AR226-1826

Laboratory Report – Revision 1

Analytical Report of Data for PFOS Dietary LC50 Study in Northern Bobwhite (Sera and Liver)

Laboratory Report No. FACT-TOX-129 (U2723)

Testing Laboratory

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LAC 03/23/01

1 *Introduction*

This study was designed to quantitatively assess perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$, CAS # 2795-39-3) levels in the sera and liver of the northern bobwhite (Colinus virginianus) exposed to PFOS through diet. Details of dosing conditions, clinical observations and other toxicity data were generated by the in-life testing facility (Wildlife International) and are not included in this report. Subsets of the eight groups of test animals were sacrificed on day 8 and day 22, post-dose. Sera and liver collected from the sacrificed animals were sent to the 3M Environmental Laboratory and analyzed quantitatively for PFOS. Tissues from animals that died before the scheduled sacrifice date were collected and analyzed also; results are included in this report.

Elucidation of data trends was complicated by the sporadic nature of the PFOS levels in sera. Within a particular dose group there was dramatic variation between individual animals. The relative standard deviation of PFOS sera levels within a dose group ranged from greater than 20% to over 200%. By comparison, the results of PFOS levels in the livers of the bobwhites were far more consistent within a particular dose level (group RSD between 10-55%)

In general, both sera and liver levels of PFOS increased with dose level, although due to animal to animal variability, this trend is not pronounced in the sera. With the exception of group 4 animals, average levels of PFOS in the sera of the bobwhites generally increased or stayed the same between day 8 and day 22. In all dose groups, the group average for PFOS concentration in liver decreased dramatically between day 8 and day 22.

Livers were collected from bobwhites that died between day 1 and day 8, prior to the scheduled sacrifice. In every case, PFOS levels in the livers of these birds were over 110 µg/g wet weight.

Although rigorous quality control measures and 3M Environmental Laboratory Standard Operating Procedures were followed, the acquisition of this data was not necessarily collected according to applicable Good Laboratory Practices. Data presented here is the highest quality data available at this time.

No data was collected for any analytes other than PFOS.

It is recommended that bobwhite quail be avoided in future studies because the small amount of tissue available causes analytical challenges that make verification difficult.

2 *Sample Receipt*

The Environmental Laboratory received 65 liver, 34 blood, and 34 serum specimens, frozen, on 05/19/99. Upon specimen receipt a 3M chain of custody was initiated.

The Environmental Laboratory received 35 liver, 35 blood, 35 serum, and 6 bile specimens, frozen, on 06/29/99. Upon specimen receipt a 3M chain of custody was initiated.

The Environmental Laboratory received 30 pooled bobwhite quail serum tubes, frozen, on 06/30/99. Upon specimen receipt a 3M chain of custody was initiated. A copy of each form can be found in an Attachment to this report.

3 *Holding Times*

There is no holding time criteria associated with these samples for LC/ESMSMS analysis.

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4 *Methods - Analytical and Preparatory*

Analytical methods

ETS-8-4.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry *with the following exceptions*:
Due to the small volume of bobwhite sera available, standard curves were prepared in methanol instead of from bobwhite sera. Four matrix spikes were prepared from the pooled bobwhite quail serum received on 06/30/99. PFOS lot 171 was used as reference material.
FACT-M-1.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry *with the following exception*:
The extraction was performed following ETS-8-6.0; this method had been validated but not approved at the time of extraction. PFOS lot 171 was used as reference material.
ETS-8-6.0, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry *with the following exception*:
Due to the small amount of bobwhite liver available, standard curves were prepared in methanol instead of from bobwhite liver. In some cases, liver samples were very limited and two or more samples had to be combined for extraction. These combinations are indicated in the results. Twenty-two matrix spikes were prepared from the bobwhite liver. PFOS lot 171 was used as reference material.
ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry.
FACT-M-2.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals on Liver Extracts Using HPLC-Electrospray/Mass Spectrometry *with the following exception*:
The analysis was performed following ETS-8-7.0, which had been validated but not approved at the time of extraction. As described in the method, all results are reported in terms of µg of analyte per gram of liver (wet weight).
ETS-8-7.0, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals on Liver Extracts Using HPLC-Electrospray/Mass Spectrometry.

Sample preparation – HPLC/ESMSMS: ion-pairing extraction

Analyte is extracted from a sample matrix with ion pairing reagent [tetrabutyl ammonium hydrogen sulfate (TBA)] in a pH-controlled environment. The cationic reagent selectively targets anionic fluorochemicals. Once the anion-TBA pair is formed, the analyte is transferred into a non-polar organic solvent (methyl-*tert*-butyl ether), dried, and reconstituted in methanol for MS analysis.

