid-level standard injected following every five samples."

To: "The calibration standard series will be injected at the beginning and end of the analytical run with, in addition, a minimum of one standard injected following every five samples."

REASON: The revised wording is compatible with current Wildlife International, Ltd. SOPs.

STUDY DIRECTOR — DATE 11-09-00

LABORATORY MANAGEMENT — DATE 11/9/00

11/30/00
DATE

Reviewed by QA
SHC 11-8-00

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Analytical Method Verification for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media

PROTOCOL NO.: 454/071700/MVAL/SUB454 AMENDMENT NO.: 3

SPONSOR: 3M Corporation PROJECT NUMBER: 454C-117

3M LAB PROJECT NO.: E00-1429 EFFECTIVE DATE: November 13, 2000

AMENDMENT: Page 2, Proposed Test Concentrations

Add: Test Concentrations: (Saltwater) 0.0, 1.00, 10.0, 100, and 1000 mg a.i./L

REASON: The above information was required to complete the saltwater portion of the protocol.

STUDY DIRECTOR — DATE 11-13-00

LABORATORY MANAGEMENT — DATE 11/13/00

DATE 11/30/00

Reviewed by QA
JHC 11-13-00

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Analytical Method Validation for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media

PROTOCOL NO.: 454/071700/MVAL/SUB454 AMENDMENT NO.: 4

SPONSOR: 3M Corporation PROJECT NUMBER: 454C-117

3M LAB PROJECT NO.: E00-1429 EFFECTIVE DATE: November 13, 2000

AMENDMENT: Amendments 1, 2, and 3 - STUDY TITLE

Change: Analytical Method Verification for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media.

To: Analytical Method Validation for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media.

REASON: Typographical error.

STUDY DIRECTOR — DATE 11-13-00

LABORATORY MANAGEMENT — DATE 11/13/00

DATE 11/30/00

Reviewed by QA
JHC 11-13-00

Appendix 2

Certificates of Analysis

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 1

Centre Analytical Laboratories COA Reference #: 023-032
3M Product: PFBS, Lot 2
Test Control Reference #: TCR-00017-71
Purity: 97.3%

Test Name	Specifications	Result
Purity[1]		97.3%
Appearance	White, crystalline solid	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium[2] 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		 1. 0.003 wt/wt.% 2. 0.001 wt/wt.% 3. 0.768 wt/wt.% 4. 10.718 wt/wt.% 5. <0.001 wt/wt.% 6. 0.002 wt/wt.% 7. <0.001 wt/wt.%
Total % Impurity (NMR)		2.07 wt/wt.%
Total % Impurity (LC/MS)		5.56 wt/wt.%
Related Compounds (POAA)		None Quantified
Total % Impurity (GC/MS)		None Quantified
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate		 1. 0.016 wt/wt.% 2. <0.005 wt/wt.% 3. <0.040 wt/wt.% 4. <0.009 wt/wt.% 5. <0.006 wt/wt.% 6. <0.030 wt/wt.% 7. <0.040 wt/wt.%
Organic Acids[3] (IC)		1. <0.1 wt/wt.% 2. <0.1 wt/wt.% 3. <0.1 wt/wt.% 4. <0.25 wt/wt.%
Elemental Analysis[4]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 Theoretical Value = 14.2% Theoretical Value = 0.0% Theoretical Value = 0.0% Theoretical Value = 9.5% Theoretical Value = 50.6%	 1. 11.0 wt/wt.% 2. 0.282 wt/wt.% 3. 0.465 wt/wt.% 4. 11.2 wt/wt.% 5. 48.2 wt/wt.%

Exact Copy of Original
MC [Initial] 03/05/01 Date

5216
Rec'd 3/5/2001 AM

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 1

Centre Analytical Laboratories COA Reference #: 023-032
3M Product: PFBS, Lot 2
Test Control Reference #: TCR-00017-71

Date of Last Analysis: 01/17/01

Expiration Date: 01/17/02

Storage Conditions: < -10 °C

Re-assessment Date: 01/17/02

[1]Purity = 100% - (Total NMR impurities, 2.07% + Total LC/MS impurities, (5.56% - 4.99%*) + Total Metal impurities, 0.006% + Total inorganic anions, 0.016%)

Total impurity from all tests = 2.662%
Purity = 100% - 2.662% = 97.3%

*1.93% of the NMR total impurity value is due to the presence of . This impurity is also observed in the LC/MS analysis at 4.99%. The impurity value is taken from the NMR analysis as it is deemed the more quantitative technique in the absence of standards (See impurity profile on page 3).

[2]Potassium and sodium are present as counterions and thus are excluded from the purity calculation.

[4]Theoretical value calculations based on the empirical formula, $C_4F_9SO_3K^+$ (MW=338.2)

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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 1

Centre Analytical Laboratories COA Reference #: 023-032
3M Product: PFBS, Lot 2
Test Control Reference #: TCR-00017-71

LC/MS Purity Profile:

Peak #	Retention Time (min)	Mass(s)	Identity	Area	% Impurity
1					0.57
2					4.99
3	9.508	298.9	PFBS	30835100	-
Total	-	-	-	32651678	5.56

NMR Purity Profile:

Component *	Molecular Weight	Relative Weight Percent
CF_3-CF_2-CF_2-CF_2-SO_3^-, Linear Product	298.9	97.9
		0.05
		1.93
		0.13
		0.01
Total Impurity		2.07

*All components are assumed to be associated with K^+ cation.
Note: NMR detected impurities in boldface type.

