25) OECD 205-OPPTS 850.2200, Analysis of fluorochemicals in Bobwhite quail samples, E02-0659 (from study 454-112)

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Analytical Report

Quantitative Analysis of Fluorochemicals in Bobwhite Quail Samples
Obtained from Wildlife International, Ltd
Amendment 1 – September 8, 2003

3M Study No./3M Laboratory LIMS No. E02-0659

Testing Laboratory

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1 Amendment

This amendment was written to update "Table 1:Target Ions Monitored in 3M Laboratory Analyses" to "Table 3: Target Ions Monitored in 3M Laboratory Analyses", to update the tables following Table 3 to the correct numbers, and to change the analyte listed in the header of Table 5 to PFBS.

2 Introduction

The purpose of the study is to determine the presence and concentration of perfluorobutanesulfonate (PFBS) in bobwhite liver and sera samples (collected from Day 8 of an acute dietary LC50 study, from birds exposed to 5620 or 10000 mg/kg in the diet) collected by Wildlife International, Ltd (E00-1429). Analyses of liver and sera samples were completed by the 3M Environmental Laboratory under study number E02-0659, and the results of these analyses are presented in this report.

This study was not intended to be performed under compliance with the Good Laboratory Practice (GLP) Standard.

3 Sample Receipt

The 3M Environmental Laboratory received liver and sera specimens on 18 June 2002 and 03 July 2002. All samples were received frozen, in good condition, and were transferred to frozen storage at -20°C ± 10°C upon arrival.

Table 1. Bobwhite Liver Samples Received

Sample	Timepoint	Dietary Exposure Concentration ug/g
S454-112-79	Day 8	5,620
S454-112-80		
S454-112-81		
S454-112-82		
S454-112-83		
S454-112-84		
S454-112-85	Day 8	10,000
S454-112-86		
S454-112-87		
S454-112-88		
S454-112-89		
S454-112-90		

Table 2. Bobwhite Sera Samples Received		
Sample	Timepoint	Dietary Exposure Concentration ug/g
S454-112-126	Day 8	5,620
S454-112-127		
S454-112-129		
S454-112-130	Day 8	10,000
S454-112-131		
S454-112-132		
S454-112-133		
S454-112-134		
S454-112-135		

4 Holding Times

No known holding times are associated with these samples.

5 Methods – Preparatory and Analytical

Preparatory/Analytical Methods

ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices". This method was used to prepare/analyze liver and sera samples.

PFBS was extracted from approximately 1.0 mL of homogenized liver or approximately 1.0 mL of diluted sera using C18 solid phase extraction (SPE) cartridges. The compounds were eluted from the C18 cartridge using 2.0 mL of methanol. Quantitative analyses were performed by monitoring a single product ion selected from a primary ion characteristic of a particular fluorochemical using HPLC-ES/MS/MS. For example, molecular ion 299, selected as the primary ion for PFBS ($C_4F_9SO_3$-) analysis, was fragmented further to produce ion 99 (FSO_3-). The characteristic product ion 99 was monitored for quantitative analysis.

The method was modified as approximately 0.16 g were weighed from each of the six liver samples, combined together, and homogenized using 9.0 mL of water (labeled as S454-112-Comp(79-84) and S454-112-Comp(85-90). For the sera samples, approximately 0.02 – 0.3 mL were measured and diluted 1/20 with water. From each of three diluted sera samples, 0.33 mL was removed for the first composite (labeled S454-112-Comp(126-129). From each of six diluted sera samples, 0.16 mL was removed for the second composite (labeled S454-112-Comp(130-135).

Note: As standards were prepared for this study, they were corrected for purity.

Analytical Equipment

The following are representative of the actual settings used during the analytical phase of this study.

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column: Keystone® Betasil™ C_{18} 2 x 50 mm (5 µm)
Column temperature: 30°C
Mobile phase components:
Component A: 2mM ammonium acetate
Component B: methanol
Flow rate: 300 µL/min
Injection volume: 10 µL
Solvent Gradient: 10.0 minutes

Time (minutes)	*%B*
0.0	10%
1.0	10%
5.5	95%
7.5	95%
8.0	10%

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.4
Cone Voltage: 15–60 V
Collision Gas Energy: 10–60 eV
Mode: Electrospray Negative
Source Block Temperature: 150°C ±10°C
Electrode: Z-spray
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 3. Target Ions Monitored in 3M Laboratory Analyses

Target Analyte	Primary Ion (AMU)	Product Ion (AMU)	Retention Time
PFBS	299	99	~ 5.8 min.