HPLC/ESMSMS

In HPLC, an aliquot of extract is injected and passed through a reverse phase liquid chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. For example, in a standard solution PFOS may elute at 8.0 minutes. Retention times between a standard PFOS solution and the analyte extracted from tissue in this analysis were matched on the HPLC system to within 1%.

Following HPLC separation, ESMSMS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray, an ionization technique used primarily for detection of molecular ions, is generally operated at relatively mild temperatures. Molecules are ionized, and a primary ion, characteristic of the analyte, is selected. This ion is bombarded with high-energy gas; subsequent collisions create smaller secondary ionic fragments unique to the primary ion, which are detected.

For example, for PFOS ($C_8F_{17}SO_3^-$) analysis, ion 499 is selected as the characteristic primary ion. This ion is fragmented into other ions such as 80 amu (corresponding to SO_3^-), and 230 amu ($C_3F_6SO_3^-$). Each of these secondary fragments is detected and can be used to differentiate PFOS from other compounds that might have the same characteristic 499 amu primary ion, but different chemical compositions and secondary ion fragmentation patterns.

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5 *Analysis*

5.1 Calibration

A mid-level calibration check standard is analyzed every 5-10 samples to monitor instrumental drift. Calibration check standards were compliant if instrumental response was within +/- 30% of the expected value.

Quantitation of the target analytes was based on linear regression analysis (weighted 1/x) of two unextracted methanol curves bracketing each group of samples. Quantitation of each analyte was based on the response of one or more specific daughter ions using the multiple response-monitoring mode of the instrument.

3M lot #217 (purity 86.9%) of PFOS was used for calibration standards. As purity information was not available at the time of data collection, reported results have not been corrected.

5.2 Extraction and Method Blanks

At least two extraction blanks, utilizing water as surrogate matrix, were extracted with each batch of samples. Additionally, two method blanks, utilizing control sample matrix (if available), were extracted along with each batch of samples and curves.

Blanks were compliant if no target analyte was detected above the limit of detection for a specific analyte. In this study, all blanks were compliant.

5.3 Surrogates

Tetra-hydroperfluorooctanesulfonate was used as a surrogate in this analysis. Surrogate response was monitored to confirm gross instrumental failure, if necessary. Surrogate response was acceptable for all analyses.

5.4 Matrix Spikes

Over the course of the analysis, twenty-six matrix spikes were prepared in the bobwhite liver matrix. All of the matrix spikes were recovered at greater than 50% of the expected value with two exceptions. The two 50 ng/g matrix spikes that were prepared on September 24, 1999 with the day 3-7 liver samples showed analyte recovery of approximately 350% of the expected value. These samples and matrix spikes were re-extracted on October 08, 1999. The re-extracted matrix spike recoveries were greater than 50% (104% – 110%), therefore day 3-7 data are reported from extraction date October 08, 1999 – refer to extraction sheet for animal numbers.

Because the bobwhite sera were so limited, only four matrix spike samples were prepared. All matrix spikes showed analyte recovery of greater than 90% of the expected value.

5.5 Laboratory Control Samples

Laboratory Control Samples were not a component of this study.

5.6 Sample Related Comments

Samples were not analyzed for any residual or potential metabolite other than PFOS. Other matrices submitted with this study will be analyzed as needed upon written request from the requestor. In the event of further analysis, an additional report will be generated.

6 *Data Summary*

Elucidation of data trends was complicated by the sporadic nature of the PFOS levels in sera. Within a particular dose group there was dramatic variation between individual animals. The relative standard deviation of PFOS sera levels within a dose group ranged from greater than 20% to over 200%. By comparison, the results of PFOS levels in the livers of the bobwhites were far more consistent within a particular dose level (group RSD between 10-55%)

In general, both sera and liver levels increased with dose level, although due to animal to animal variability, this trend is not pronounced in the sera. With the exception of group 4 animals, average levels of PFOS in the sera of the bobwhites generally increased or stayed the same between day 8 and day 22. In all dose groups, the group average for PFOS levels in liver decreased dramatically between day 8 and day 22.

Livers were collected from bobwhites that died between day 1 and day 7, prior to the scheduled sacrifice. In every case, PFOS levels in the livers of these birds were over 110 µg/g.