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

Prepared By: _____ 2/26/01
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ 2/26/01
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

Original Certificate of Analysis

$C_4F_9SO_3^-K^+$ (Lot 1; PFBS)
. Lot 2 (1999)

April 17, 2000
(Supercedes Certificate of Analysis dated April 11, 2000)

This sample was analyzed using , and elemental analyses techniques. The results of these tests show the sample to contain the following weight percent composition:

$C_3F_7SO_3^-K^+$	0.13 %
$C_4F_9SO_3^-K^+$	97.90%
	1.96 %

Additionally, the isomer distribution of the sample was determined using . techniques and found to contain the following weight percent composition:

$CF_3(CF_2)_3$-$SO_3^-K^+$ (Normal chain)	97.86 %
	0.04 %

Analytical Study Number: 60488

Appendix 3

Matrix Water Parameters

Appendix 3.1

Salinity and pH of Saltwater Measured During the 4-Week Period Immediately Preceding the Test

	Mean	Range
Salinity (ppt)	20 (N = 4)	20 - 20
pH	8.3 (N = 4)	8.2 - 8.5

Appendix 3.2
Analyses of Pesticides, Organics and Metals
in Wildlife International, Ltd. Well Water[1]

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

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

Appendix 3.2 (Continued)
Analyses of Pesticides, Organics and Metals
in Wildlife International, Ltd. Well Water[1]

Pesticides and Organics			
Component	Measured Concentration	Component	Measured Concentration
Malathion	<0.02 µg/L	Methoxychlor	<0.01 µg/L
Metalaxyl	<0.05 µg/L	Metolachlor	<0.01 µg/L
Metamitron	<0.05 µg/L	Metribuzin	<0.02 µg/L
Metazachlor	<0.02 µg/L	Mevinphos	<0.01 µg/L
Methidathion	<0.02 µg/L	Nitrothal-Isopropyl	<0.05 µg/L
Paclobutazole	<0.05 µg/L	Pyrifenox-1	<0.01 µg/L
Parathion	<0.01 µg/L	Pyrifenox-2	<0.01 µg/L
Parathion-methyl	<0.01 µg/L	Pyrimethanil	<0.01 µg/L
Penconazole	<0.05 µg/L	Quizalofop-ethyl	<0.02 µg/L
Pendimethalin	<0.03 µg/L	Simazine	<0.01 µg/L
Permethrin-cis	<0.01 µg/L	Sulfotep	<0.02 µg/L
Permethrin-trans	<0.01 µg/L	Tebuconazole	<0.05 µg/L
Phosalone	<0.05 µg/L	Tebufenpyrad	<0.05 µg/L
Phosmet	<0.02 µg/L	Terbutryn	<0.01 µg/L
Phosphamidon-cis	<0.05 µg/L	Terbuthylazine	<0.01 µg/L
Pirimicarb	<0.01 µg/L	Tetrachlorvinphos	<0.01 µg/L
Pirimiphos-ethyl	<0.01 µg/L	Tetrahydroftalimide	<0.05 µg/L
Pirimiphos-methyl	<0.01 µg/L	Tetramethrin	<0.01 µg/L
Prochloraz	<0.02 µg/L	Thiabendazole	<0.05 µg/L
Procymidon	<0.01 µg/L	Thiometon	<0.04 µg/L
Prometryn	<0.01 µg/L	Tolclofos-methyl	<0.01 µg/L
Propachlor	<0.01 µg/L	Tolylfluanid	<0.04 µg/L
Propazine	<0.01 µg/L	Triadimefon	<0.05 µg/L
Propham	<0.02 µg/L	Triadimenol	<0.05 µg/L
Propiconazole	<0.05 µg/L	Triallate	<0.02 µg/L
Propoxur	<0.03 µg/L	Triazophos	<0.02 µg/L
Propyzamide	<0.02 µg/L	Trifluralin	<0.02 µg/L
Prosulfocarb	<0.02 µg/L	Vamidothion	<0.01 µg/L
Pyrazophos	<0.03 µg/L	Vinclozolin	<0.01 µg/L
Metals			
Magnesium	13.2 mg/L	Nickel	<1.4 µg/L
Sodium	21 mg/L	Copper	<1.0 µg/L
Calcium	35 mg/L	Zinc	<2.3 µg/L
Iron	<0.02 mg/L	Molybdenum	<0.7 µg/L
Potassium	6.2 mg/L	Silver	<0.3 µg/L
Aluminum	<0.09 mg/L	Cadmium	<0.3 µg/L
Manganese	0.72 µg/L	Arsenic	<0.25 µg/L
Beryllium	<0.3 µg/L	Mercury	<0.025 µg/L
Chromium	<0.6 µg/L	Selenium	<1 µg/L
Cobalt	<0.4 µg/L		

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

Appendix 3.3
Analyses of Pesticides, Organics and Metals
in Wildlife International, Ltd. Saltwater[1]

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

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

Appendix 3.3 (Continued)
Analyses of Pesticides, Organics and Metals
in Wildlife International, Ltd. Saltwater[1]

Pesticides and Organics			
Component	Measured Concentration	Component	Measured Concentration
Malathion	<0.02 µg/L	Methoxychlor	<0.01 µg/L
Metalaxyl	<0.05 µg/L	Metolachlor	<0.01 µg/L
Metamitron	<0.05 µg/L	Metribuzin	<0.02 µg/L
Metazachlor	<0.02 µg/L	Mevinphos	<0.01 µg/L
Methidathion	<0.02 µg/L	Nitrothal-Isopropyl	<0.05 µg/L
Paclobutazole	<0.05 µg/L	Pyrifenox-1	<0.01 µg/L
Parathion	<0.01 µg/L	Pyrifenox-2	<0.01 µg/L
Parathion-methyl	<0.01 µg/L	Pyrimethanil	<0.01 µg/L
Penconazole	<0.05 µg/L	Quizalofop-ethyl	<0.02 µg/L
Pendimethalin	<0.03 µg/L	Simazine	<0.01 µg/L
Permethrin-cis	<0.01 µg/L	Sulfotep	<0.02 µg/L
Permethrin-trans	<0.01 µg/L	Tebuconazole	<0.05 µg/L
Phosalone	<0.05 µg/L	Tebufenpyrad	<0.05 µg/L
Phosmet	<0.02 µg/L	Terbutryn	<0.01 µg/L
Phosphamidon-cis	<0.05 µg/L	Terbuthylazine	<0.01 µg/L
Pirimicarb	<0.01 µg/L	Tetrachlorvinphos	<0.01 µg/L
Pirimiphos-ethyl	<0.01 µg/L	Tetrahydroftalimide	<0.05 µg/L
Pirimiphos-methyl	<0.01 µg/L	Tetramethrin	<0.01 µg/L
Prochloraz	<0.02 µg/L	Thiabendazole	<0.05 µg/L
Procymidon	<0.01 µg/L	Thiometon	<0.04 µg/L
Prometryn	<0.01 µg/L	Tolclofos-methyl	<0.01 µg/L
Propachlor	<0.01 µg/L	Tolylfluanid	<0.04 µg/L
Propazine	<0.01 µg/L	Triadimefon	<0.05 µg/L
Propham	<0.02 µg/L	Triadimenol	<0.05 µg/L
Propiconazole	<0.05 µg/L	Triallate	<0.02 µg/L
Propoxur	<0.03 µg/L	Triazophos	<0.02 µg/L
Propyzamide	<0.02 µg/L	Trifluralin	<0.02 µg/L
Prosulfocarb	<0.02 µg/L	Vamidothion	<0.01 µg/L
Pyrazophos	<0.03 µg/L	Vinclozolin	<0.01 µg/L
Metals			
Magnesium	730 mg/L	Nickel	<14 µg/L
Sodium	5,800 mg/L	Copper	<10.0 µg/L
Calcium	270 mg/L	Zinc	<20.0 µg/L
Iron	<0.03 mg/L	Molybdenum	<7.0 µg/L
Potassium	200 mg/L	Silver	<3.0 µg/L
Aluminum	<0.18 mg/L	Cadmium	<3.0 µg/L
Manganese	<1.0 µg/L	Arsenic	1.2 µg/L
Beryllium	<3.0 µg/L	Mercury	<0.025 µg/L
Chromium	<6.0 µg/L	Selenium	<1 µg/L
Cobalt	<4.0 µg/L		

