Study Title

ANALYTICAL PHASE REPORT FOR
PFOS: A REPRODUCTION STUDY WITH THE NORTHERN BOBWHITE

Data Requirement

FIFRA Subdivision E, Section 71-4
OECD Guideline 206

Author

Lisa A. Stevenson

Analytical Phase Initiation Date

January 31, 2002

Analytical Phase Completion Date

April 18, 2003

Performing Laboratory

3M Environmental Laboratory
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Project Identification

3M Environmental Laboratory Study # E01-1245
Wildlife International Ltd: 454-108

Total Number of Pages

226

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

GLP COMPLIANCE STATEMENT

Study Title: Analytical Phase Report for PFOS: A Reproduction Study with The Northern Bobwhite

Study Identification Number: E01-1245

This analytical phase was conducted in compliance with Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards with the exceptions listed below:

Exceptions to GLP compliance:

- Automated data collection systems are not validated. The hardcopy printouts are considered as the original raw data.
- The stability of the reference substances and samples has not been determined.
- Not all data generated were recorded directly or promptly, or initiated and dated on date of entry.

Lisa A. Stevenson 04/18/03

Lisa A. Stevenson, Principal Analytical Investigator *Date*

Susan A. Beach 4/18/03

Susan A. Beach, Sponsor Representative *Date*

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

QUALITY ASSURANCE STATEMENT

Study Title: Analytical Phase Report for PFOS: A Reproduction Study with The Northern Bobwhite

Study Identification Number: E01-1245

The analytical phase was audited by the 3M Environmental Laboratory Quality Assurance Unit (QAU), as indicated in the following table. The findings were reported to the study director and laboratory management.

INSPECTION DATES	PHASE	DATE REPORTED TO	
		MANAGEMENT	STUDY DIRECTOR
10/08/01-10/09/01	Protocol	10/09/01	10/09/01
02/01/02	In-phase	02/04/02	02/04/02
04/03/02-04/08/02, 04/17/02	Data	04/08/02, 05/16/02	04/08/02, 05/16/02
05/20/02-05/21/02	Final Report	05/21/02	05/21/02

Matthew W. Mauk 04/18/03

Quality Assurance Representative *Date*

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

TABLE OF CONTENTS

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

LIST OF TABLES

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

STUDY INFORMATION

Study Director

Sean P. Gallagher
Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601

Sponsor

3M Environmental Technology and Safety Services
3M Environmental Laboratory
Building 2-3E-09
St. Paul, MN 55106
Susan A. Beach, *Sponsor Representative*

Analytical Chemistry Laboratory

3M Environmental Technology and Safety Services (ET&SS)
3M Environmental Laboratory (3M Lab)
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55106
Lisa Stevenson, *Principal Analytical Investigator*

Study Personnel

Sean P. Gallagher, *Wildlife International LTD., Study Director*
Joann B. Beavers, *Wildlife International LTD., Laboratory Management*
William K. Reagen, *3M Environmental Laboratory Management*
Susan A. Beach, *Sponsor Representative*
Harold O. Johnson, III, *Principal Analytical Investigator*
Lisa A. Stevenson, *Principal Analytical Investigator*
Kent R. Lindstrom, *Senior Research Chemist*
Ognjenka Krupljanin, *Analytical Chemist*
Marlene Heying, *Analytical Chemist*

Experimental Dates

Sample Analysis Initiation: 31 January 2002
Sample Analysis Completion: 15 April 2002

Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory according to 40 CFR Part 792. The analytical reference standard reserve samples, as well as the samples pertaining to the analytical phase of this study, are archived at the 3M Environmental Laboratory according to 40 CFR Part 792.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

SUMMARY

The concentration of the $C_8F_{17}SO_3^-$ anion of perfluorooctane sulfonate, potassium salt (PFOS, $C_8F_{17}SO_3^-K^+$) was determined in the liver and sera of adult bobwhite quail and their offspring in both a control group and a 10 ppm, dosed group from Wildlife feeding study #454-108. The following tables summarize the $C_8F_{17}SO_3^-$ concentrations found in the liver and sera of study samples. These results only relate to the samples tested. Matrix-extracted PFOS standards were used to establish the HPLC-MS/MS instrument calibration curve for $C_8F_{17}SO_3^-$. Concentrations of $C_8F_{17}SO_3^-$ are reported after correction for the purity and the potassium contribution to the mass of the PFOS reference substance.

Table 1 summarizes all study sample data. Table 2 summarizes study data after removing certain results that have been statistically determined to be outliers utilizing the Dixon Q-Test.

Table 1. Summary Table, $C_8F_{17}SO_3^-$ Concentration in Sera and Liver

Sampling Event	Number of Sera Samples	Average $C_8F_{17}SO_3^-$ Conc. Sera	Range of Results	Number of Liver Samples	Average $C_8F_{17}SO_3^-$ Conc. Liver	Range of Results
Male Control Group	19	NA	<400 ng/mL (19 of 19)	20	<50.2 ng/g*	<50.2 ng/g (19 of 20)* 209 ng/g (1 of 20)*
Female Control Group	17	NA	<400 ng/mL (16 of 17) 2110 ng/mL (1 of 17)	20	<50.2 ng/g*	<50.2 ng/g (20 of 20)*
Male 10ppm Group	20	141000 ng/mL	94400 - 226000 ng/mL	20	88500 ng/g*	50100 - 147000 ng/g*
Female 10ppm Group	18	9990 ng/mL	4710 - 31900 ng/mL	20	4900 ng/g*	3290 - 7550 ng/g*
Male Offspring Control Group	6	NA	<400 ng/mL (6 of 6)	6	<20.0 ng/g*	<20.0 ng/g (6 of 6)*
Female Offspring Control Group	4	NA	<400 ng/mL (4 of 4)	4	<20.0 ng/g*	<20.0 ng/g (4 of 4)*
Male Offspring 10ppm Group	5	12600 ng/mL	8220 - 19200 ng/mL	5	7350 ng/g*	4940 - 13700 ng/g*
Female Offspring 10ppm Group	5	12400 ng/mL	7640 - 18500 ng/mL	5	7180 ng/g*	4590 - 13900 ng/g*

For individual sample values see Tables 10 to 13 and Attachment B.
Accuracy: Sample results are accurate to 100% +/- 30%.
Sera QC samples met the acceptance criteria for the method.
* Liver QC samples did not meet the ½ of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Table 2. Summary Table, $C_8F_{17}SO_3^-$ Concentration in Sera and Liver with Outliers Removed

Sampling Event	Number of Sera Samples	Average $C_8F_{17}SO_3^-$ Conc. Sera	Range of Results	Number of Liver Samples	Average $C_8F_{17}SO_3^-$ Conc. Liver	Range of Results
Male Control Group	19*	*	*	19	<50.2 ng/g**	<50.2 ng/g (19 of 19)**
Female Control Group	16	<400 ng/mL	<400 ng/mL (16 of 16)	20*	*	*
Male 10ppm Group	20*	*	*	20*	*	*
Female 10ppm Group	17	8700 ng/mL	4710 - 14700 ng/mL	20*	*	*
Male Offspring Control Group	6*	*	*	6*	*	*
Female Offspring Control Group	4*	*	*	4*	*	*
Male Offspring 10ppm Group	5*	*	*	4	5760 ng/g**	4940 - 6520 ng/g**
Female Offspring 10ppm Group	5*	*	*	4	5490 ng/g**	4590 - 7030 ng/g**

* No outliers from this data set. Refer to Table 1 for results. For individual sample values see Tables 10 to 13 and Attachment B.
Accuracy: Sample results are accurate to 100% +/- 30%.
Sera QC samples met the acceptance criteria for the method.
** Liver QC samples did not meet the ½ of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

Purpose

The purpose of this analytical phase of the study is to quantify $C_8F_{17}SO_3^-$ in the liver and sera of both control and dosed Northern Bobwhite Quail and their offspring.

Reference Substances

Table 3. Reference Substances

Reference Substance	PFOS	PFDA
Source	3M Internal	Oakwood Products, Inc.
Expiration Date	08/31/2006	12/01/2010
Storage Conditions	Frozen -20°C +/- 10°C	Frozen -20°C +/- 10°C
3M Laboratory Identification Number	SD-018, Lot # 217	SD-036
Physical Description	White Crystalline Powder	White Solid
Purity	86.9%	98%

PFDA is nonadecafluorodecanoic acid, or Perfluorodecanoic acid, $C_{10}F_{19}O_2H$ and is used solely to monitor for gross instrument failure and not as an internal standard for quantitation.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

SPECIMEN RECEIPT AND STORAGE

Northern Bobwhite serum and liver samples were obtained from the sacrifice of the test animals upon the conclusion of the in-life phase of the study (#454-108) conducted at Wildlife International, Ltd. and sent to 3M Environmental Laboratory for analysis. The specimens were received at 3M Environmental Laboratory on 09/05/01, 09/25/01, and 09/27/01; assigned a laboratory tracking (LIMS) number (E01-1245, E01-1342, and E01-1365, respectively), and were stored at -20°C +/- 10°C until extraction and analysis.

METHOD SUMMARY

The determination of $C_8F_{17}SO_3^-$ concentration in the liver and sera was performed according to 3M Environmental Laboratory method ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices".

The sera samples were prepared by measuring a volume of serum and diluting with water in a 1/20 ratio. The liver samples were prepared by measuring a weight of liver and diluting with water to a 1/10 ratio prior to homogenizing. Five mL of acetonitrile was added to 1.0mL of the diluted sera or 1.0mL of the homogenate of liver and was shaken for 20 minutes. These samples were then centrifuged for 10 minutes; the supernatant was decanted into 40 mL of water and passed through a conditioned solid phase extraction (SPE) column. Upon drying the column 2.0mL of methanol was passed through the column to elute the analyte. The samples were analyzed for $C_8F_{17}SO_3^-$ using HPLC-ES/MS/MS.

PREPARATORY METHOD

- **ETS-8-231.1 "Solid Phase Extraction and Analysis of Fluorochemical Components from Biological matrices."** This method describes the extraction of target analytes from biological matrices, such as sera and liver, using solid phase extraction (SPE). Sera samples were diluted with Kandiyohi (commercially bottled) water 1/20, before extraction. Liver samples were diluted 1/10, approximately 1g of liver and 9ml of Kandiyohi water, and then homogenized. A 1.0 mL aliquot of the diluted sera or homogenate of liver was extracted by adding 5mL of acetonitrile, shaking for 20 minutes, and centrifuging for 10 minutes. The supernatant was transferred to a clean tube, diluted with 40 ml of Kandiyohi water, and filtered through a conditioned C18 (SPE) column. Analytes were eluted off the column, and analyzed by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ES/MS/MS).

Table 4. SPE Extraction Summary

STEP	SAMPLE PREPARATION
1	Measure out volume of sera or weigh portion of liver
2	Dilute sera (1/20) or liver (1/10) with water
3	Mix sera/water or homogenize liver/water
4	Aliquot 1.0ml of diluted/homogenized sample and add 5.0ml of ACN. Spike samples accordingly. Shake for 20 minutes, centrifuge for 10 minutes.
5	Dilute supernatant with 40 ml of water
6	Filter sample through conditioned SPE C18 column.
7	Elute target analytes from the column with 2.0 mL methanol (MeOH).

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

SAMPLE COLLECTION AND ANALYSIS

All sera and liver samples were received frozen and remained frozen until thawed prior to preparing samples for analysis. After dilution or homogenization steps, unused samples and diluted sera or homogenized liver were frozen again if extractions were not immediately performed.

On each day of extraction, a water blank, a sera or liver blank, and a matrix spike and matrix spike duplicate were prepared along with each set of samples. Frozen diluted sera/homogenized liver samples were allowed to thaw prior to transferring a representative sample for extraction. Samples were prepared and analyzed according to ETS-8-231.1.

The following table describes the sample collection and preparation regimen. For clarity, a reference to "Day 1" refers only to the group of samples associated with the first batch of samples extracted.