7 *Data Quality Objectives and Data Integrity*

No circumstances existed during the present study that would have adversely affected the quality or integrity of the data. The following data quality objectives were followed during the study:

- Linearity – The coefficient of determination (r^2) of the standard curve was equal to or greater than 0.985 using 1/x weighting
- Limits of quantitation - PFOS in sera is 0.010 µg/mL; PFOS in liver is approximately 0.070 µg/g
- Acceptable duplicate precision - < 30%, assessed by analysis of the MS/MSDs.
- Acceptable spike recoveries – sera: 70% to 130%; liver: 50% to 150%
- Use of confirmatory methods – Given the selectivity of the analytical tool used (HPLC-ESMSMS) and lack of a viable alternative for analysis, no confirmatory methods were used.
- Demonstration of specificity – Specificity was demonstrated by chromatographic retention time (matched to standards to within 3%) and the response of at least one characteristic product ion arising from collisions of an analyte-specific parent ion.
- The characterization of the reference material (PFOS, lot #171), with respect to purity, stability, and homogeneity, was not complete on the date this report was issued. Once the characterization is complete, a copy of the report will on file in the 3M Environmental Lab archives.

Given the parameters listed above and assuming spike recoveries form a suitable indication of endogenous analyte recovery, sera data are quantitative to +/-30 % and liver data are quantitative to +/-50 %. The validity of these assumptions has not been verified by other techniques. If a greater degree of accuracy is required, additional matrix should be provided so samples can be evaluated versus a standard curve extracted from the same matrix as the samples.

It is recommended that bobwhite quail be avoided in future studies because the small amount of tissue available causes analytical challenges.

03/23/01

8 - Attachments

- Attachment A: Table of results of liver analysis
- Attachment B: Table of results of sera analysis
- Attachment C: Sample Receipt
- Attachment D: In-life protocol from Wildlife Int., LTD
- Attachment E: Extraction and analytical data

LAC 03/23/01

9 Signatures

Lisa A Clemen — 03/23/01

Lisa Clemen, Advanced Chemist — Date

Kris Hansen — 03/23/01

Kris Hansen, Ph.D., Principal Analytical Investigator — Date

Quality Assurance Unit Representative — Date

LC 03/23/01

9 Signatures

Lisa A. Clemen — 06/06/00
Lisa Clemen, Advanced Chemist — Date

Kris Hansen — 06/06/00
Kris Hansen, Ph.D., Principal Analytical Investigator — Date

6-9-00
Quality Assurance Unit Representative — Date

Signature page from original report

 NA 03/23/01

Laboratory Report

Analytical Report of Data for PFOS Dietary LC50 Study in Northern Bobwhite (Sera and Liver)

Laboratory Report No. FACT-TOX-129 (U2723)

Attachment A

Table of Results - Liver Analysis

- Day 3-7
- Day 8
- Day 22

FACT-TOX-129

Study:	454-103 Bobwhite Quail LC50		
Product Number(Test Substance):	PFOS		
Matrix:	Bobwhite Quail Liver	Filename:	See Attachments
Method/Revision:	ETS-8-6.0 & ETS-8-7.0	R-Squared Value:	See Attachments
Analytical Equipment System Number:	Amelia 062498	Slope:	See Attachments
Instrument Software/Version:	Masslynx 3.3	Y-Intercept:	See Attachments
Date of Extraction/Analyst:	10/08/99 SEE/MCH		
Date of Analysis/Analyst:	10/21/99, 01/18/00 IAS/MMH		
Date of Data Reduction/Analyst:	10/22/99, 01/19/00 IAS/MMH		

Sample Data

BOBWHITE LIVER DAY 3-7

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g or %Rec	RSD (%) Std. Dev.(ug/g) MS/MSD RPD
Method Blk	BWL10089-H2O Blk-11	0.00	<LOQ		
	BWL10089-H2O Blk-12	0.00	<LOQ	<LOQ	NA
QC - 50 ppb	R342--0-Day8-50ppb-MS-11	68.7	110%		
	R342-0-Day8-50ppb-MSD-11	64.6	104%	107%	6%
QC - 1000 ppb	B439-0-Day8-1ppm-MS-12	980	82%		
	B439-0-Day8-1ppm-MSD-12	1034	86%	84%	5%
Group1	R342-0-Day8	229	0.229		35.7
0.0 mg/kg food	B439-0-Day8	383	0.383	0.306	0.109
Group5 146 mg/kg food	G355-Day7	110527	111	111	NA
Group6	R352, B450-Day5	248693	249		
293 mg/kg food	Y417, R353-Day6	201523	202		
	G357, G358-Day7	153461	153		30.2
	B449, Y416-Day7	123521	124	182	55.0
Group7	AL354-Day3	NE	NE		
586 mg/kg food	R354-Day4	NE	NE		
	R355, Y401, B452-Day5	125957	126		
	Y418, B451, G360-Day6	235179	235		43.0
	G359, AL355-Day7	111299	111	157	67.7
Group8	AL356, R357, Y419-Day3	133852	134		
1171 mg/kg food	AL357, B453, G361-Day4	131788	132		
	B454, G362-Day4	122934	123		4.21
	R356, Y420-Day4	124333	124	128	5.40