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

Appendix 3.4

Freshwater Algal Medium Constituents[1]

Compound	Nominal Concentration	
$MgCl_2 \bullet 6H_2O$	12.16	mg/L
$CaCl_2 \bullet 2H_2O$	4.40	mg/L
H_3BO_3	0.1856	mg/L
$MnCl_2 \bullet 4H_2O$	0.416	mg/L
$ZnCl_2$	3.28	µg/L
$FeCl_3 \bullet 6H_2O$	0.1598	mg/L
$CoCl_2 \bullet 6H_2O$	1.428	µg/L
$Na_2MoO_4 \bullet 2H_2O$	7.26	µg/L
$CuCl_2 \bullet 2H_2O$	0.012	µg/L
$Na_2EDTA \bullet 2H_2O$	0.300	mg/L
$NaNO_3$	25.50	mg/L
$MgSO_4 \bullet 7H_2O$	14.70	mg/L
K_2HPO_4	1.044	mg/L
$NaHCO_3$	15.0	mg/L

[1]The pH was adjusted to 7.5 ± 0.1 after preparation.

Appendix 4

Analytical Method Validation Data

Appendix 4.1

Analytical Method Flow Chart for the Analysis of PFBS in Filtered Saltwater and Freshwater Algal Medium.

METHOD OUTLINE FOR THE ANALYSIS OF PFBS IN FILTERED SALTWATER AND FRESHWATER ALGAL MEDIUM

Prepare matrix fortification samples in algal medium by weighing the requisite amount of PFBS test substance on an analytical balance and transferring directly into a Class A volumetric flask partially filled with algal medium. Rinse weighing paper and sides of the flask with repeat algal medium rinses. Swirl the flask to dissolve the test substance and then bring to final volume with algal medium. Sonicate as appropriate and mix with several repeat inversions. The matrix blank is unfortified algal medium.

↓

Prepare matrix fortification samples in saltwater as follows: For target PFBS concentrations ≤ 100 mg a.i./L, fortify the saltwater matrix with the appropriate stock solution of PFBS. For target PFBS concentrations >100 mg a.i./L, prepare by weighing the requisite amount of PFBS on an analytical balance and transferring directly into a Class A volumetric flask partially filled with saltwater matrix. Rinse weighing paper and the sides of the flask with repeat saltwater rinses. Swirl the flask to dissolve the test substance and then bring to final volume with saltwater. Sonicate as appropriate and mix with several repeat inversions. The matrix blank is unfortified saltwater.

↓

Dilute samples into the calibration range of the PFBS LC/MS methodology: Partially fill Class A volumetric flasks with dilution solvent (50% methanol: 50% NANOpure® water). Add appropriate volume of sample and bring to volume with dilution solvent. Process matrix blank samples using the same dilution and aliquot volumes as for the lowest fortification level. Mix well by several repeat inversions.

↓

Ampulate samples and submit for LC/MS analysis.