Table 5. Sample Analysis

STEP	PROCEDURE	DATE PERFORMED
1	Preparation of method blanks and spikes, calibration curve, qc samples, and sera samples, control group, adults (day 1 sera samples), see Attachment D for samples prepped	31 January 2002
2	Analysis of day 1 sera samples	31 January 2002
3	Preparation of method blanks and spikes, and sera samples, control and 10 ppm adults, control offspring, and 10ppm offspring (day 2 sera samples), see Attachment D for samples prepped	01 February 2002
4	Analysis of day 2 sera samples	01 February 2002
5	Analysis of 10ppm female sera samples	03 February 2002
6	Analysis of control, 10 ppm and offspring, from day 1 and day 2 sera samples	07 February 2002
7	Weighing out of liver samples	07, 08 February 2002
8	Preparation of method blanks and spikes, calibration curve, qc samples, and samples, control group (day 1 liver samples), see Attachment D for samples prepped	11 February 2002
9	Analysis of day 1 liver samples	11 February 2002
10	Preparation of method blanks and spikes, and liver samples, control group (day 2 liver samples), see Attachment D for samples prepped	12 February 2002
11	Analysis of day 2 liver samples	12 February 2002
12	Preparation of method blanks and spikes, and liver samples, 10ppm group (day 3 liver samples) , see Attachment D for samples prepped	13 February 2002
13	Preparation of method blanks and qc samples at 5 and 200 ppb	13 February 2002
14	Analysis of qc samples	13 February 2002
15	Analysis of day 3 liver samples	14 February 2002

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Step	Procedure	Date Performed
16	Preparation of method blanks and spikes, and liver samples, 10ppm group, control offspring, 10ppm offspring (day 4 liver samples), see Attachment D for samples prepped	15 February 2002
17	Analysis of day 4 liver samples	18 February 2002
18	Re-analysis of day 3 liver samples	01 March 2002
19	Re-analysis of QC samples	05 April 2002
20	Re-analysis of QC samples	10 April 2002

Analytical Method

- Samples were analyzed using HPLC-ES/MS/MS. $C_8F_{17}SO_3^-$ concentrations were determined using matrix-extracted calibration standards ranging in concentration from 0.25-1000 ng $C_8F_{17}SO_3^-$/mL methanol. This result was then used to back calculate the ng $C_8F_{17}SO_3^-$/mL sera or ng $C_8F_{17}SO_3^-$/gram liver as appropriate. An internal standard, Nonadecafluorodecanoic acid (PFDA), was used to monitor instrument performance for gross malfunction, but was not used to quantitate results. HPLC-EC/MS/MS analytical conditions are summarized as follows:
  - The HPLC used for the study was a Hewlett-Packard 1100. The system consists of a vacuum degasser, binary pump, autosampler and temperature controlled column compartment. The run time is 10 minutes per sample. HPLC conditions include the use of a Keystone Scientific, Betasil C18 HPLC analytical column (2.1mm x 50mm, 5µm particle size), a 40°C column compartment temperature, a 10µL injection volume and a 0.300mL/min pump flow rate. The HPLC gradient is listed below:

Time, Minutes	% 2 mM Ammonium Acetate	% Methanol
0	90	10
1.0	90	10
5.5	5	95
7.5	5	95
8	90	10
10	90	10

  - The mass spectrometer used for the study was a Micromass Ultima. The system consists of Z-Spray interface equipped with an electrospray probe operated in negative mode. The instrument was setup to operate in MS/MS mode, passing the $C_8F_{17}SO_3^-$ molecular anion (m/z 499) into the collision cell, where it is collided with inert gas to further fragment to the characteristic ion m/z 99, FSO_3^-. Similarly the PFDA transition from m/z 513 to m/z 299 was monitored. The collision gas, 95% Argon / 5% Methane, was maintained at a pressure of approximately $3.0x10^{-3}$ mBar.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

ANALYTICAL RESULTS

Data quality objectives outlined in the 3M Environmental Laboratory method and protocol were met, except as noted and discussed below (see Attachment A, ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices" and Attachment E, Study Protocol, Protocol Amendments, and Deviations).

- **Regressions.** Quadratic curve fit, weighted 1/x, was applied to calibration standards and sample data. Each calibration curve had a coefficient of determination of ≥ 0.985.
- **Calibration Standards.** Instrument response was calibrated daily prior to sample analysis using matrix-extracted standards ranging in concentration in the final solvent extract from 0.20 to 1000 ng $C_8F_{17}SO_3^-$/mL methanol, corresponding to a concentration of 201 to 40160 ng/mL of sera or 3.99 to 19953 ng/g liver. The calibration curve analyzed consisted of nine sera-extracted standards and fourteen liver-extracted standards bracketing a wide concentration of analyte. The accuracy of each level was evaluated. Any level outside 75%-125% of nominal was deactivated with the exception of the lowest curve point, which needed to meet 70%-130% of nominal. In some cases the lowest concentration curve point, although outside the 30% nominal requirement, was included when constructing the calibration curve, but it was not used to determine the limit of quantitation. The limit of quantitation was then the next point on the curve meeting the 30% nominal requirement. Due to the quadratic response of matrix-extracted standards using LC/MS/MS instrumentation, the calculated concentration of higher-level standards was often indeterminate, that is a single result could not be calculated using the quadratic equation. The accepted upper concentration range for study samples was determined by the highest calibration standard meeting the 75%-125% accuracy criteria when back calculated to the original concentration spiked into the matrix-extracted standards. All analytical results that are reported are within the accepted calibration range of the instrument.
- **System Suitability Samples.** System suitability was demonstrated prior to the start and at the end of each analytical run. Prior to the calibration curve and after the last sample, three mid-level unextracted calibration standards were analyzed. The peak area precision and retention time precision was monitored at the beginning and end of the run separately. The peak area precision was equal to or less than 5.0% RSD, the precision of the retention time was equal to or less than 2.5% RSD, meeting the stated quality objectives to show that the instrumentation was providing suitable results throughout each analytical sequence.
- **Continuing Calibration Verification (CCV).** A mid-level matrix calibration check was analyzed every ten samples or less to monitor instrumental drift, with a limit of ±25% deviation of the target concentrations. Only data bracketed by passing CCV's were reported. Samples bracketed by one or more failing CCV's were reanalyzed.
- **Limit of Quantitation (LOQ).** The lower limit of quantitation (LLOQ) was defined in liver (ng/g) and in serum (ng/mL) as the lowest acceptable extracted calibration curve point that is within the 70-130% nominal criteria and with an analyte peak area at least 2 times the blank. The upper limit of quantitation (ULOQ) was defined as the concentration of the highest acceptable curve point used to calibrate the response of the instrument.
- **Blanks.** Method blanks (purchased Kandiyohi drinking water, sera and/or liver taken through the entire sample preparation and analysis process) provided a measure of background contamination.
- **Quality Control (QC) and Matrix Spikes.** One set of nine QC samples in each matrix was extracted and analyzed and consisted of three levels of analyte in triplicate. Additionally, two matrix spike samples at the lowest concentration were prepared with each extraction set. Results are as follows:

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Table 6. Serum QC Summary Table

Serum QC Samples	Concentration in Diluted Serum	Mean recovery of triplicates	Relative Standard Deviation
MS-5ppb*	201 ng/mL	75.2%	3.0%
QC-25ppb*	1000 ng/mL	115%	8.3%
MS-200ppb	8030 ng/mL	90.5%	2.5%
QC-40ppm	1610000 ng/mL	76.2%	2.8%
QC-80ppm*	3230000 ng/mL	114%	5.7%

Sera samples labeled as MS and QC were prepared following the same procedure on a different day and at different concentrations. These MS samples were inadvertently not labeled as QC.

*QC samples were reanalyzed to confirm original results. Data from reanalysis were not included in calculating these results.

Table 7. Serum Matrix Spike Summary Table

Serum Matrix Spike Samples*	Concentration in Diluted Serum	Average Recoveries
Three Matrix Spikes	10000 ng/mL	93.9%
Three Matrix Spikes Duplicates	10000 ng/mL	91.8%

*One matrix spike sample was injected in a second analytical batch. Both results are included in the average recoveries.

Table 8. Liver QC Summary Table

Liver QC Samples	Concentration in Diluted Liver	Mean recovery of triplicates	Relative Standard Deviation
QC-10ppb*	200 ng/g	138%	2.9%
QC-75ppb*	1500 ng/g	115%	2.9%
QC-150ppb*	2990 ng/g	104%	2.0%

*QC samples were reanalyzed to confirm original results. Data from reanalysis were not included in calculating these results.

Table 9. Liver Matrix Spike Summary Table

Liver Matrix Spike Samples*	Concentration in Diluted Liver	Average Recoveries
Four Matrix Spikes	499 ng/g	110%
Four Matrix Spikes Duplicates	499 ng/g	112%

The protocol and method state that 2/3 of all QC and a minimum of ½ of QC at each level must be within 75% - 125% criteria. All three of the liver "QC-10ppb" spikes exceeded the stated criteria, therefore the liver data reported in the low range of the curve (Day 0 samples)

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

may be biased high. Since all study samples that were not "Day 0" show analyte levels significantly above the low range of the curve, the overall impact (or bias) on data is minimal or none at all.

- **Absolute Recoveries.** The absolute recovery of the QC samples is determined by comparing the results of the extracted quality control spikes quantitated versus an matrix-extracted curve and a solvent curve. The sera spikes quantitate higher using a solvent curve than when using a matrix-extracted curve. The low spikes are 37.8% higher, the medium spikes 31.7% higher, and the high spikes 28.6% higher. The 32.5% average higher quantitation of the three levels of spikes using a solvent curve versus a matrix-extracted curve demonstrates that it is appropriate to use matrix-extracted standards. The same trend is observed for liver samples. The liver spikes quantitate at 4.8% lower, 6.4% higher, and 8.4% higher respectively for the low, medium and high spikes of the liver matrix with an average of 3.0% higher using a solvent curve versus an extracted liver matrix curve, again demonstrating that it is appropriate to use matrix-extracted standards.
- **Internal Standard:** The internal standard (PFDA) was added to all samples and standards. PFDA was not used for quantitation, but was used to monitor for gross instrument failure.
- **Instrument Response Calibration.** HPLC-ES/MS/MS instrument response was calibrated using the concentration of the $C_8F_{17}SO_3$ anion of perfluorooctane sulfonate, potassium salt (PFOS) that was adjusted for purity and the potassium ion contribution to the mass of PFOS used to prepare stock solutions. Any future calculations requiring concentrations to be expressed as a percentage of dietary in-take must adjust the dosed amounts as well.

DATA SUMMARY

Tables 10 through 13 summarize individual sample data. Representative chromatograms are presented in Attachment C. The tables display $C_8F_{17}SO_3^-$ concentrations for individual injections. All data are corrected for purity of the PFOS reference substance used for the matrix-extracted calibration curve. All results relate only to the samples tested.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Table 10. $C_8F_{17}SO_3^-$ Data Summary, Female Quail Sera (Adult and Offspring)

3M Sample Number	Sample Description	Amount of Sera Used (mL)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/mL of Sera)	Average of Dup. Samples
* E01-1245-28444	454-108-330, 0ppm female, adult, 01-31-02	0.4	1	<400	<400
		0.4	1	<400	
* E01-1245-28446	454-108-332, 0ppm female, adult, 01-31-02	0.05	1	<400	<400
		0.05	1	<400	
* E01-1245-28449	454-108-336, 0ppm female, adult, 01-31-02	0.3	1	<400	<400
		0.3	1	<400	
* E01-1245-28451	454-108-338, 0ppm female, adult, 01-31-02	0.3	1	<400	<400
		0.3	1	<400	
* E01-1245-28453	454-108-340, 0ppm female, adult, 01-31-02	0.2	1	<400	<400
		0.2	1	<400	
* E01-1245-28456	454-108-344, 0ppm female, adult, 01-31-02	0.2	1	<400	<400
		0.2	1	<400	
* E01-1245-28458	454-108-346, 0ppm female, adult, 02-01-02	0.1	1	<400	<400
		0.1	1	<400	
* E01-1245-28460	454-108-348, 0ppm female, adult, 02-01-02	0.3	1	<400	<400
		0.3	1	<400	
* E01-1245-28462	454-108-350, 0ppm female, adult, 02-01-02	0.4	1	<400	<400
		0.4	1	<400	
* E01-1245-28464	454-108-352, 0ppm female, adult, 02-01-02	0.4	1	<400	<400
		0.4	1	<400	
* E01-1245-28466	454-108-354, 0ppm female, adult, 02-01-02	0.3	1	<400	<400
		0.3	1	<400	
* E01-1245-28468	454-108-356, 0ppm female, adult, 02-01-02	0.3	1	<400	<400
		0.3	1	<400	
* E01-1245-28470	454-108-358, 0ppm female, adult, 02-01-02	0.3	1	<400	<400
		0.3	1	<400	
* E01-1245-28472	454-108-360, 0ppm female, adult, 02-01-02	0.3	1	2050	2110 ●
		0.3	1	2180	
* E01-1245-28474	454-108-362, 0ppm female, adult, 02-01-02	0.2	1	<400	<400
		0.2	1	<400	
* E01-1245-28477	454-108-366, 0ppm female, adult, 02-01-02	0.2	1	<400	<400
		0.2	1	<400	
* E01-1245-28479	454-108-368, 0ppm female, adult, 02-01-02	0.3	1	<400	<400
		0.3	1	<400	
E01-1245-28481	454-108-370, 10ppm female, adult, 02-01-02	0.3	1	9170	
E01-1245-28483	454-108-372, 10ppm female, adult, 02-01-02	0.4	1	9140	
E01-1245-28485	454-108-374, 10ppm female, adult, 02-01-02	0.2	1	8400	
E01-1245-28487	454-108-376, 10ppm female, adult, 02-01-02	0.3	1	9280	
E01-1245-28489	454-108-378, 10ppm female, adult, 02-01-02	0.1	1	4980	
E01-1245-28491	454-108-380, 10ppm female, adult, 02-01-02	0.1	1	5790	