PFOS = Perfluorooctanesulfonate
Limit of Quantitation (LOQ): PFOS is approximately 0.070 ug/g
NA = not applicable G1 Day 8 data is included to support QC
NE = not extracted
Date Entered/By: 01/18/00, 01/26/00 LAC
Date Verified/ By: 04/12/00 KJH

FACT-TOX-129

Study:	454-103 Bobwhite Quail LC50		
Product Number(Test Substance):	PFOS		
Matrix:	Bobwhite Quail Liver	Filename:	See Attachments
Method/Revision:	FACT-M-1.1 and FACT-M-2.1	R-Squared Value:	See Attachments
Analytical Equipment System Number:	Amelia 062498	Slope:	See Attachments
Instrument Software/Version:	Masslynx 3.2	Y-Intercept:	See Attachments
Date of Extraction/Analyst:	06/30/99 SAH		
Date of Analysis/Analyst:	07/02/99, 07/22/99, 09/01/99 MEE/PTF		
Date of Data Reduction/Analyst:	07/20/99, 07/23/99, 09/02/99 DRB/MEE/PTF		

Sample Data

BOBWHITE LIVER DAY 8

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g or %Rec	RSD (%) Std. Dev.(ug/g) MS/MSD RPD
Method Blk	BWL-H2O Blk-6/30-1	0.00	<LOQ		
	BWL-H2O Blk-6/30-2	0.00	<LOQ		
	BWL-H2O Blk-6/30-3	0.00	<LOQ		
	BWL-H2O Blk-6/30-4	0.00	<LOQ	<LOQ	NA
QC - 1000 ppb	G347-MS-1	977	82%		
	G347-MSD-1	972	81%	81%	0%
	Y405-MS-2	920	76%		
	Y405-MSD-2	872	72%	74%	5%
QC - 50 ppb	G346-0-MS11-1	47.2	79%		
	G348-0-MS11-2	72.1	120%		
	G347-0-MS11-3	42.8	71%		
	Y405-0-MS11-4	51.7	86%	89%	21%
QC - 1000 ppb	G346-0-MS11-1	841	70%		
	G348-0-MS11-2	955	79%		
	G347-0-MS11-3	680	57%		
	Y405-0-MS11-4	804	67%	68%	9%
Group1	G347	0.00	<LOQ		
0.0 mg/kg food	B438/AL341	34.7	<LOQ		
	Y405	0.00	<LOQ		
	AL343	86.2	0.0862		
	B440	39.1	<LOQ		
	Y406	0.00	<LOQ		
	Y407	60.6	<LOQ		
	R341	9.72	<LOQ		
	R342	16.5	<LOQ		
	B439	0.00	<LOQ		
	G346/R346 Day6	147	0.147		NA
	G348/AL342	0.00	<LOQ	<LOQ	NA
Group2	B442/AL345	22780	22.8		
18.3 mg/kg food	R340	15691	15.7		20.3
	G350/Y409	17080	17.1	18.5	3.76
Group3	Y411	28909	28.9		
36.6 mg/kg food	B444/R347	20463	20.5		18.0
	AL347/G352	28049	28.0	25.8	4.65
Group4	R348/B446	43414	43.4		
73.2 mg/kg food	G354/AL349	48741	48.7		10.1
	Y413	39880	39.9	44.0	4.46
Group5	AL351/Y415	65404	65.4		9.94
146 mg/kg food	G356/B448	75293	75.3	70.3	6.99
Group 6 293 mg/kg food	AL353-Day8	NE	NE	NA	NA

PFOS = Perfluorooctanesulfonate
Limit of Quantitation (LOQ): PFOS is approximately 0.070 ug/g
NA = not applicable
NE = not extracted
Date Entered/By: 07/21/99, 07/23/99, 09/03/99 LAC
Date Verified/ By: 08/09/99 GML, 04/12/00 KJH