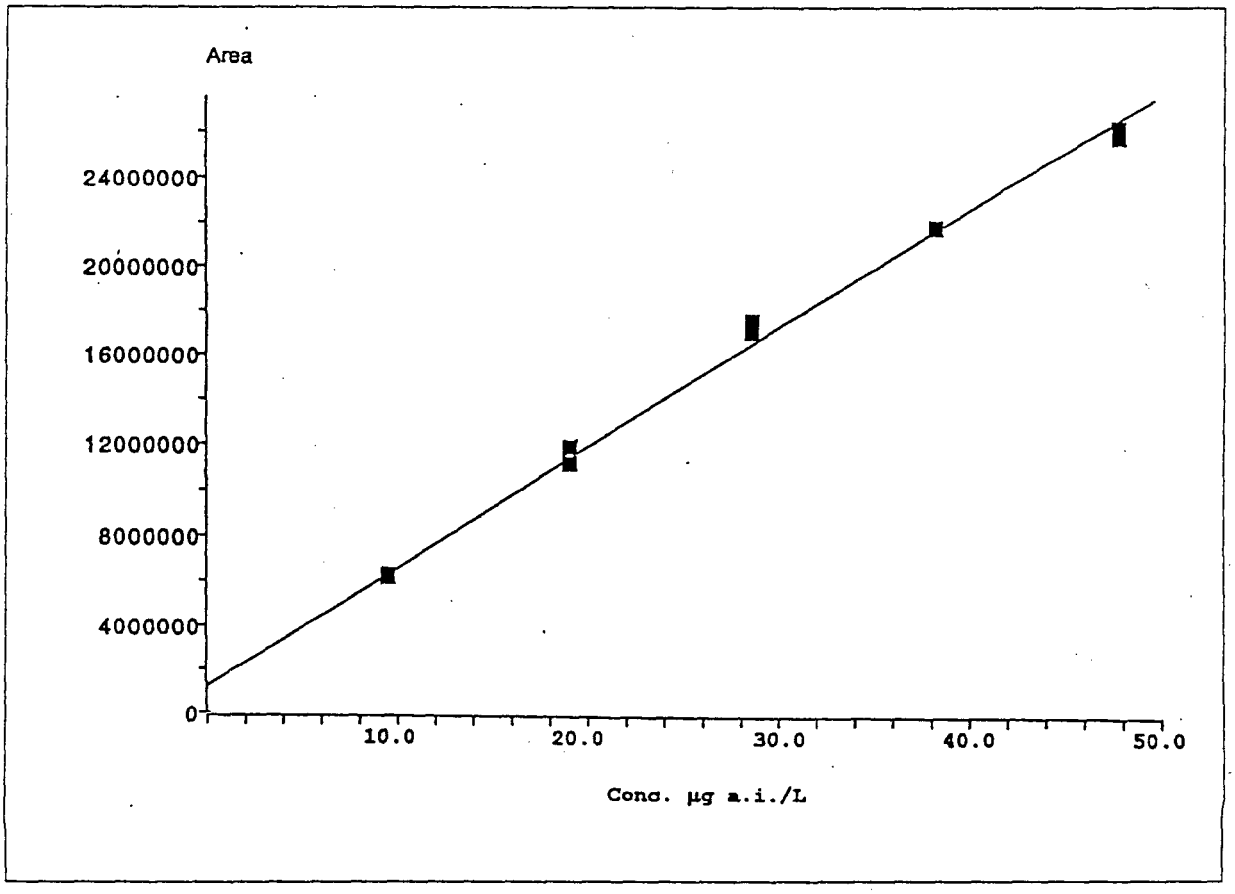
Appendix 4.2

Typical LC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 3000LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in selective ion monitoring mode (SIM).
ANALYTICAL COLUMN:	Keystone Prism Column (30 mm x 1.5 mm)
GUARD COLUMN:	Keystone Prism (20 mm x 2 mm)
OVEN TEMPERATURE:	40°C
STOP TIME:	3.00 minutes
FLOW RATE:	0.200 mL/minute
MOBILE PHASE:	75.0% Methanol : 25.0% NANOpure® Water containing 0.1% Ammonium Formate
INJECTION VOLUME:	5.0 µL
PFBS RETENTION TIME:	Approximately 2.2 minutes
PFBS MONITORED MASS:	299.0 amu

Appendix 4.3

A Typical Calibration Curve for PFBS



Slope = 508806048.00; Intercept = 1216876.63; r = 0.99863.

Appendix 4.4

Example Calculations for a Representative Sample

Sample number 454C-117-VMAS-1, nominal concentration of 100 mg a.i./L in freshwater algal medium.

First Initial Volume: 0.500 mL

First Final Volume: 25.0 mL

Second Initial Volume: 0.500 mL

Second Final Volume: 50.0 mL

Dilution Factor: 5000

PFBS Peak Area: 11667144

Calibration curve equation.

Slope: 508806048.00

Intercept: 1216876.63

Curve is weighted (1/x).

$$\text{PFBS (mg a.i./L) in sample} = \frac{\text{peak area - (y-intercept)}}{\text{slope}} \times \text{dilution factor}$$

$$= \frac{11667144 - 1216876.63}{508806048.00} \times 5000$$

$$= 102.7$$

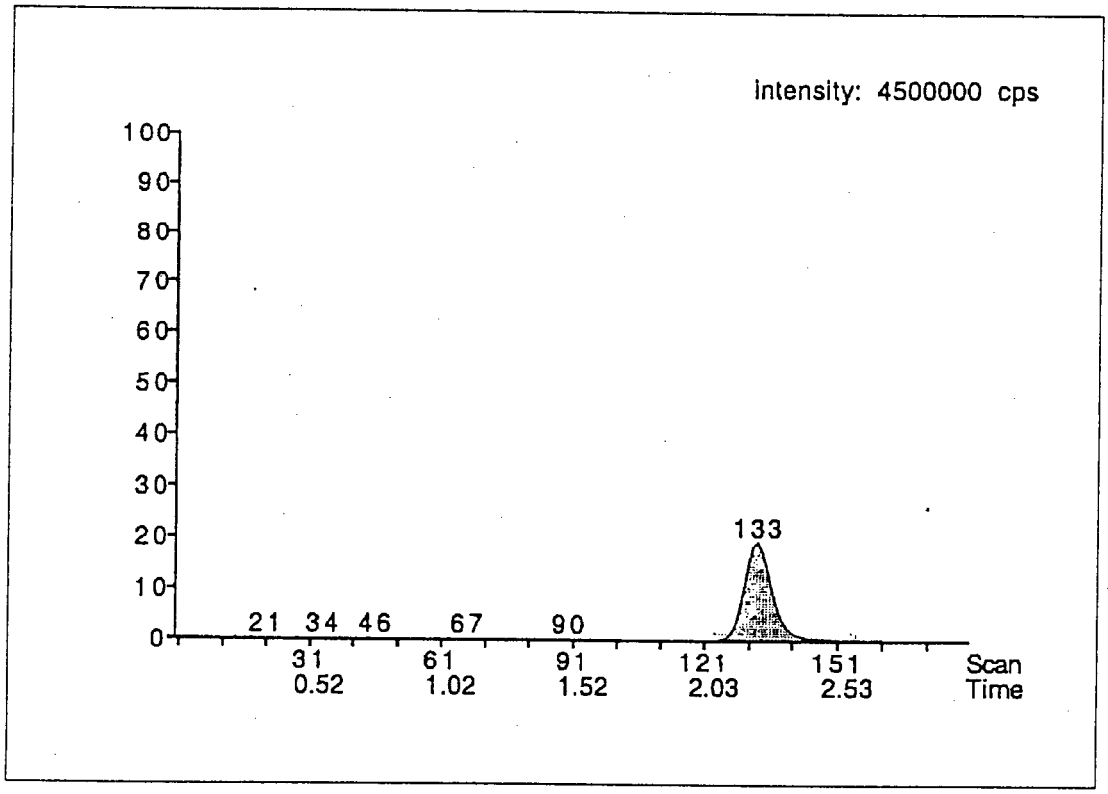
$$\text{Percent of Nominal Concentration} = \frac{\text{PFBS (mg a.i./L) in sample}}{\text{PFBS (mg a.i./L) nominal}} \times 100$$

$$= \frac{102.7}{100} \times 100 = 103\%$$

Instrument Software: MacQuan, version 1.6.