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

3M Sample Number	Sample Description	Amount of Sera Used (mL)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/mL of Sera)	Average of Dup. Samples
E01-1245-28493	454-108-382, 10ppm female, adult, 02-01-02	0.3	10	31900 •	
E01-1245-28496	454-108-386, 10ppm female, adult, 02-01-02	0.4	1	14700	
E01-1245-28498	454-108-388, 10ppm female, adult, 02-01-02	0.3	1	11600	
E01-1245-28500	454-108-390, 10ppm female, adult, 02-01-02	0.2	1	6310	
E01-1245-28502	454-108-392, 10ppm female, adult, 02-01-02	0.3	1	9400	
E01-1245-28504	454-108-394, 10ppm female, adult, 02-01-02	0.3	1	11400	
E01-1245-28506	454-108-396, 10ppm female, adult, 02-01-02	0.09	1	4710	
E01-1245-28508	454-108-398, 10ppm female, adult, 02-01-02	0.1	1	10500	
E01-1245-28511	454-108-402, 10ppm female, adult, 02-01-02	0.4	1	6720	
E01-1245-28513	454-108-404, 10ppm female, adult, 02-01-02	0.1	1	9060	
E01-1245-28515	454-108-406, 10ppm female, adult, 02-01-02	0.2	1	8980	
E01-1245-28517	454-108-408, 10ppm female, adult, 02-01-02	0.2	1	7840	
* E01-1245-28518	454-108-246, 0ppm female, offspring, 02-01-02	0.1 0.1	1 1	<400 <400	<400
* E01-1245-28521	454-108-259, 0ppm female, offspring, 02-01-02	0.1 0.1	1 1	<400 <400	<400
* E01-1245-28524	454-108-273, 0ppm female, offspring, 02-01-02	0.08 0.08	1 1	<400 <400	<400
* E01-1245-28525	454-108-277, 0ppm female, offspring, 02-01-02	0.2 0.2	1 1	<400 <400	<400
* E01-1245-28528	454-108-517, 10ppm female, offspring, 02-01-02	0.02 0.02	1 1	18500 16600	17600
* E01-1245-28530	454-108-525, 10ppm female, offspring, 02-01-02	0.03 0.03	1 1	12800 12100	12500
* E01-1245-28532	454-108-538, 10ppm female, offspring, 02-01-02	0.08 0.08	1 1	14600 12200	13400
* E01-1245-28535	454-108-557, 10ppm female, offspring, 02-01-02	0.06 0.06	1 1	8330 7460	7900
* E01-1245-28536	454-108-561, 10ppm female, offspring, 02-01-02	0.05 0.05	1 1	11500 10100	10800

* There are two results reported for these samples. Both values are included and averaged in the "Average of Dup Sample" column.

• These values were rejected based on the results of the Dixon's Q-Test, 90% confidence interval, for the rejection of outliers.

Accuracy: Sample results are accurate to 100% +/- 30%.

Sera QC samples met the acceptance criteria for the method.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Table 11. $C_8F_{17}SO_3^-$ Data Summary, Male Quail Sera (Adult and Offspring)

3M Sample Number	Sample Description	Amount of Sera Used (mL)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/mL of Sera)	Average of Dup. Samples
E01-1245-28443	454-108-329, 0ppm male, adult, 01-31-02	0.2	1	<400	<400
		0.2	1	<400	
E01-1245-28445	454-108-331, 0ppm male, adult, 01-31-02	0.4	1	<400	<400
		0.4	1	<400	
E01-1245-28447	454-108-333, 0ppm male, adult, 01-31-02	0.4	1	<400	<400
		0.4	1	<400	
E01-1245-28448	454-108-335, 0ppm male, adult, 01-31-02	0.2	1	<400	<400
		0.2	1	<400	
E01-1245-28450	454-108-337, 0ppm male, adult, 01-31-02	0.1	1	<400	<400
		0.1	1	<400	
E01-1245-28452	454-108-339, 0ppm male, adult, 01-31-02	0.2	1	<400	<400
		0.2	1	<400	
E01-1245-28454	454-108-341, 0ppm male, adult, 01-31-02	0.2	1	<400	<400
		0.2	1	<400	
E01-1245-28455	454-108-343, 0ppm male, adult, 01-31-02	0.1	1	<400	<400
		0.1	1	<400	
E01-1245-28457	454-108-345, 0ppm male, adult, 01-31-02	0.2	1	<400	<400
		0.2	1	<400	
E01-1245-28459	454-108-347, 0ppm male, adult, 02-01-02	0.2	1	<400	<400
		0.2	1	<400	
E01-1245-28461	454-108-349, 0ppm male, adult, 02-01-02	0.02	1	<400	<400
		0.02	1	<400	
E01-1245-28465	454-108-353, 0ppm male, adult, 02-01-02	0.3	1	<400	<400
		0.3	1	<400	
E01-1245-28467	454-108-355, 0ppm male, adult, 02-01-02	0.2	1	<400	<400
		0.2	1	<400	
E01-1245-28469	454-108-357, 0ppm male, adult, 02-01-02	0.07	1	<400	<400
		0.07	1	<400	
E01-1245-28471	454-108-359, 0ppm male, adult, 02-01-02	0.2	1	<400	<400
		0.2	1	<400	
E01-1245-28473	454-108-361, 0ppm male, adult, 02-01-02	0.2	1	<400	<400
		0.2	1	<400	
E01-1245-28475	454-108-363, 0ppm male, adult, 02-01-02	0.1	1	<400	<400
		0.1	1	<400	
E01-1245-28476	454-108-365, 0ppm male, adult, 02-01-02	0.4	1	<400	<400
		0.4	1	<400	
E01-1245-28478	454-108-367, 0ppm male, adult, 02-01-02	0.1	1	<400	<400
		0.1	1	<400	

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

3M Sample Number	Sample Description	Amount of Sera Used (mL)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/mL of Sera)	Average of Dup. Samples
E01-1245-28480	454-108-369, 10ppm male, adult, d100,02-01-02	0.2	100	107000	112000
		0.2	100	117000	
E01-1245-28482	454-108-371, 10ppm male, adult, d100,02-01-02	0.2	100	181000	180000
		0.2	100	179000	
E01-1245-28484	454-108-373, 10ppm male, adult, d100,02-01-02	0.1	100	144000	150000
		0.1	100	156000	
E01-1245-28486	454-108-375, 10ppm male, adult, d100,02-01-02	0.3	100	138000	138000
		0.3	100	138000	
E01-1245-28488	454-108-377, 10ppm male, adult, d100,02-01-02	0.07	100	94400	95300
		0.07	100	96100	
E01-1245-28490	454-108-379, 10ppm male, adult, d100,02-01-02	0.1	100	150000	158000
		0.1	100	166000	
E01-1245-28492	454-108-381, 10ppm male, adult, d100,02-01-02	0.1	100	141000	145000
		0.1	100	149000	
E01-1245-28494	454-108-383, 10ppm male, adult, d100,02-01-02	0.3	100	147000	151000
		0.3	100	156000	
E01-1245-28495	454-108-385, 10ppm male, adult, d100,02-01-02	0.1	100	119000	121000
		0.1	100	123000	
E01-1245-28497	454-108-387, 10ppm male, adult, d100,02-01-02	0.3	100	174000	185000
		0.3	100	196000	
E01-1245-28499	454-108-389, 10ppm male, adult, d100,02-01-02	0.1	100	129000	132000
		0.1	100	136000	
E01-1245-28501	454-108-391, 10ppm male, adult, d100,02-01-02	0.06	100	141000	144000
		0.06	100	147000	
E01-1245-28503	454-108-393, 10ppm male, adult, d100,02-01-02	0.2	100	160000	162000
		0.2	100	165000	
E01-1245-28505	454-108-395, 10ppm male, adult, d100,02-01-02	0.2	100	127000	131000
		0.2	100	136000	
E01-1245-28507	454-108-397, 10ppm male, adult, d100,02-01-02	0.3	100	99600	103000
		0.3	100	107000	
E01-1245-28509	454-108-399, 10ppm male, adult, d100,02-01-02	0.1	100	138000	148000
		0.1	100	159000	
E01-1245-28510	454-108-401, 10ppm male, adult, d100,02-01-02	0.1	100	203000	215000
		0.1	100	226000	
E01-1245-28512	454-108-403, 10ppm male, adult, d100,02-01-02	0.2	100	127000	132000
		0.2	100	138000	
E01-1245-28514	454-108-405, 10ppm male, adult, d100,02-01-02	0.1	100	108000	112000
		0.1	100	117000	
E01-1245-28516	454-108-407, 10ppm male, adult, d100,02-01-02	0.1	100	99200	105000
		0.1	100	112000	

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

3M Sample Number	Sample Description	Amount of Sera Used (mL)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/mL of Sera)	Average of Dup. Samples
* E01-1245-28519	454-108-253, 0ppm male, offspring, 02-01-02	0.02	1	<400	<400
		0.02	1	<400	
* E01-1245-28520	454-108-256, 0ppm male, offspring, 02-01-02	0.02	1	<400	<400
		0.02	1	<400	
* E01-1245-28522	454-108-266, 0ppm male, offspring, 02-01-02	0.07	1	<400	<400
		0.07	1	<400	
* E01-1245-28523	454-108-270, 0ppm male, offspring, 02-01-02	0.2	1	<400	<400
		0.2	1	<400	
* E01-1245-28526	454-108-283, 0ppm male, offspring, 02-01-02	0.02	1	<400	<400
		0.02	1	<400	
* E01-1245-28527	454-108-291, 0ppm male, offspring, 02-01-02	0.05	1	<400	<400
		0.05	1	<400	
E01-1245-28529	454-108-522, 10ppm male, offspring, 02-01-02	0.07	10	19300	19300
* E01-1245-28531	454-108-533, 10ppm male, offspring, 02-01-02	0.07	1	9310	8760
		0.07	1	8220	
* E01-1245-28533	454-108-545, 10ppm male, offspring, 02-01-02	0.04	1	15500	14600
		0.04	1	13600	
* E01-1245-28534	454-108-552, 10ppm male, offspring, 02-01-02	0.07	1	13600	12500
		0.07	1	11500	
* E01-1245-28537	454-108-568, 10ppm male, offspring, 02-01-02	0.1	1	12100	11200
		0.1	1	10400	

* There are two results reported for these samples. Both values are included and averaged in the "Average of Dup Sample" column.

Accuracy: Sample results are accurate to 100% +/- 30%.