Liver samples were homogenized beginning on 08 October 2002. Sera samples were diluted beginning on 14 October 2002. All were extracted beginning on 14 October 2002 and analyzed beginning on 17 October 2002 following method ETS-8-231.1 using high-performance liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple reaction mode versus extracted matrix curves. PFBS levels were quantitated by external calibration.

6 Data Quality Objectives

The following data quality objectives were indicated in the method performance section of ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices". The data quality objectives were followed loosely in this analysis. Practiced procedures are adequate to support the stated data quality for this data set.

System Suitability: System suitability will be determined prior to the start and at the completion of each analytical run. Prior to the calibration curve and after the last sample of the run three (3) mid-level unextracted calibration standards will be analyzed. As applicable, the peak area precision, retention time precision, resolution, and peak asymmetry will be monitored at the beginning and the end of the run separately. The peak area precision must be equal to or less than 5.0% RSD, the precision of the retention time must be equal to or less than 2.5% RSD, the resolution must be > 2.0, and the peak asymmetry (fronting or tailing) must be $0.5<AF<2.0$, where AF is the asymmetry factor.

Calibration: One calibration curve will be prepared from extracted matrix standards, in the same matrix as the samples. It will consist of 8 levels. The curve will be plotted by quadratic regression, weighted 1/x, and not forced through the origin, using MassLynx or other suitable software. The coefficient of determination (r^2) equal to or greater than 0.990. All active calibration curve points must be within 25% of the theoretical value with the exception of the LOQ point, which may deviate up to 30%.

Calibration standards with peak areas less than two times the curve matrix blank will be deactivated to disqualify a data range that may be affected by background levels of the analyte.

A valid calibration curve must contain at least 6 active points above and including the LOQ

Blanks: Water and matrix blanks must have a peak area $\leq$ ½ the peak area of the lowest acceptable standard (LOQ).

Surrogate Standards: Surrogates will be added to all samples prior to the start of the preparatory procedure. The surrogate to be used is nonadecafluorodecanoic acid (PFDA) and it will be used to determine method performance. Surrogates standards must have a percent deviation < 50%.

Limits of Quantitation (LOQ): The LOQ is equal to the lowest acceptable standard in the calibration curve.

Matrix Spikes: Matrix spikes are not a component of this study.

Quality Control (QC) Samples: Three (3) quality control samples (QC) will be prepared for each matrix during the course of the study. QC samples will consist of one sample at each of three levels of analyte. The levels listed below may be used:

Low level: 3X to 5X the LLOQ,
Mid-level: equivalent to a point near the middle of the calibration curve,
High level: 80% of the ULOQ

Two QC sample levels are analyzed after every tenth sample injection starting after the last calibration standard injection, with a minimum of three QC per analysis. Solvent blanks are not considered samples but may be included as such for determining when QC samples will be analyzed.

Each QC is expected to show an accuracy of 75-125% of expected. A minimum of 2/3 of all QC samples analyzed within an analytical run must meet this criteria, and a minimum of 1/2 of the QC samples at each level within an analytical run must meet this criteria. If not, the set must either be re-analyzed or re-extracted

7 Analysis

System Suitability: System suitability was determined prior to the start and at the completion of each analytical run. The peak area precision was less than 5.0% RSD (0.5 – 3.0%), the precision of the retention time was less than 2.5% RSD (0.0 – 0.34%).

Calibration: Quantitation of the target analyte was based on quadratic regression fit analysis weighted 1/x of a single extracted curve for each group of liver or sera samples. Points on the curve which weren't within +/- 25% criteria or low curve points with peak areas less than 2 times that of the matrix blanks were deactivated. Quantitation of each analyte was based on the response of one specific product ion using the multiple reaction-monitoring mode of the instrument. Extracted calibration standards were prepared to run, undiluted, at approximately 10 ng/g or ng/mL – 1000 ng/g or ng/mL. The coefficient of determination (r^2) of each standard curve was ≥0.990.