Appendix 4.5

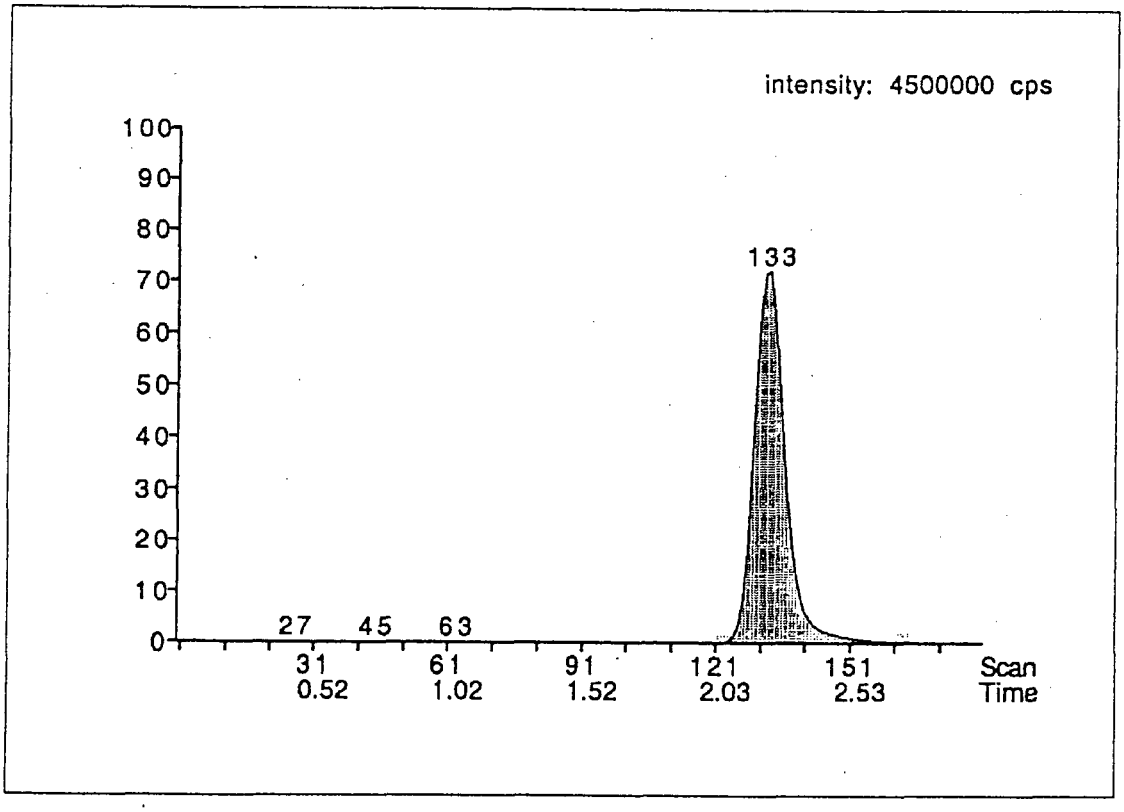
Ion Chromatogram of a Low-level PFBS Standard



Nominal concentration: 0.0100 mg a.i./L

Appendix 4.6

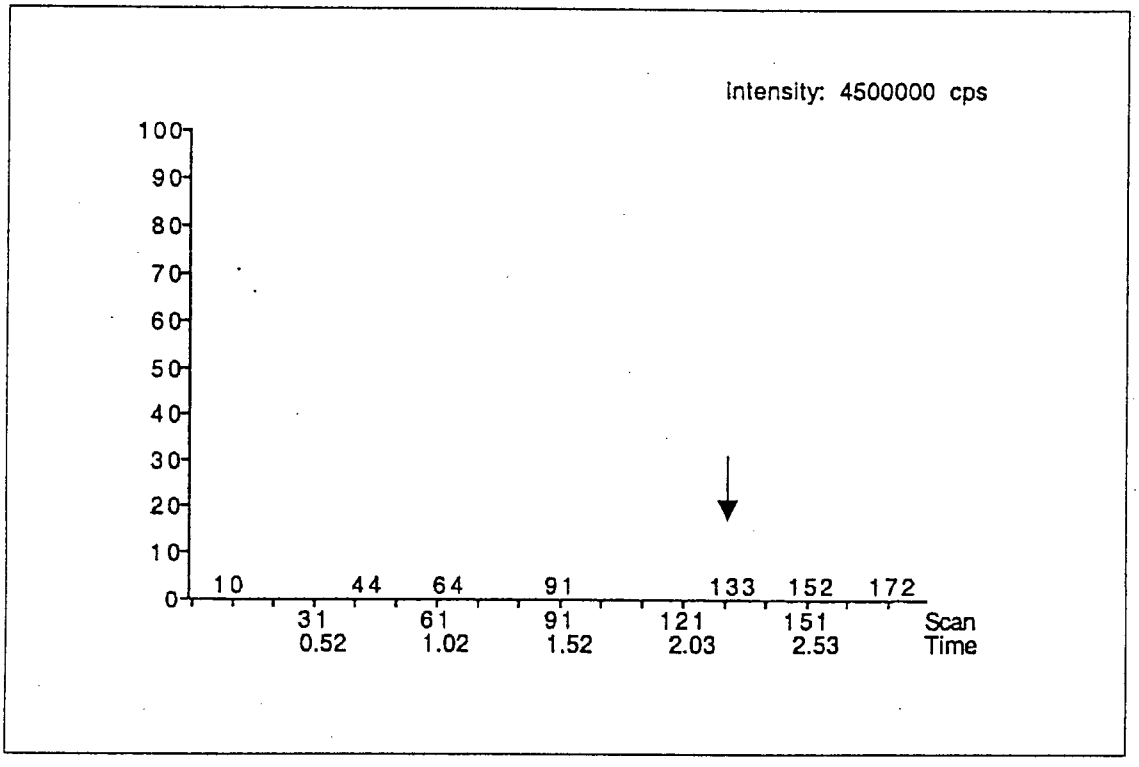
Ion Chromatogram of a High-level PFBS Standard