Sera QC samples met the acceptance criteria for the method.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Table 12. $C_8F_{17}SO_3^-$ Data Summary, Female Quail Liver (Adult and Offspring)

3M Sample Number	Sample Description	Amount of Liver Weighed (g)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/g of Liver)
E01-1342-30321	0 ppm a.i. 330 F Liver	1.0749	1	<50.2*
E01-1342-30339	0 ppm a.i. 332 F Liver	1.0469	1	<50.2*
E01-1342-30357	0 ppm a.i. 334 F Liver	1.0232	1	<50.2*
E01-1342-30375	0 ppm a.i. 336 F Liver	1.0188	1	<50.2*
E01-1342-30393	0 ppm a.i. 338 F Liver	1.0543	1	<50.2*
E01-1342-30411	0 ppm a.i. 340 F Liver	1.0182	1	<50.2*
E01-1342-30429	0 ppm a.i. 342 F Liver	1.0904	1	<50.2*
E01-1342-30447	0 ppm a.i. 344 F Liver	1.0087	1	<50.2*
E01-1342-30465	0 ppm a.i. 346 F Liver	1.0998	1	<50.2*
E01-1342-30483	0 ppm a.i. 348 F Liver	1.1036	1	<50.2*
E01-1342-30501	0 ppm a.i. 350 F Liver	1.0237	1	<50.2*
E01-1342-30519	0 ppm a.i. 352 F Liver	1.1509	1	<50.2*
E01-1342-30537	0 ppm a.i. 354 F Liver	1.0578	1	<50.2*
E01-1342-30555	0 ppm a.i. 356 F Liver	1.0312	1	<50.2*
E01-1342-30573	0 ppm a.i. 358 F Liver	1.1031	1	<50.2*
E01-1342-30591	0 ppm a.i. 360 F Liver	1.0586	1	<50.2*
E01-1342-30609	0 ppm a.i. 362 F Liver	1.0424	1	<50.2*
E01-1342-30627	0 ppm a.i. 364 F Liver	1.0364	1	<50.2*
E01-1342-30645	0 ppm a.i. 366 F Liver	1.0577	1	<50.2*
E01-1342-30663	0 ppm a.i. 368 F Liver	1.0920	1	<50.2*
E01-1342-30681	10 ppm a.i. 370 F Liver	1.0868	1	5920*
E01-1342-30699	10 ppm a.i. 372 F Liver	1.0748	1	4430*
E01-1342-30717	10 ppm a.i. 374 F Liver	1.0790	1	5430*
E01-1342-30735	10 ppm a.i. 376 F Liver	1.1036	1	3980*
E01-1342-30753	10 ppm a.i. 378 F Liver	1.0021	1	3750*
E01-1342-30771	10 ppm a.i. 380 F Liver	1.0825	1	3990*
E01-1342-30789	10 ppm a.i. 382 F Liver	1.0122	1	3290*
E01-1342-30820	10 ppm a.i. 384 F Liver	0.6864	1	4190*
E01-1342-30838	10 ppm a.i. 386 F Liver	1.1215	1	7550*
E01-1342-30856	10 ppm a.i. 388 F Liver	1.0878	1	3490*
E01-1342-30874	10 ppm a.i. 390 F Liver	1.0078	1	4870*
E01-1342-30892	10 ppm a.i. 392 F Liver	1.0060	1	5940*
E01-1342-30910	10 ppm a.i. 394 F Liver	1.0238	1	5030*
E01-1342-30928	10 ppm a.i. 396 F Liver	1.0370	1	4910*
E01-1342-30946	10 ppm a.i. 398 F Liver	1.0240	1	4400*
E01-1342-30964	10 ppm a.i. 400 F Liver	1.0181	1	5040*
E01-1342-30982	10 ppm a.i. 402 F Liver	1.0876	1	5900*
E01-1342-31000	10 ppm a.i. 404 F Liver	1.0173	1	5420*
E01-1342-31018	10 ppm a.i. 406 F Liver	1.0047	1	5870*
E01-1342-31036	10 ppm a.i. 408 F Liver	1.0044	1	4560*

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

3M Sample Number	Sample Description	Amount of Liver Weighed (g)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/g of Liver)
E01-1365-31184	0 ppm a.i. 246 F Liver, Offspring	0.7302	1	<20.0*
E01-1365-31204	0 ppm a.i. 259 F Liver, Offspring	0.4434	1	<20.0*
E01-1365-31219	0 ppm a.i. 273 F Liver, Offspring	0.5004	1	<20.0*
E01-1365-31224	0 ppm a.i. 277 F Liver, Offspring	0.4326	1	<20.0*
E01-1365-31240	10 ppm a.i. 517 F Liver, Offspring	0.6570	1	5690*
E01-1365-31250	10 ppm a.i. 525 F Liver, Offspring	0.5720	1	7030*
E01-1365-31260	10 ppm a.i. 538 F Liver, Offspring	0.5211	10	13900•*
E01-1365-31275	10 ppm a.i. 557 F Liver, Offspring	0.4654	1	4680*
E01-1365-31280	10 ppm a.i. 561 F Liver, Offspring	0.9417	1	4590*

• These values were rejected based on the results of the Dixon's Q-Test, 90% confidence interval, for the rejection of outliers.

Accuracy: Sample results are accurate to 100% +/- 30%.

* Liver QC samples did not meet the ½ of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Table 13. $C_8F_{17}SO_3^-$ Data Summary, Male Quail Liver (Adult and Offspring)

3M Sample Number	Sample Description	Amount of Liver Weighed (g)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/g of Liver)
E01-1342-30312	0 ppm a.i. 329 M Liver	1.0050	1	<50.2*
E01-1342-30330	0 ppm a.i. 331 M Liver	1.0530	1	<50.2*
E01-1342-30348	0 ppm a.i. 333 M Liver	1.0338	1	209 •*
E01-1342-30366	0 ppm a.i. 335 M Liver	1.0062	1	<50.2*
E01-1342-30384	0 ppm a.i. 337 M Liver	1.0269	1	<50.2*
E01-1342-30402	0 ppm a.i. 339 M Liver	0.0886	1	<50.2*
E01-1342-30420	0 ppm a.i. 341 M Liver	1.0235	1	<50.2*
E01-1342-30438	0 ppm a.i. 343 M Liver	1.0090	1	<50.2*
E01-1342-30456	0 ppm a.i. 345 M Liver	1.1046	1	<50.2*
E01-1342-30474	0 ppm a.i. 347 M Liver	1.0586	1	<50.2*
E01-1342-30492	0 ppm a.i. 349 M Liver	1.0358	1	<50.2*
E01-1342-30510	0 ppm a.i. 351 M Liver	1.0583	1	<50.2*
E01-1342-30528	0 ppm a.i. 353 M Liver	1.0183	1	<50.2*
E01-1342-30546	0 ppm a.i. 355 M Liver	1.0899	1	<50.2*
E01-1342-30564	0 ppm a.i. 357 M Liver	1.0382	1	<50.2*
E01-1342-30582	0 ppm a.i. 359 M Liver	1.0941	1	<50.2*
E01-1342-30600	0 ppm a.i. 361 M Liver	1.0084	1	<50.2*
E01-1342-30618	0 ppm a.i. 363 M Liver	1.0818	1	<50.2*
E01-1342-30636	0 ppm a.i. 365 M Liver	1.0916	1	<50.2*
E01-1342-30654	0 ppm a.i. 367 M Liver	1.0327	1	<50.2*
E01-1342-30672	10 ppm a.i. 369 M Liver	1.0010	10	78600*
E01-1342-30690	10 ppm a.i. 371 M Liver	1.0856	10	78500*
E01-1342-30708	10 ppm a.i. 373 M Liver	1.0276	10	86300*
E01-1342-30726	10 ppm a.i. 375 M Liver	1.1008	10	70000*
E01-1342-30744	10 ppm a.i. 377 M Liver	1.0236	10	80300*
E01-1342-30762	10 ppm a.i. 379 M Liver	1.0312	10	90700*
E01-1342-30780	10 ppm a.i. 381 M Liver	1.0501	10	80000*
E01-1342-30798	10 ppm a.i. 383 M Liver	1.0110	10	73500*
E01-1342-30829	10 ppm a.i. 385 M Liver	1.0678	10	83400*
E01-1342-30847	10 ppm a.i. 387 M Liver	1.0315	10	79300*
E01-1342-30865	10 ppm a.i. 389 M Liver	1.0310	10	70300*
E01-1342-30883	10 ppm a.i. 391 M Liver	1.0947	10	70400*
E01-1342-30901	10 ppm a.i. 393 M Liver	1.0834	10	86800*
E01-1342-30919	10 ppm a.i. 395 M Liver	1.0489	10	65100*
E01-1342-30937	10 ppm a.i. 397 M Liver	1.0309	10	50100*
E01-1342-30955	10 ppm a.i. 399 M Liver	1.0276	10	63000*
E01-1342-30973	10 ppm a.i. 401 M Liver	1.0361	100	147000*
E01-1342-30991	10 ppm a.i. 403 M Liver	1.0019	100	146000*
E01-1342-31009	10 ppm a.i. 405 M Liver	1.0336	100	138000*
E01-1342-31027	10 ppm a.i. 407 M Liver	1.0956	100	132000*

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

3M Sample Number	Sample Description	Amount of Liver Weighed (g)	Additional Dilution	Conc. of $C_8F_{17}SO_3^-$ in Sample (ng/g of Liver)
E01-1365-31194	0 ppm a.i. 253 M Liver, Offspring	0.3806	1	<20.0*
E01-1365-31199	0 ppm a.i. 256 M Liver, Offspring	0.5947	1	<20.0*
E01-1365-31209	0 ppm a.i. 266 M Liver, Offspring	0.2673	1	<20.0*
E01-1365-31214	0 ppm a.i. 270 M Liver, Offspring	0.5900	1	<20.0*
E01-1365-31229	0 ppm a.i. 283 M Liver, Offspring	0.6666	1	<20.0*
E01-1365-31234	0 ppm a.i. 291 M Liver, Offspring	0.6072	1	<20.0*
E01-1365-31245	10 ppm a.i. 522 M Liver, Offspring	0.7325	10	13700 •*
E01-1365-31255	10 ppm a.i. 533 M Liver, Offspring	0.6903	1	4940*
E01-1365-31265	10 ppm a.i. 545 M Liver, Offspring	0.4624	1	5580*
E01-1365-31270	10 ppm a.i. 552 M Liver, Offspring	0.5353	1	5990*
E01-1365-31285	10 ppm a.i. 568 M Liver, Offspring	0.7581	1	6520*

• These values were rejected based on the results of the Dixon's Q-Test, 90% confidence interval, for the rejection of outliers.

Attachment B contains data summary tables.

Accuracy: Sample results are accurate to 100% +/- 30%.

* Liver QC samples did not meet the ½ of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

Statement of Conclusion

Sera results from the male control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 19 of 19 quail (<400 ng/mL). The sera results from the female control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 16 of 16 birds (<400 ng/mL) (Table 15). Analytical results for twenty sera samples from the 10ppm dosed male group showed an average of 141000 ng/mL $C_8F_{17}SO_3^-$ (range 94400 – 226000 ng/mL, Table 14). Analytical results for seventeen sera samples from the 10ppm dosed female group showed an average of 8670 ng/mL $C_8F_{17}SO_3^-$ (range 4710 – 14700 ng/mL, Table 15).

Sera results from the male offspring control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 6 of 6 birds (<400 ng/mL). The sera results from the female offspring control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 4 of 4 birds (<400 ng/mL, Table 14). Analytical results for five sera samples from the 10ppm dosed male offspring group showed an average of 12600 ng/mL (range 8220 - 19200 ng/mL, Table 14). Analytical results for five sera samples from the 10ppm dosed female offspring group showed an average of 12400 ng/mL (range 7640 - 18500 ng/mL, Table 14).

Liver results from the male control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 19 of 19 birds (<50.2 ng/g, Table 15) and quantifiable concentrations of $C_8F_{17}SO_3^-$ in 1 of 20 (209 ng/g, Table 14). The liver results from the female control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 20 of 20 birds (<50.2 ng/g, Table 14). Analytical results for twenty liver samples from the 10ppm dosed male group showed an average of 88500 ng/g $C_8F_{17}SO_3^-$ (range 50100 - 147000 ng/g, Table 14). Analytical results for twenty liver samples from the 10ppm dosed female group showed an average of 4900 ng/g $C_8F_{17}SO_3^-$ (range 3290 – 7550 ng/g, Table 14).

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Liver results from the male offspring control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 6 of 6 birds (<50.2 ng/g, Table 14). The liver results from the female offspring control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 4 of 4 birds (<20.0 ng/g, Table 14). Analytical results for four liver samples from the 10ppm dosed male offspring group showed an average of 5760 ng/g (range 4940 – 6520 ng/g, Table 15). Analytical results for four liver samples from the 10ppm dosed female offspring group showed an average of 5490 ng/g (range 4590 - 7030 ng/g, Table 15).