Demonstration of Specificity: Specificity for analyte identification was demonstrated by chromatographic retention time (~5.8 minutes) and mass spectral daughter ion characterization.

Blanks: Kandiyohi water was used for all water blanks. Bobwhite liver and Bobwhite sera were used as matrix blanks. All blank peak areas were ≤ ½ the peak area of the limit of quantitation for the compounds of interest.

Surrogate Standards: PFDA surrogate standard was spiked into all samples during the course of this study. The percent difference of each surrogate standard was < 50%.

Limits of Quantitation (LOQ): The LOQ was equal to the lowest acceptable standard in the calibration curve (defined as a standard within ±30% of the theoretical value), and had a peak area 2 times greater than the analyte peak area detected in the average of the surrogate matrix blanks. Because low levels of the target analytes are ubiquitous in the laboratory, it is imperative that these criteria for LOQ determination be observed. The approximate LOQ for each analyte (reported as <LOQ) in each matrix is listed on the results summary tables.

Matrix Spikes: Matrix spikes were not a component of this study.

Quality Control (QC) Samples: Three (3) quality control samples (QC) were prepared for each matrix during the course of the study at approximately 25 ppb, 250 ppb, and 750 ppb. All were within +/-25% criteria (range: 3-25%).

Laboratory Control Samples: Laboratory Control Samples were not a component of this study.

Sample Related Comments: It is not possible to verify true recovery of endogenous analyte from liver/sera without radiolabeled reference material. The only measurement of accuracy available at this time, QC samples, indicate that these data can be reported with a stated accuracy of +/-25%.

These data were corrected for purity of the target analytes. Available purity information was included in the raw data.

8 Data Summary

PFBS was detected in the Bobwhite liver and sera samples. Refer to Tables 4 and 5 for more information.

Table 4. PFBS Data Summary of Bobwhite Liver Samples

Sample	Timepoint	Dietary Exposure Concentration ug/g	PFBS Liver Concentration ug/g	Mean ug/g
S454-112-Comp(79-84)-1	Day 8	5,620	1.29	1.36
S454-112-Comp(79-84)-2			1.43	
S454-112-Comp(85-90)-1		10,000	1.40	1.34
S454-112-Comp(85-90)-2			1.27	

Table 5. PFBS Data Summary of Bobwhite Sera Samples

Sample	Timepoint	Dietary Exposure Concentration ug/g	PFBS Sera Concentration ug/mL	Mean ug/mL
S454-112-Comp(126-129)-1	Day 8	5,620	2.55	2.67
S454-112-Comp(126-129)-2			2.78	
S454-112-Comp(130-135)-1		10,000	1.30	1.26
S454-112-Comp(130-135)-2			1.22	

Example Calculation:

$$AR\ (ng/mL) \times DF \times \frac{\partial\ curve\ (mL/mL)}{\partial\ sample\ (mL/mL)} \times \frac{VR\ (mL)\ Curve}{VR\ (mL)\ Matrix} \times \frac{FV\ (mL)\ in\ Curve}{FV\ (mL)\ in\ Matrix} \times \frac{1.0\ \mu g}{1000\ ng} = (\mu g/mL)$$

AR = Analytical result from MassLynx summary
DF = Dilution factor
MA = Matrix amount
∂ curve = MA of tissue/fluid calibration curve, assumed to be 1 g or 1 mL/5 mL water
∂ sample = MA of tissue/fluid sample (___g or mL of sample/5 mL water)
VR = Volume removed for extraction
FV = Final volume

Example calculation for sample S454-112-Comp(126-129)-1:

$$101.13\ \text{ng/mL} \times 25 \times \frac{1\ \text{mL}}{5\ \text{mL}} \times \frac{1.0\ \text{mL}}{0.99\ \text{mL}} \times \frac{2.0\ \text{mL}}{2.0\ \text{mL}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = 2.55\ \mu\text{g/mL}$$

Refer to Attachment A for data summary tables.

9 Data / Sample Retention

All original raw data and analytical report will be archived at the 3M Environmental Laboratory. The analytical reference standard reserve sample and remaining specimens pertaining to the analytical phase of this study will be archived at the 3M Environmental Laboratory according to applicable standard operating procedures.