Nominal concentration: 0.0500 mg a.i./L

Appendix 4.7

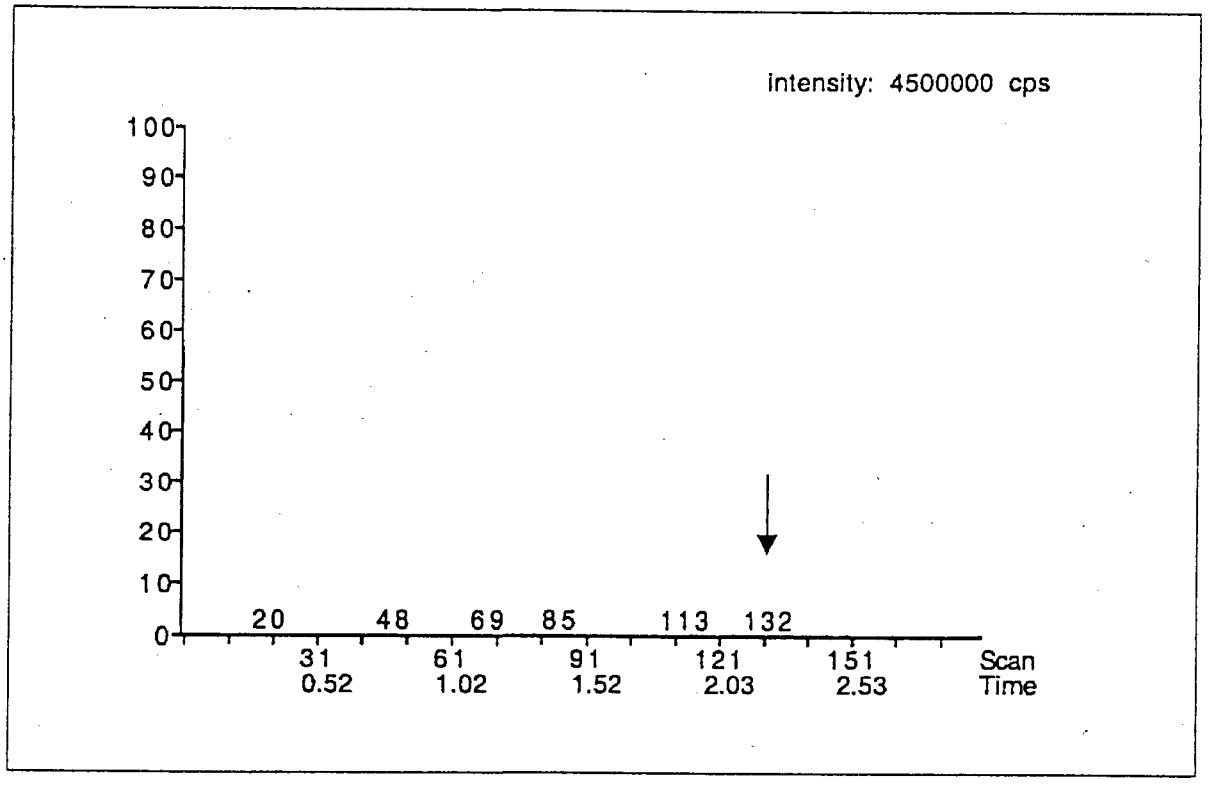
Ion Chromatogram of an Algal Medium Matrix Blank



Sample number 454C-117-VMAB-1. The arrow indicates the approximate retention time of PFBS.

Appendix 4.8

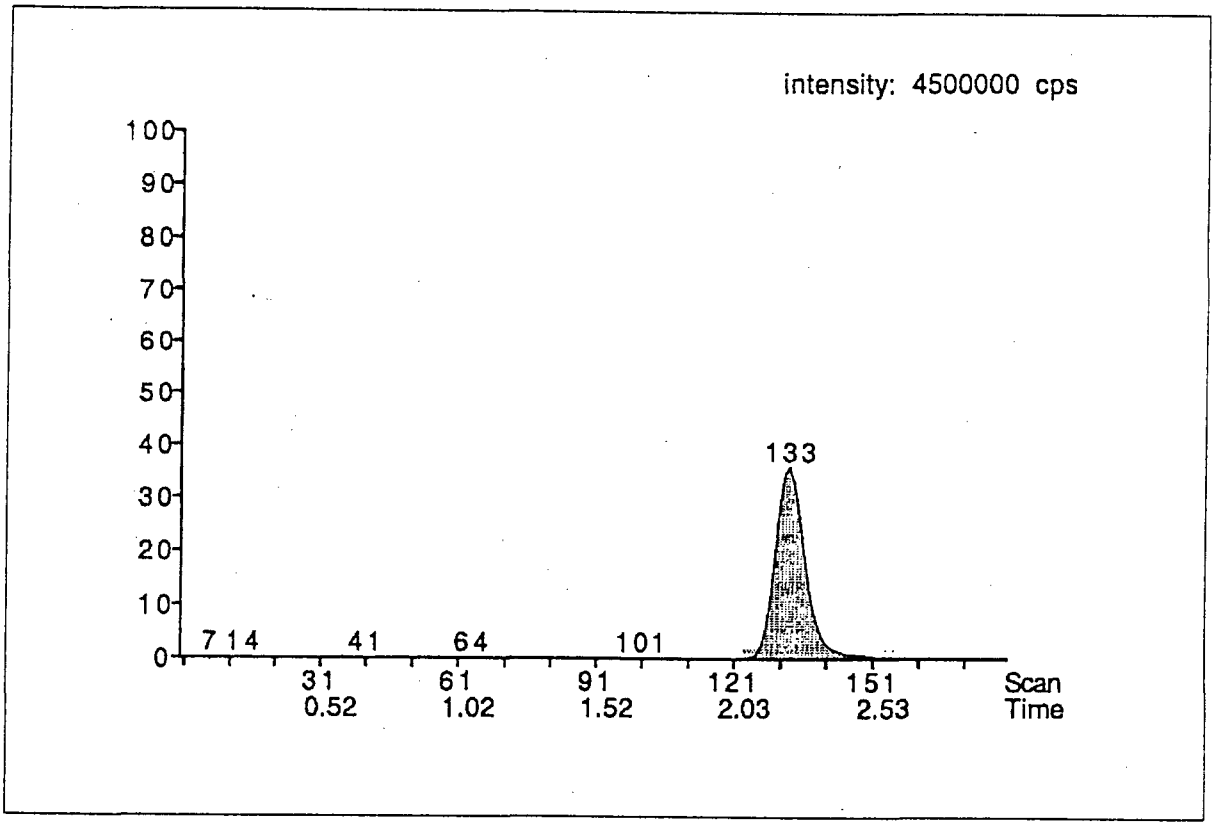
Ion Chromatogram of a Saltwater Matrix Blank