Table 14. Summary Table, $C_8F_{17}SO_3^-$ Concentration in Sera and Liver

Sampling Event	Number of Sera Samples	Average $C_8F_{17}SO_3^-$ Conc. Sera	Range of Results	Number of Liver Samples	Average $C_8F_{17}SO_3^-$ Conc. Liver	Range of Results
Male Control Group	19	NA	<400 ng/mL (19 of 19)	20	<50.2 ng/g*	<50.2 ng/g (19 of 20)* 209 ng/g (1 of 20)*
Female Control Group	17	NA	<400 ng/mL (16 of 17) 2110 ng/mL (1 of 17)	20	<50.2 ng/g*	<50.2 ng/g (20 of 20)*
Male 10ppm Group	20	141000 ng/mL	94400 - 226000 ng/mL	20	88500 ng/g*	50100 - 147000 ng/g*
Female 10ppm Group	18	9990 ng/mL	4710 - 31900 ng/mL	20	4900 ng/g*	3290 - 7550 ng/g*
Male Offspring Control Group	6	NA	<400 ng/mL (6 of 6)	6	<20.0 ng/g*	<20.0 ng/g (6 of 6)*
Female Offspring Control Group	4	NA	<400 ng/mL (4 of 4)	4	<20.0 ng/g*	<20.0 ng/g (4 of 4)*
Male Offspring 10ppm Group	5	12600 ng/mL	8220 - 19200 ng/mL	5	7350 ng/g*	4940 - 13700 ng/g*
Female Offspring 10ppm Group	5	12400 ng/mL	7640 - 18500 ng/mL	5	7180 ng/g*	4590 - 13900 ng/g*

For individual sample values see Tables 10 to 13 and Attachment B.
Accuracy: Sample results are accurate to 100% +/- 30%.
Sera QC samples met the acceptance criteria for the method.
* Liver QC samples did not meet the ½ of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Table 15. Summary Table, $C_8F_{17}SO_3^-$ Concentration in Sera and Liver with Outliers Removed

Sampling Event	Number of Sera Samples	Average $C_8F_{17}SO_3^-$ Conc. Sera	Range of Results	Number of Liver Samples	Average $C_8F_{17}SO_3^-$ Conc. Liver	Range of Results
Male Control Group	19*	*	*	19	<50.2 ng/g**	<50.2 ng/g (19 of 19)**
Female Control Group	16	<400 ng/mL	<400 ng/mL (16 of 16)	20*	*	*
Male 10ppm Group	20*	*	*	20*	*	*
Female 10ppm Group	17	8700 ng/mL	4710 - 14700 ng/mL	20*	*	*
Male Offspring Control Group	6*	*	*	6*	*	*
Female Offspring Control Group	4*	*	*	4*	*	*
Male Offspring 10ppm Group	5*	*	*	4	5760 ng/g**	4940 - 6520 ng/g**
Female Offspring 10ppm Group	5*	*	*	4	5490 ng/g**	4590 - 7030 ng/g**

* No outliers from this data set. Refer to Table 1 for results. For individual sample values see Tables 10 to 13 and Attachment B.
Accuracy: Sample results are accurate to 100% +/- 30%.
Sera QC samples met the acceptance criteria for the method.
** Liver QC samples did not meet the ½ of QC at each level must be within 75% - 125% criteria for the method. All liver QC samples (3) spiked at 10 ppb were >125% recovery. All other liver QC samples (6) were within criteria, therefore the second QC criteria was met in that 2/3 of all QC were within 75% - 125%.

Statistical Methods

Statistical methods were limited to calculating means, standard deviations, % coefficient of variation and the Dixons Q-Test. The Dixons Q-Test, used for the rejection of outliers, has values ordered from lowest to highest. A Q-value is determined from the difference of a result and the next closest result which is divided by the difference of the highest and lowest value in the group of values. A critical Q-value is determined based on the number of samples in the group and the desired confidence interval. If the Q-value is greater than the critical Q-value then the value will be rejected.

Statement of Data Quality

The measurement of accuracy available at this time is the quality control and matrix spike results. For sera data, the accuracy (the observed amount in the QC and MS samples, divided by the amount spiked, expressed as a percent) was found to range from 74.1% to 126%; while precision, expressed as the standard deviation of the recoveries, was found to range from 2.14% to 9.56%. For liver data, the accuracy was found to range from 72.6% to 141%; while precision was found to range from 2.11% to 5.51%.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

The protocol and method state that 2/3 of all QC and a minimum of ½ of QC at each level must be within 75% - 125% criteria. All three of the liver "QC-10ppb" spikes exceeded the stated criteria, therefore the liver data reported in the low range of the curve (Day 0 samples) may be biased high. Since all study samples that were not "Day 0" show analyte levels significantly above the low range of the curve, the overall impact (or bias) on data is minimal or none at all.

REFERENCES

1. Wildlife International, Ltd. Protocol No. 454-108 "PFOS: A Reproduction Study with the Northern Bobwhite".
2. Wildlife International, Ltd. Protocol No. 454/120700/QR/SUB454 (3M Environmental Laboratory Project Number U2723) "PFOS: A Reproduction Study with the Northern Bobwhite".
3. Centre Analytical Laboratories, Inc. Study 023-044, "Validation of Analytical Methods "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC Electrospray/Mass Spectrometry" and "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC Electrospray/Mass Spectrometry in Quail Serum and Liver."
4. 3M Environmental Laboratory Standard Operating Procedure ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds form Biological Matrices".

LIST OF ATTACHMENTS

- Attachment A: Extraction and Analytical Method
- Attachment B: Data Summary Tables
- Attachment C: Sample Chromatograms, Calibration Information, and Instrument Parameters
- Attachment D: Sample Preparation Sheets
- Attachment E: Study Protocol, Protocol Amendments, and Deviations
- Attachment F: Example Calculations

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

SIGNATURE PAGE

We certify that this report is a true and complete representation of the data for this study:

Lisa A. Stevenson 04/18/03

Lisa A. Stevenson
Principal Analytical Investigator

Date

William K. Reagen 04/18/03

William K. Reagen
Laboratory Management

Date

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

ATTACHMENT A: EXTRACTION AND ANALYTICAL METHOD, ETS-8-231.1, "SOLID PHASE EXTRACTION AND ANALYSIS OF FLUOROCHEMICAL COMPOUNDS FROM BIOLOGICAL MATRICES"

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

3M Environmental Laboratory

Method

Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices

Method Number: ETS-8-231.1

Adoption Date: 11/13/01

Revision Date: 2/18/02

Effective Date: 2/18/02

Approved By:

2/18/02

Willliam K. Reagen
Laboratory Manager

Date

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

1 Scope and Application

This method describes the extraction of target analytes from fish, rat liver, rat sera, mouse liver, and mouse sera using solid phase extraction (SPE). This method may also be extended to other biological matrices provided that the data quality objectives are met.

2 Method Summary

An amount of biological material, determined by the analyst, is prepared (fluids diluted and tissues homogenized) at a 1/6 dilution, or other dilution as determined by the analyst using reagent grade water. An aliquot of the dilution/homogenate is spiked with the appropriate surrogate or analyte mixture. Acetonitrile (ACN) is added as an extraction solvent and also serves to precipitate the proteins. The sample is capped, mixed, and put on the centrifuge to clarify the supernatant. The supernatant is transferred to a clean tube, diluted with water, and passed through a pre-conditioned C_{18} SPE cartridge. Finally, the analytes of interest are eluted from the SPE cartridge and analyzed by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ES/MS/MS).

3 Definitions

3.1 Dilution

A dilution expressed as 1:5 or 1/6 is defined as: 1 mL of sample + 5 mLs of diluent for a total of 6 mLs combined, unless otherwise noted.

3.2 SPE cartridge

A column containing an open solvent reservoir at one end and packed with bonded silica sorbents at the other end. It is designed to retain the compounds of interest under some solvent conditions and elute them under others. A separation is thus achieved; compounds can be removed from difficult biological matrices and introduced into appropriate solvents for analysis.

3.3 Reagent grade water

Water with no detectable concentration(s) of the target analyte(s).

3.4 Quality control sample

Sample used to monitor the extraction efficiency (as a matrix spike) and to verify the continued accuracy of the initial calibration curve (as a continuing calibration verification).

4 Warnings and Cautions

4.1 Health and Safety Warnings

Always wear appropriate gloves, eyewear, and clothing when working with solvents, samples and/or equipment.

Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 volts.

4.2 Cautions

Take care not to allow the SPE column to run to dryness after the methanol and water washes. After washing is complete, add sample then allow all of the liquid to pass through the SPE column to dryness.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HPLC will initiate automatic shutdown.

Do not run solvent pumps to dryness.

5 Interferences

To minimize interferences, Teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6 Instrumentation, Supplies, and Materials

The following instrumentation, supplies, and materials are used while performing this method. Equivalent instrumentation, supplies, and materials may be used in place of those listed.

6.1 Instrumentation

Vortex mixer, VWR, Vortex Genie 2

Ultra-Turrax T25 tissue homogenizer

Vacuum Pump

SPE Extraction Manifold

Centrifuge, Mistral 1000 or IEC

Shaker, Eberbach or VWR

Balance (+/- 0.1000 g)

Micromass, Quattro II or Ultima triple quadrupole Mass Spectrometer equipped with an electrospray ionization source

HP1100 or Agilent low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

6.2 Supplies and Materials

Eppendorf or disposable pipettes, plastic or glass

Dissecting scalpels

Polypropylene bottles, capable of holding 50 mL to 1 L (Nalgene)

Volumetric flasks, glass, type A

40 mL glass vials (ICHEM)

Plastic sampule vials, Wheaton, 6 mL (or other appropriate size)

Centrifuge tubes, polypropylene, 15 mL and 50 mL

Labels

Graduated pipettes, glass

Syringes, capable of measuring 5 µL to 1000 µL

Bottle-Top Dispenser (capable of dispensing 5mL of solvent)

SPE extraction cartridge, 1 g, Sep-Pak 6 cc tri-functional C_{18} (Waters)

75 mL sample reservoir (or other appropriate size)

Crimp cap glass autovials and caps

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Crimpers

HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.

7 Reagents and Standards

Reagent grade water, Milli-Q™, Nanopure II, or equivalent

Acetonitrile, HPLC grade or equivalent

Methanol, HPLC grade or equivalent

Ammonium acetate, reagent grade or equivalent

Biological fluids or tissues, frozen from supplier

7.1 Reagents preparation

2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing reagent grade water, mix until all solids are dissolved, bring to volume using reagent grade water. Store at room temperature.

Note: When preparing different volumes than those listed in reagents preparation, target analyte standard preparation, and surrogate standard preparation, adjust accordingly.

7.2 Target analyte standard preparation

Prepare target analyte standard(s) for the standard curve. Multicomponent analyte standards are acceptable. The following is an example only and may or may not be appropriate for all standard preparations.

Weigh approximately 100 mg of target analyte into a 100 mL volumetric flask and record the actual weight in the standard logbook or other appropriate location.

Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/mL).

Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm. Example calculation: 1000 µg/mL x 5 mL/100 mL = 50 µg/mL.

Dilute working standard 1 with methanol to produce a working standard 2 solution of approx. 5.0 ppm. Example calculation: 50 µg/mL x 10 mL/100mL = 5.0 µg/mL.

Dilute working standard 1 with methanol to produce a working standard 3 solution of approx. 0.50 ppm. Example calculation: 50 µg/mL x 1.0 mL/100 mL = 0.5 µg/mL.

7.3 Surrogate standard preparation

Prepare surrogate standard(s). The following is an example only and may or may not be appropriate for all surrogate standard preparations.

Weigh approximately 90-110 mg of surrogate standard into a 100-mL volumetric flask and record the actual weight.

Bring to volume with methanol for a surrogate standard stock of approximately 900 - 1100 ppm.

Prepare a surrogate standard working standard. Transfer approximately 1 mL of surrogate standard stock to a 10-mL volumetric flask and bring to volume with methanol for a working standard of 90-110ppm. Record the actual volume transferred and standard concentrations in the standards logbook or other appropriate location.