10 Attachments

10.1 *Attachment A:* Data Summary Tables

11 Signatures

09/08/03
Date

Laboratory Management — 09/08/03
Date

Quality Assurance Representative — 9/8/03
Date

Analytical Report

Quantitative Analysis of Fluorochemicals in Bobwhite Quail Samples Obtained from Wildlife International, Ltd

Amendment 1 – September 8, 2003

3M Study No./3M Laboratory LIMS No. E02-0659

Attachment A

Data Summary Tables

Study:	E02-0659		
Product Number(Test Substance):	NA		
Matrix:	Bobwhite Liver versus Bobwhite Liver extracted curv	Filename:	See Attachments
Method/Revision:	ETS-8-231.1	R-Squared Value:	See Attachments
Analytical Equipment System Number:	Amelia062498	Slope:	See Attachments
Instrument Software/Version:	MassLynx 3.4	Y-Intercept:	See Attachments
Date of Extraction/Analyst:	10/14/02 RWW		
Date of Analysis/Analyst:	10/17/02 LAS		
Date of Data Reduction/Analyst:	10/23/02 LAS		

Sample Data

Various Tissues

Group Dose	Sample #	Concentration of PFBS ug/g or % Rec	Mean PFBS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blank	WB101402-H2O Blank-3	<LOQ (0.0256 ug/g)		
	WB101402-H2O Blank-4	<LOQ (0.0256 ug/g)	<LOQ	NA
Matrix Blank	BWL101402-Liver Blank-1	<LOQ (0.0256 ug/g)		
	BWL101402-Liver Blank-2	<LOQ (0.0256 ug/g)		
	BWL101402-Liver Blank-3	<LOQ (0.0256 ug/g)		NA
	BWL101402-Liver Blank-4	<LOQ (0.0256 ug/g)	<LOQ	NA
Samples	S-454-112-Comp(79-84)-1, 5620 ng/mL	1.29		
	S-454-112-Comp(79-84)-2, 5620 ng/mL	1.43	1.36	NA
	S-454-112-Comp(85-90)-1, 10,000 ng/mL	1.40		
	S-454-112-Comp(85-90)-2, 10,000 ng/mL	1.27	1.34	NA

PFBS = Perfluorobutanesulfonate

Date Entered/Analyst:	11/13/02 LAS
Date Verified/Analyst:	0

Study:	E02-0659		
Product Number(Test Substance):	NA		
Matrix:	Bobwhite Sera versus Bobwhite Sera extracted cu	Filename:	See Attachments
Method/Revision:	ETS-8-231.1	R-Squared Value:	See Attachments
Analytical Equipment System Number:	Amelia062498	Slope:	See Attachments
Instrument Software/Version:	MassLynx 3.4	Y-Intercept:	See Attachments
Date of Extraction/Analyst:	10/14/02 RWW		
Date of Analysis/Analyst:	10/17/02 LAS		
Date of Data Reduction/Analyst:	10/23/02 LAS		

Sample Data

Various Tissues

Group Dose	Sample #	Concentration of PFBS ug/mL or % Rec	Mean PFBS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	WB101402-H2O Blank-1	<LOQ (0.0254 ng/mL)		
	WB101402-H2O Blank-2	<LOQ (0.0254 ng/mL)	<LOQ	NA
Matrix Blk	BWS101402-Sera Blank-1	<LOQ (0.0254 ng/mL)		
	BWS101402-Sera Blank-2	<LOQ (0.0254 ng/mL)		
	BWS101402-Sera Blank-3	<LOQ (0.0254 ng/mL)		NA
	BWS101402-Sera Blank-4	<LOQ (0.0254 ng/mL)	<LOQ	NA
Samples	S-454-112-Comp(126-129)-1, 5620 ng/mL	2.55		
	S-454-112-Comp(126-129)-2, 5620 ng/mL	2.78	2.67	NA
	S-454-112-Comp(130-135)-1, 10,000 ng/mL	1.30		
	S-454-112-Comp(130-135)-2, 10,000 ng/mL	1.22	1.26	NA

PFBS = Perfluorobutanesulfonate

Date Entered/Analyst:	11/13/02 LAS
Date Verified/Analyst:	0