Sample number 454C-117-VMAB-4. The arrow indicates the approximate retention time of PFBS.

Appendix 4.9

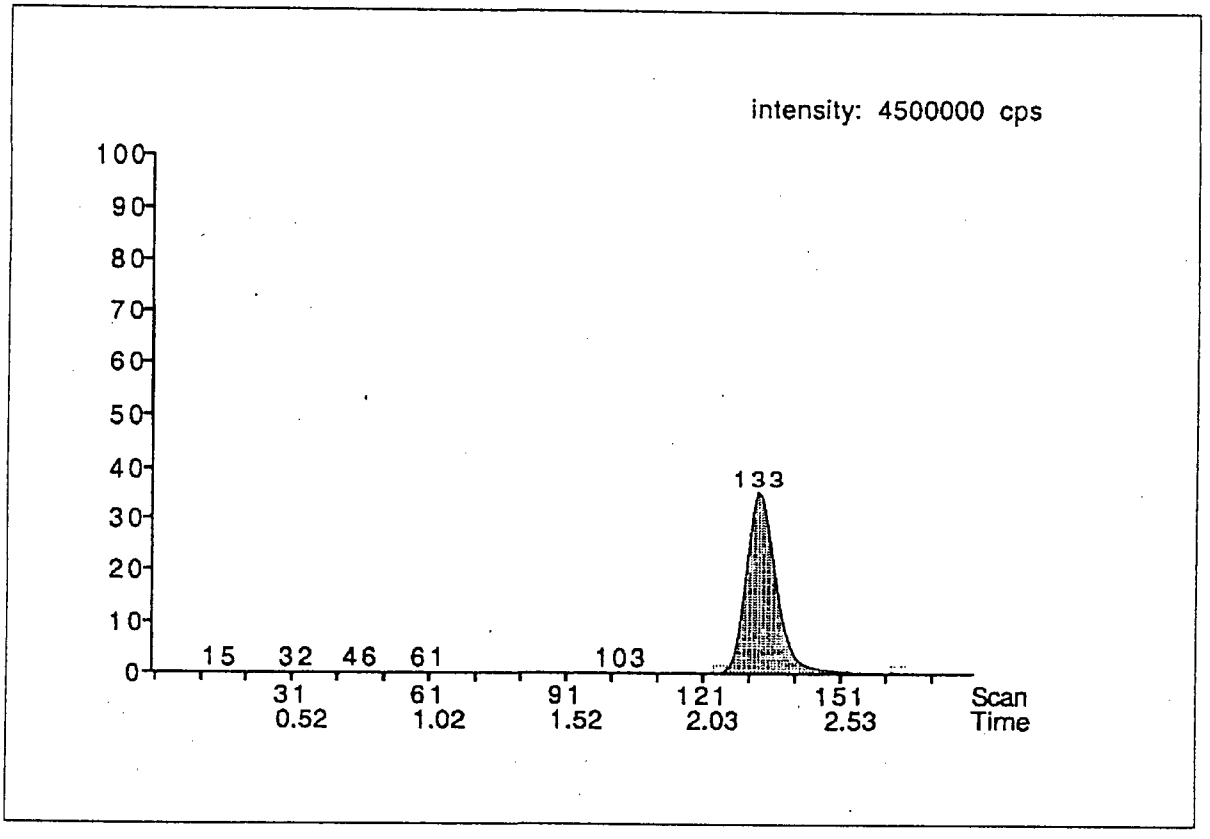
Ion Chromatogram of a Low-level Algal Medium Fortification Sample



Sample number 454C-117-VMAS-1, 100 mg a.i./L nominal concentration, dilution factor = 5000.