7.4 Internal standard preparation

Prepare internal standard(s). The following is an example only and may or may not be appropriate for all internal standard preparations.

Weigh approximately 90-110 mg of internal standard into a 100-mL volumetric flask and record the actual weight.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Bring to volume with methanol for an internal standard stock of approximately 900 - 1100 ppm.

Prepare an internal standard working standard. Transfer approximately 1 mL of internal standard stock to a 10-mL volumetric flask and bring to volume with methanol for a working standard of 90-110ppm. Record the actual volume transferred and standard concentrations in the standards logbook or other appropriate location.

8 Sample Handling

All samples are received frozen and must be kept frozen until the extraction is performed.

Allow samples to thaw to room temperature prior to extraction.

Typically fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials until analysis.

If analysis will be delayed, extracted standards and samples may be refrigerated at approximately 4°C indefinitely or may be stored at room temperature until analysis can be performed.

9 Quality Control

9.1 Blanks

9.1.1 Solvent Blank

An aliquot of methanol is used as a solvent blank. Solvent blanks are not extracted.

9.1.2 Method Blank

An aliquot of 1.0 mL of water, or other appropriate amount, is used as a method blank. Four method blanks are extracted and analyzed with each set following this procedure (two are spiked with surrogate and two are not spiked).

9.1.3 Matrix Blank

An aliquot of 1.0 mL or 1.0 g of matrix (diluted or homogenized) is used as a matrix blank. Other amounts may be used, as appropriate. Matrix blanks are prepared from one of three sources: 1) a study control matrix from a study control animal received with a sample set; 2) a commercially obtained sample of the same species as the study animals; or 3) a surrogate matrix, also obtained commercially, but of a different species than the study animal. (eg. if rat is used to generate standard curves and CCVs for a mouse study). The matrix to use is dependent on the matrix used for the curve.

9.1.3.1 Study control matrix curve - if the study control matrix is used for the curve, prepare four (4) matrix blanks using the study control matrix (two spiked with surrogate and two not spiked).

9.1.3.2 Commercially obtained (same species) matrix curve – if the commercially obtained matrix is used for the curve, prepare four (4) matrix blanks using the same commercially available matrix (two spiked with surrogate and two not spiked).

9.1.3.3 Surrogate matrix curve – if a surrogate matrix is used for the curve, prepare four (4) matrix blanks using the same commercially available matrix and prepare four (4) matrix blanks using a commercially available matrix of the same species as the study animals (two spiked with surrogate and two not spiked).

9.1.3.4 If limited matrix is available, the number of method and matrix blanks may be adjusted and will be noted in the study protocol or in the raw data.

9.2 Sample Replicate

Samples replicates are prepared according to each study protocol or project outline.

9.3 Surrogate standard

If surrogate standard is a component of the study, all samples are spiked with surrogate standard prior to extraction to obtain a concentration in the mid-range of the calibration curve, with the exception of blank samples as described above.

Typically surrogate standard is spiked into the 1.0 mL diluted/homogenized sample removed for extraction. However, surrogate may be spiked directly into the matrix prior to diluting with water, into the diluted/homogenized sample prior to removing the 1.0 mL sample, or into the 1.0 mL diluted/homogenized sample removed for extraction.

9.4 Internal standard

If internal standard is a component of the study, all samples are spiked with internal standard after extraction to obtain a concentration in the mid-range of the calibration curve.

Typically internal standard is spiked into the 2.0 mL of extract in the 15 mL centrifuge tube, before transferring to the autovial.

9.5 Lab Control Sample

Lab control samples are not a component of this method.

9.6 Quality Control (QC) Sample

Prepare quality control (QC) samples to monitor extraction efficiency and to verify the continued accuracy of the initial calibration curve. Typically 1.0 mL, or other appropriate amount, of the same matrix used to prepare the initial calibration curve is used for each QC sample.

Twelve (12) quality control samples (QC) will be prepared for each matrix during the course of a study. A minimum of 3 QC samples must be prepared (one at each level) on each day of sample extraction. (e.g. If the study is such that samples will be extracted on three different days then four QC samples must be prepared on each day of extraction for a total of twelve.)

QC samples will consist of four samples at each of three levels of analyte. The levels listed below may be used and may represent sample concentrations diluted into the range of the calibration curve:

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.

QC samples extracted with a particular sample set must be analyzed in the same analytical run. Any QC samples extracted during the course of the study may be included in subsequent analyses.

If samples from multiple extraction dates are analyzed in one analytical run, then QC samples from the same sample extraction dates must be included in that analysis.

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

9.7 Sample Dilution

Any sample with an area greater than that of the highest acceptable standard will need to be diluted into the range of the calibration curve. If samples are diluted into the range of the curve during analyses and enough sample remains, a post-run dilution validation will be performed to verify sample values.

To perform the dilution validation, one sample will be separated into two representative samples (i.e. two 1.0 mL aliquots for fluid samples or two 1.0 gram amounts for tissue samples, or other amount as determined by the analyst

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

and documented in a note to file) then diluted using two procedures. The first procedure consists of diluting the sample with additional matrix prior to extraction (fluid adding fluid), while the second procedure consists of diluting the extract with solvent post-extraction (methanol extract adding additional methanol solvent.)

If the relative percent difference is not within 15% for these two samples; additional testing will be required to determine which value is a correct representation of the sample concentration.

10 Calibration and Standardization

10.1 Instrument Calibration

One calibration curve will be prepared from extracted matrix standards, in the same matrix as the samples, per study. It will consist of a minimum of nine (9) levels. Additional calibration curves may be extracted on separate sample extraction dates, as determined by the analyst and documented in a note to file.

Transfer 1.0 mL, or other appropriate amount, of diluted control fluid or homogenized control tissue to a 15 mL centrifuge tube using a disposable plastic pipette. This will be repeated while preparing aliquots for the standard curve. Be sure to mix or shake the control matrix container between aliquots to ensure a homogenous sample is removed.

Record each standard volume on the weight/volumes sheet or extraction worksheet, as appropriate.

Four 1.0 mL aliquots, or other appropriate amount, of control matrix serve as matrix blanks.

The standard concentrations and spiking amounts listed in Table 1 may be used, when appropriate, to spike one standard curve. A total of 9 standards, four matrix blanks, and four method blanks are prepared in addition to the QC samples and test samples. The number of standards and blanks may be adjusted as determined by the analyst and documented in a note to file.

Use Attachment C, or other appropriate form, as an aid in calculating the concentrations of the working standards. Refer to section 12 to calculate the actual concentration of analyte in each calibration standard and QC sample.

Typically the target analyte standard is spiked into the 1.0 mL diluted/homogenized sample removed for extraction. However, it may be spiked directly into the matrix prior to diluting with water, into the diluted/homogenized sample prior to removing the 1.0 mL sample, or into the 1.0 mL diluted/homogenized sample removed for extraction.

Analyze the extracted matrix standard curve prior to each set of extracts. The curve equation will be determined by regression analysis using the peak areas of the target analyte(s) using MassLynx or other suitable software.

Any level outside 75% - 125% of nominal must be deactivated, and regression re-calculated, except the LLOQ which must be within 30% of nominal. All levels must show a response greater than twice that of the blank. A maximum of three (3) levels may be deactivated in any one set, or the set will be re-analyzed.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

TABLE 1 APPROXIMATE SPIKING AMOUNTS FOR STANDARDS AND SPIKES USING 1.0 mL OF MATRIX			
Working standard (approximate concentration)	µL	Approximate final concentration of analyte **in Matrix diluted 1:5**	Approximate final concentration of analyte **in Final 2.0 mL volume**
-	-	Blank	Blank
0.500 ug/mL	1.5	5.00 ng/g or ng/mL	0.375 ng/mL
0.500 ug/mL	3.0	10.0 ng/g or ng/mL	0.750 ng/mL
0.500 ug/mL	8.0	25.0 ng/g or ng/mL	2.00 ng/mL
0.500 ug/mL	16	50.0 ng/g or ng/mL	4.00 ng/mL
0.500 ug/mL	32	100 ng/g or ng/mL	8.00 ng/mL
5.00 ug/mL	5.6	175 ng/g or ng/mL	14.0 ng/mL
5.00 ug/mL	8.0	250 ng/g or ng/mL	20.0 ng/mL
5.00 ug/mL	16	500 ng/g or ng/mL	40.0 ng/mL
5.00 ug/mL	24	750 ng/g or ng/mL	60.0 ng/mL
5.00 ug/mL	32	1000 ng/g or ng/mL	80.0 ng/mL
5.00 ug/mL	40	1250 ng/g or ng/mL	100 ng/mL
50.0 ug/mL	5.0	1500 ng/g or ng/mL	125 ng/mL
50.0 ug/mL	6.0	1750 ng/g or ng/mL	150 ng/mL
Surrogate Std 100 ug/mL	10	6500 ng/g or ng/mL	500 ng/mL

11 Procedures

11.1 Tissue Sample Preparation

Obtain frozen tissue samples

Cut approximately 1.0000 g of tissue (+/- 0.1000 g), or other appropriate amount, using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the tissue to thaw.

Weigh the tissue directly into a tared plastic sampule vial.

Record the weight on the weight/volume sheet, extraction worksheet, or other appropriate location.

Return unused tissue to the freezer after extraction amounts have been removed.

Add 2.5 mL of reagent water to sampule vial, or other volume as determined by the analyst and documented in a note to file.

Homogenize the sample. Put the Ultra-Turrax grinder probe in the sample and grind for approximately 2 minutes, or until the sample is homogeneous.

Rinse the probe into the tube containing the sample with 2.5 mL of reagent grade water, or other volume as determined by the analyst and documented in a note to file, using a pipette.

Take the grinder apart and clean it with methanol after each sample. Refer to ETS-9-52 for more information.

If an amount other than 1.0000 g (not within +/- 0.1000 g) is removed for an initial weight, adjust the water volume accordingly to maintain a 1/6 dilution. (e.g. if 0.5 g is removed for extraction, add a total of 2.5 mL of water.), or other ratio as determined by the analyst and documented in a note to file.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

11.2 Fluid Sample Preparation

Obtain frozen fluid sample and allow it to thaw at room temperature or in lukewarm water.

Label a 15 mL polypropylene centrifuge tube with the study number, sample ID, extraction date and analyst initials. See attached worksheet (Attachment A or similar worksheet) for documenting the remaining steps.

Vortex mix the fluid sample for approximately 15 seconds, then transfer 1.0 mL of fluid, or other appropriate amount to a plastic sampule vial, or other appropriate container.

Return unused samples to freezer after extraction amounts have been removed.

Add 5.0 mL of reagent water to the 1.0 mL of fluid for a 1/6 dilution, or other dilution as determined by the analyst and documented in a note to file.

If a volume other than 1.0 mL is removed for an initial volume, adjust the water volume accordingly to maintain the same dilution as above.

11.3 Tissue and Fluid Sample Extraction

After tissue or fluid samples have been prepared according to sections 11.1 and 11.2, vortex mix or shake by hand the diluted/homogenized sample for approximately 15 seconds then transfer 1.0 mL, or other appropriate volume, to a clean 15 mL polypropylene centrifuge tube.

Return unused diluted/homogenized portions to the freezer after extraction amounts have been removed.

Record the volume removed on the extraction worksheet, (Attachment A or similar worksheet).

Spike blanks, samples, and standards, ready for extraction with surrogate standard as described in this method.

Spike each calibration standard matrix with the appropriate amount of standard as described in this method for the calibration curve standards and each QC sample.

Vortex mix the standard curve samples and QC samples for approximately 5 seconds.

To each sample and standard, add 5.0 mL of acetonitrile, cap, and vortex mix or shake by hand approximately 15 seconds.

Place all samples on the shaker at an appropriate speed for 20 minutes to adequately mix (a setting of approximately 300 rpm on the models listed in section 6.1).

Remove from the shaker and centrifuge at an appropriate speed for 10 minutes to adequately pellet the precipitate (a setting of approximately 2000 rpm on the models listed in section 6.1).

Add 40.0 mL of reagent grade water to a clean 50 mL centrifuge tube. Remove samples from the centrifuge and decant the supernatant into the water in the 50 mL tube, taking care not to introduce any of the matrix solids into the solution. Cap and mix by inverting several times. In this step the order of addition may be changed (i.e. the sample may be put into the centrifuge tube and then the water added).