Appendix 4.10

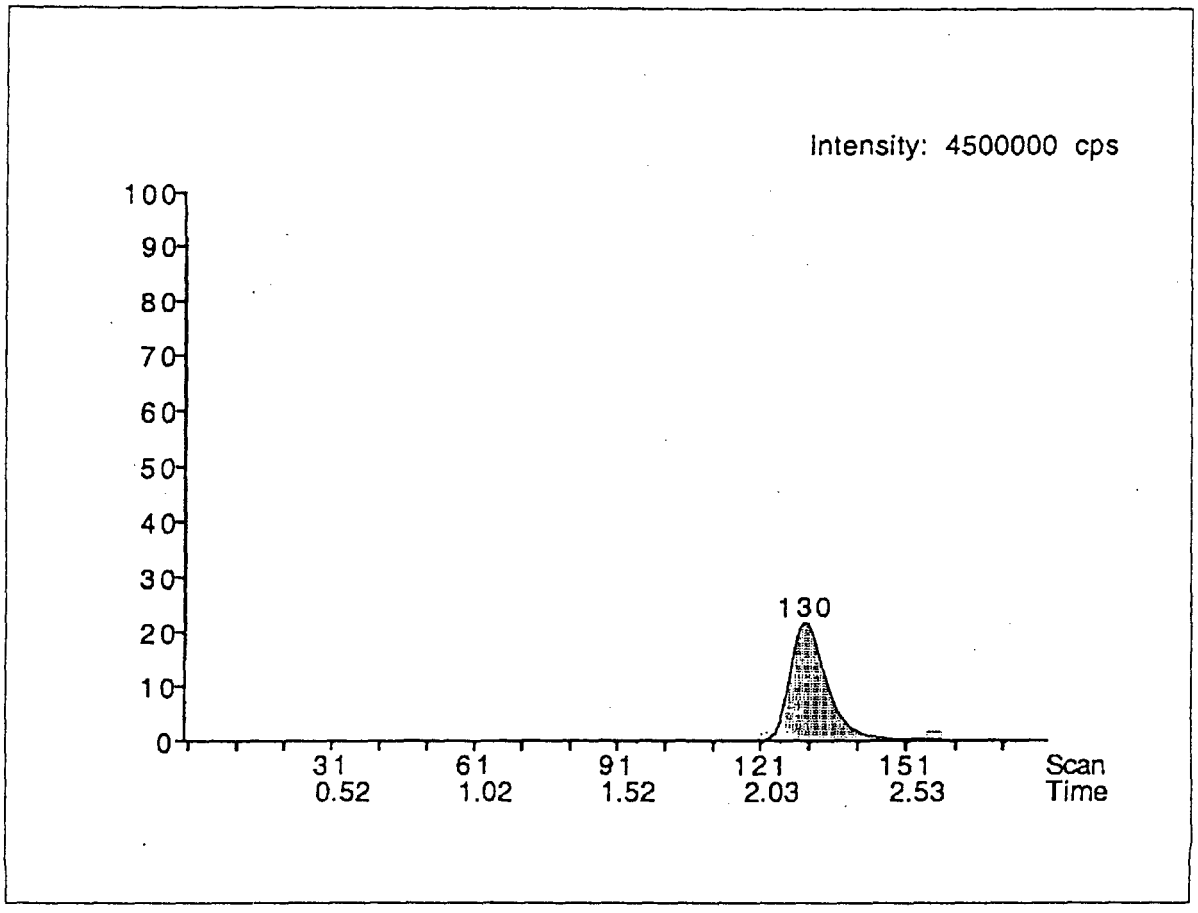
Ion Chromatogram of a High-level Algal Medium Fortification Sample



Sample number 454C-117-VMAS-10, 10000 mg a.i./L nominal concentration, dilution factor = 500000

Appendix 4.11

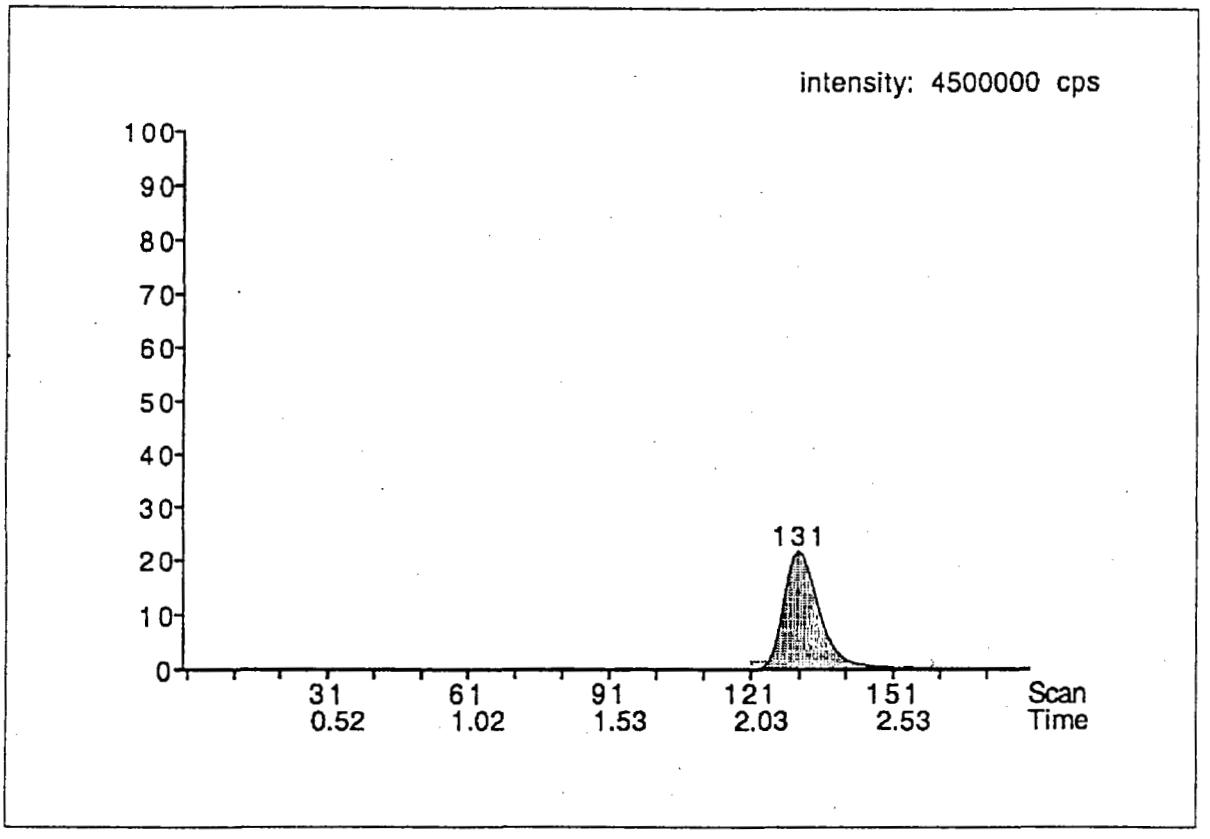
Ion Chromatogram of a Low-level Saltwater Fortification Sample



Sample number 454C-117-VMAS-25, 1.00 mg a.i./L nominal concentration, dilution factor = 50

Appendix 4.12

Ion Chromatogram of a High-level Saltwater Fortification Sample



Sample number 454C-117-VMAS-22, 1000 mg a.i./L nominal concentration, dilution factor = 50000

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 A. MacGregor, B.S., Scientist
4. Frank J. Lezotte, B.S., Chemist