Attach the reservoir to the SPE cartridge and attach this reservoir/cartridge unit to a vacuum manifold.

NOTE: When running the vacuum, set the vacuum chamber at approximately 15 kPA - to give an approximate elution flow of 5-7 mL/min. Flows may vary through cartridges and the kPA may be raised for slow tubes and drying after most have been drawn down.

Prepare the SPE cartridge by washing twice with approximately 5.0 mL of methanol, followed by approximately two 5.0 mL aliquots of water, **taking care not to allow the column to run to dryness after each wash**.

After washing is complete, pour the sample into the reservoir/cartridge unit and **allow all of the liquid to pass through the column to dryness**.

Run the vacuum on high for approximately 5 minutes to adequately dry each SPE cartridge.

Place a collection 15 mL polypropylene centrifuge tube under each cartridge and elute with 2.0 mL of methanol.

Spike extracted blanks, samples, and standards with internal standard as described in this method.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Label each glass autovial, as appropriate, with the study number, vial file archive number, animal number/gender/timepoint or LIMS number, matrix, final solvent, analyte components (if needed), extraction type, extraction date, and analyst(s) performing the extraction.

Transfer each eluant to a glass autovial and cap.

11.4 Extract Analysis

11.4.1 Software set-up

On the MassLynx main page, set up a sample list name. Save the list as instrument designator letter, last 2 digits of test year-month-day, and a letter that will increase through the alphabet with each additional list for that day.

Example Sample List: IYYMMDDa or A020204a

I = Initial of the instrument name (A = "Amelia")

YY = Test year (02)

MM = Test month (02)

DD = Test day (04)

a = First sample list (run) of the day (the next sample list will end with 'b', the next 'c',

and so on.)

Assign a filename using the instrument designator letter, the last 2 digits of the test year-month-day, and a 3-digit sequential file number that starts with 1 and increases by one for each filename.

Example filename: IYYMMDD### or A020204001

I = Initial of instrument name

YY = Test year

MM = Test month

DD = Test day

= 3-digit sequential file number starting with 1 through 999 (001)

Also, as part of the samplelist, assign a method (MS) for acquiring, an inlet file, a bottle number, an injection volume, and sample descriptions.

To create a method, click on Method Editor button in the MS Status Pane and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate mass(es). Also set the acquisition start and stop times. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx "Guide to Data Acquisition" for additional information on MRM.

Typically the analytical batch run sequence begins with system suitability, solvent blanks, and a set of extracted matrix standards.

Sample extracts are analyzed with two QC samples injected after every tenth sample injection. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered sample extracts but may be included as such.

11.4.2 HPLC set-up

Set up sample tray according to the sample list prepared above.

Set up the HPLC to the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook, or other appropriate location:

Sample size = 10 µL injection

Inject/sample = 1

Cycle time = 10.0 minutes

Flow rate = 300 µL/min

Mobile phase: Solvent A = 2 mM Ammonium Acetate, Solvent B = Methanol

Solvent gradient program:

Time	% Solvent B
0.00	10%
1.00	10%
5.50	95%
7.50	95%
8.00	10%

11.4.3 Instrument set-up

Refer to ETS-9-24, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source," for details.

Check the solvent level in HPLC reservoirs and refill if necessary.

Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

Turn on the nitrogen.

Open the tune page. Click on operate to initiate source block and desolvation heaters.

Open the Inlet Editor.

Download the HPLC method and initiate solvent flow to begin system equilibrium.

Set the flow to 10–500uL/min or as appropriate

Set HPLC pump to "On"

Observe droplets or mist coming out of the tip of the probe. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed

Allow to equilibrate for approximately 10 minutes.

Typical instrument parameters include:

Drying gas 250–400 liters/hour

ES nebulizing gas 10–15 liters/hour

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

HPLC constant flow mode, flow rate 10–500 µL/min

Pressure <400 bar (this parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

Source block temperature approximately 150°C

Desolvation temperature approximately 250°C

These settings may change in order to optimize the response

Print the tune page, sample list, and acquisition method from MassLynx and store it in the study binder with a copy taped into the instrument log.

Click on start button in the Acquisition Control Panel (the location of the start button may vary among MassLynx versions, refer to appropriate MassLynx User's Guide).

12 Data Analysis and Calculations

12.1 Calculations

If other calculations are used than those listed, they will be documented in the raw data.

Calculate the matrix amount contained in the initial dilution using the following equation:

$$\text{Matrix Amount (g/mL or mL/mL)} = \frac{\text{IW (g) (or IV (mL))}}{\text{(IW(g) (or IV(mL))+ DV (mL)}}$$

Calculate actual concentrations of analyte in calibration standards using the following equation:

$$\text{Concentration (ng/g or ng/mL)} = \frac{\text{Spike Concentration (ug/mL)} \times \text{Spiked Amount (mL)}}{\text{SV (mL)} \times \text{Matrix Amount (g/mL or mL/mL)}} \times \frac{1000\,\text{ng}}{1\,\text{ug}}$$

IW = Initial weight (where 1.0 g = 1.0 mL)

IV = Initial volume

DV = Diluent volume (reagent grade water)

SV = Sample volume removed for extraction (typically 1.0 mL)

AR = Analytical result from MassLynx summary

DF = Dilution factor

FV = Final volume

MA = Matrix amount

∂ curve = MA of tissue/fluid standard 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)

Calculate spike percent recoveries using the following equation:

$$\%\ \text{Recovery} = \frac{\text{Observed Result} - \text{Matrix Blank Result}}{\text{Spiking Level}} \times 100$$

Calculate relative standard deviation using the following equation:

$$\text{Relative Standard Deviation} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

Calculate percent deviation using the following equation:

$$\%\ \text{Deviation} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

Calculate actual concentration of analyte in fluid (µg/mL):

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

Calculate actual concentration of analyte in tissue (µg/g):

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

13 Method Performance

13.1 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.

If any item of the system suitability fails, system maintenance must be completed prior to running a second set of system suitability samples and the system suitability must pass before starting the calibration. If system suitability fails at the completion of a run, the sample set must be reanalyzed.

13.2 Quantitation

The coefficient of determination value for the calibration curve, plotted by regression using the peak areas of the analyte(s), must be 0.990 or better.

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.

If the curve cannot meet these criteria, the sample set must be reanalyzed or reextracted.

13.3 Accuracy

Two thirds of all quality control samples and 1/2 of each quality control sample at each level are expected to show an accuracy of 75-125%.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Surrogates and internal standards must have a percent deviation < 50%. Deviations outside this range will be reanalyzed to confirm. If the second analysis confirms the original, the deviation will be documented in the raw data. If the second analysis is within 50%, then the second value will replace the original value.

14 Pollution Prevention and Waste Management

Sample waste is disposed of in noninfectious biohazard waste containers.

Flammable solvent waste is disposed of in high BTU containers.

Glass pipette waste is disposed of in broken glass containers located in the laboratory.

15 Records

Complete the extraction worksheet attached to this method. or other applicable worksheet, and store with the study raw data.

Each page generated for a study must contain the following information (if applicable): study/project or instrument number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst. Other information may be added if applicable to the study.

Print the tune page, sample list, and acquisition method from MassLynx to include with the study raw data. Copy these pages and tape into the instrument runlog.

Plot the calibration curve by the appropriate regression. Print these graphs and store with the study raw data.

Print data integration summary, integration method, and chromatograms from MassLynx, and store with the study raw data.

Summarize data using suitable software (Excel 7.0 or LIMS) and store in the study folder.

Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

16 Attachments

Attachment A: Extraction Worksheet

Attachment B: Sample Weight/Volume Worksheet

Attachment C, Calibration Standard Concentration Worksheet

Attachment D, Dilutions Summary Worksheet

17 References

ETS-9-24, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source"

ETS-9-52, "Operation and Maintenance of a Tissue Grinder"

18 Affected Documents

None

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

19 Revisions

Revision Number	Revision Description	Revision Date
1	Minor formatting changes. Added detailed information to all sections concerning the extraction procedure, analytical procedure, and calculations. Added attachments and references.	02/18/02

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Attachment A – Extraction Worksheet

Study Number: Method Revision:ETS-8-231.1
Prep Date: Matrix:
Analysts initials: Sample Timepoint:
Box#:

Sample Number	Sample Number or description	Volume of diluted sample removed	Amount and spike mix used	Amount and surrogate spike mix used	Type of column used and lot	Elution solvent and volume	Comments

Blank matrix _____ TN-A-_____; Amount weighed/aliquoted: _____ g/mL

1. Homogenize sample
2. Aliquot 1 mL of diluted matrix into 15 mL polypropylene tube
3. Spike samples accordingly
4. Add ____ mL of ACN (TN-A-_____) to each diluted sample and shake or vortex mix
5. Shake sample for 20 min @ _____ rpm (Shaker _____)
6. Centrifuge sample for 10 min @ _____ rpm (Centrifuge _____)
7. Add 40 mL of _____ water to 50 mL polypropelene centrifuge tube.
8. Decant extract into centrifuge tubes with water
9. Shake sample slightly to ensure proper mixing
10. Attach 6 mL C18 SPE cartridges and 75 mL reservoirs to vacuum manifold
11. Condition column with two washes of ~5 mL MeOH (TN-A-_____) - do not allow column to go to dryness
12. Wash column with two washes of ~5 mL _____ water - do not allow column to go to dryness
13. Filter sample through conditioned column, discarding filtrate
14. Allow column to go to dryness. After dripping stops, draw a high vacuum through column for at least 5 minutes.
15. Elute column with solvent (_____TN-A-_____) into appropriate 15 mL centrifuge tube
16. Spike samples with _____ uL of internal standard # _____, conc._____
17. Transfer sample into appropriately labeled autovial and cap

Note: In vacuum steps above set the vacuum chamber at approximately 15 kPA - this should give approximately 5-7 mL/min elution flow
Flows may vary through cartridges - kPA may be raised for slow tubes and drying after most have been drawn down and shut off.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Attachment B - Sample Weight/Volume Worksheet

Prep Date(s):
Analyst(s):
Sample Matrix:
Method/Revision:

Study Number:
Equipment Number:
Final Solvent & TN Number:

Sample ID	Initial Wt./Vol. g/mL/L	Water Volume added (mL)	Volume Removed (mL)		Comments

Form Completion Verified By:_____

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Attachment C: Calibration Standard Concentration Worksheet

Prep date(s): **Standard number:**
Analyte(s): **Equipment number:**
Sample matrix: **Final solvent and TN:**
Method/revision: **Blank Tissue or Fluid/identifier:**

Analyte mix std approx. 0.500 ug/mL:
Analyte mix std approx. 5.00 ug/mL:
Analyte mix std approx. 50.0 ug/mL:
Surrogate std approx. 100 ug/mL:

Actual concentrations of standards in the analyte mix

Analyte Std conc ug/mL		**All** Am't spiked mL	**All** Final Volume: mL	**All** Initial Fluid Dilution mL/mL	**All** Initial Tissue Density g/mL
0.500		0.0015	2.00	0.1667	0.1600
0.500		0.0030	2.00	0.1667	0.1600
0.500		0.0080	2.00	0.1667	0.1600
0.500		0.0160	2.00	0.1667	0.1600
0.500		0.0320	2.00	0.1667	0.1600
5.00		0.0056	2.00	0.1667	0.1600
5.00		0.0080	2.00	0.1667	0.1600
5.00		0.0160	2.00	0.1667	0.1600
5.00		0.0240	2.00	0.1667	0.1600
5.00		0.0320	2.00	0.1667	0.1600
5.00		0.0400	2.00	0.1667	0.1600
50.0		0.005	2.00	0.1667	0.1600
50.0		0.006	2.00	0.1667	0.1600

Calculated concentrations of standards in relation to the final 2.0 mL solvent and initial matrix

2.0 mL Final Volume		**Fluid Matrix**		**Tissue Matrix**	
Analyte Final conc. ng/mL	**Surrogate** Std conc ng/mL	**Analyte** Final conc. ng/mL	**Surrogate** Std conc ng/mL	**Analyte** Final conc. ng/g	**Surrogate** Std conc ng/mL
0.375	100	5.00	100	5.00	100
0.750		10.0		10.0	
2.00	**Surrogate**	25.0	**Surrogate**	25.0	**Surrogate**
4.00	Final conc	50.0	Final conc	50.0	Final conc
8.00	ng/mL	100	ng/mL	100	ng/g
14.0	0.500	175	6500	175	6500
20.0		250		250	
40.0		500		500	
60.0		750		750	
80.0		1000		1000	
100		1250		1250	
125		1500		1500	
150		1750		1750	

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Attachment D: Dilutions Summary Worksheet

Study: **Solvent/TN Number:**
Dilution Date/Analyst: **Extraction Date/Analyst:**
Box Number: **Matrix/Timepoint:**

Sample Number or Description	Dilutions								
	1/	1/	1/	1/	1/	1/	1/	Comments	Verified By:

Notes:
1/10 dilution = _____ of sample + _____ of solvent

Form Completion Verified By: _____

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

ATTACHMENT B: DATA SUMMARY TABLES

Study number E01-1245 - QUAIL SERA DATA SUMMARY, BLANKS AND SPIKES

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (homoginate)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for control samples	Conc. of PFOS in Sample (ng/mL of Sera)
E01-1245-35766	1/31/2002	Water blank-day 1	d020207031	0.05	40	1	10.0	400	400
E01-1245-35825	2/1/2002	Blank H2O-day 2	d020203019	0.05	40	1	10.0	400	400
E01-1245-35825	2/1/2002	Blank H2O-day 2	d020207035	0.05	40	1	10.0	400	400
E01-1245-35767	1/31/2002	quail sera blank-day 1	d020207032	0.05	40	1	10.0	400	400
E01-1245-35826	2/1/2002	Blank quail sera-day 2	d020203020	0.05	40	1	10.0	400	400
E01-1245-35826	2/1/2002	Blank quail sera-day 2	d020207036	0.05	40	1	10.0	400	400

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (homoginate)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples	Conc. of PFOS in Sample (ng/mL of Sera)	% Rec	
E01-1245-35768	1/31/2002	quail Sera MS-day 1	d020207033	0.05	40	1	225.29	400	9012	89.76	**true value 10040**
E01-1245-35827	2/1/2002	quail Sera MS-day 2	d020203021	0.05	40	1	254.33	400	10173	101.3	**avg %rec 93.9**
E01-1245-35827	2/1/2002	quail Sera MS-day 2	d020207037	0.05	40	1	227.43	400	9097	90.61	**avg conc 9427**
											std dev of conc 647.4
E01-1245-35769	1/31/2002	quail Sera MSD-Day1	d020207034	0.05	40	1	217.03	400	8681	86.47	**true value 10040**
E01-1245-35828	2/1/2002	quail Sera MSD-Day2	d020203022	0.05	40	1	254.55	400	10182	101.4	**avg %rec 91.8**
E01-1245-35828	2/1/2002	quail Sera MSD-Day2	d020207038	0.05	40	1	219.54	400	8782	87.47	**avg conc 9215**
											std dev of conc 839.0

Conc of PFOS in Sample (ng/ml) = (Inst conc(ng/ml)) x (additional dilution) x (dilution(diluted sera))

dilution(diluted sera) = final volume(mL)/amount of sera in diluted sample(mL) = 2.0 mL/0.05 mL = 40
amount of sera in diluted sample = amount of sera used.

for both ms and msd

avg % rec 92.84
std dev of % rec 6.980
avg conc. 9321
std dev of conc. 680.2

Study number E01-1245 - QUAIL SERA

Absolute Recoveries

versus unextracted curve original

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples	Conc. of PFOS in Sample (ng/mL of Sera)		% Rec	
E01-1245-35779	01/31/2002	qc-25ppb-1, 01-31-02 (1004 ng/mL diluted sera)	d020213101	0.05	40	1	38.69	201	1548	A	154.1	**avg** 163.3
E01-1245-35780	01/31/2002	qc-25ppb-2, 01-31-02 (1004 ng/mL diluted sera)	d020213102	0.05	40	1	39.12	201	1565	A	155.9	**std dev** 14.46
E01-1245-35781	01/31/2002	qc-25ppb-3, 01-31-02 (1004 ng/mL diluted sera)	d020213103	0.05	40	1	45.18	201	1807	A	180.0	**%RSD** 8.853
E01-1245-36155	02/13/2002	ms-5ppb-1, 02-13-02 (201 ng/mL diluted sera)	d020213104	0.05	40	1	5.54	201	222		110.2	**avg** 108.0
E01-1245-36156	02/13/2002	ms-5ppb-2, 02-13-02 (201 ng/mL diluted sera)	d020213105	0.05	40	1	5.51	201	220		109.7	**std dev** 3.403
E01-1245-36157	02/13/2002	ms-5ppb-3, 02-13-02 (201 ng/mL diluted sera)	d020213106	0.05	40	1	5.23	201	209		104.1	**%RSD** 3.151
E01-1245-36158	02/13/2002	ms-200ppb-1, 02-13-02 (8032 ng/mL diluted sera)	d020213107	0.05	40	1	248.13	201	9925		123.6	**avg** 125.4
E01-1245-36159	02/13/2002	ms-200ppb-2, 02-13-02 (8032 ng/mL diluted sera)	d020213108	0.05	40	1	254.19	201	10168	A	126.6	**std dev** 1.636
E01-1245-36160	02/13/2002	ms-200ppb-3, 02-13-02 (8032 ng/mL diluted sera)	d020213109	0.05	40	1	253.36	201	10134	A	126.2	**%RSD** 1.304

for all in original analysis (absolute recovery)

avg %rec 132.3
std dev 25.62

Study number E01-1245 - QUAIL SERA

versus unextracted curve rerun

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of diluted sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples	Conc. of PFOS in Sample (ng/mL of Sera)		% Rec	
E01-1245-35779	01/31/2002	qc-25ppb-1, 01-31-02 (1004 ng/mL diluted sera)	d020405082	0.05	40	1	36.06	201	1442	A	143.7	**avg** 163.0
E01-1245-35780	01/31/2002	qc-25ppb-2, 01-31-02 (1004 ng/mL diluted sera)	d020405083	0.05	40	1	41.69	201	1668	A	166.1	**std dev** 17.97
E01-1245-35781	01/31/2002	qc-25ppb-3, 01-31-02 (1004 ng/mL diluted sera)	d020405084	0.05	40	1	44.98	201	1799	A	179.2	**%RSD** 11.03
E01-1245-36155	02/13/2002	ms-5ppb-1, 02-13-02 (201 ng/mL diluted sera)	d020405085	0.05	40	1	6.17	201	247		122.8	**avg** 120.9
E01-1245-36156	02/13/2002	ms-5ppb-2, 02-13-02 (201 ng/mL diluted sera)	d020405086	0.05	40	1	5.88	201	235		117.0	**std dev** 3.332
E01-1245-36157	02/13/2002	ms-5ppb-3, 02-13-02 (201 ng/mL diluted sera)	d020405087	0.05	40	1	6.17	201	247		122.8	**%RSD** 2.757
E01-1245-36158	02/13/2002	ms-200ppb-1, 02-13-02 (8032 ng/mL diluted sera)	d020405088	0.05	40	1	248.12	201	9925		123.6	**avg** 126.7
E01-1245-36159	02/13/2002	ms-200ppb-2, 02-13-02 (8032 ng/mL diluted sera)	d020405089	0.05	40	1	260.27	201	10411	A	129.6	**std dev** 3.027
E01-1245-36160	02/13/2002	ms-200ppb-3, 02-13-02 (8032 ng/mL diluted sera)	d020405090	0.05	40	1	254.58	201	10183	A	126.8	**%RSD** 2.390

A = Not within +/-25% criteria

for all in rerun analysis (absolute recovery)

avg %rec 136.8
std dev 21.84

Conc of PFOS in Sample (ng/ml) = (Inst conc(ng/ml)) x (additional dilution) x (dilution(diluted sera))

dilution(diluted sera) = final volume(mL)/amount of sera in diluted sample(mL) = 2.0 mL/0.05 mL = 40
amount of sera in diluted sample = amount of sera used.

Study number E01-1245 - QUAIL SERA

QC Sample Recoveries

versus extracted curve

original of all and rerun of 80ppm

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples	Conc. of PFOS in Sample (ng/mL of Sera)		% Rec	
E01-1245-35785	01/31/2002	qc-80ppm-1, 01-31-02 (3227040 ng/mL diluted sera)	d020201035	0.05	40	1000	95.65	400	3826000		118.6	**avg** 113.6
E01-1245-35786	01/31/2002	qc-80ppm-2, 01-31-02 (3227040 ng/mL diluted sera)	d020201036	0.05	40	1000	93.45	400	3738000		115.8	**std dev** 6.444
E01-1245-35787	01/31/2002	qc-80ppm-2, 01-31-02 (3227040 ng/mL diluted sera)	d020201037	0.05	40	1000	85.75	400	3430000		106.3	**%RSD** 5.674
E01-1245-35779	01/31/2002	qc-25ppb-1, 01-31-02 (1004 ng/mL diluted sera)	d020207044	0.05	40	1	26.99	400	1080		107.5	**avg** 114.7
E01-1245-35780	01/31/2002	qc-25ppb-2, 01-31-02 (1004 ng/mL diluted sera)	d020207045	0.05	40	1	27.89	400	1116		111.1	**std dev** 9.555
E01-1245-35781	01/31/2002	qc-25ppb-2, 01-31-02 (1004 ng/mL diluted sera)	d020207046	0.05	40	1	31.52	400	1261	A	125.6	**%RSD** 8.327
E01-1245-35782	01/31/2002	qc-40ppm-1, 01-31-02 (1613520 ng/mL diluted sera)	d020207047	0.05	40	100	299.0	400	1196120	A	74.13	**avg** 76.16
E01-1245-35783	01/31/2002	qc-40ppm-2, 01-31-02 (1613520 ng/mL diluted sera)	d020207048	0.05	40	100	316.2	400	1264880		78.39	**std dev** 2.138
E01-1245-35784	01/31/2002	qc-40ppm-2, 01-31-02 (1613520 ng/mL diluted sera)	d020207049	0.05	40	100	306.4	400	1225440		75.95	**%RSD** 2.808
E01-1245-35785	01/31/2002	qc-80ppm-1, 01-31-02 (3227040 ng/mL diluted sera)	d020207050	0.05	40	1000	88.66	400	3546400		109.9	**avg** 104.9
E01-1245-35786	01/31/2002	qc-80ppm-2, 01-31-02 (3227040 ng/mL diluted sera)	d020207051	0.05	40	1000	85.99	400	3439600		106.6	**std dev** 5.956
E01-1245-35787	01/31/2002	qc-80ppm-2, 01-31-02 (3227040 ng/mL diluted sera)	d020207052	0.05	40	1000	79.33	400	3173200		98.33	**%RSD** 5.676

for all in original analysis (recovery)

avg %rec 101.5
std dev 19.89

Study number E01-1245 - QUAIL SERA

versus extracted curve

rerun

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples	Conc. of PFOS in Sample (ng/mL of Sera)		% Rec	
E01-1245-35779	01/31/2002	qc-25ppb-1, 01-31-02 (1004 ng/mL diluted sera)	d020405082	0.05	40	1	24.57	201	983		97.89	**avg** 111.3
E01-1245-35780	01/31/2002	qc-25ppb-2, 01-31-02 (1004 ng/mL diluted sera)	d020405083	0.05	40	1	28.49	201	1140		113.5	**std dev** 12.51
E01-1245-35781	01/31/2002	qc-25ppb-3, 01-31-02 (1004 ng/mL diluted sera)	d020405084	0.05	40	1	30.78	201	1231		122.6	**%RSD** 11.24
E01-1245-36155	02/13/2002	ms-5ppb-1, 02-13-02 (201 ng/mL diluted sera)	d020405085	0.05	40	1	3.85	201	154		76.62	**avg** 75.22
E01-1245-36156	02/13/2002	ms-5ppb-2, 02-13-02 (201 ng/mL diluted sera)	d020405086	0.05	40	1	3.65	201	146	A	72.64	**std dev** 2.243
E01-1245-36157	02/13/2002	ms-5ppb-3, 02-13-02 (201 ng/mL diluted sera)	d020405087	0.05	40	1	3.84	201	154		76.42	**%RSD** 2.981
E01-1245-36158	02/13/2002	ms-200ppb-1, 02-13-02 (8032 ng/mL diluted sera)	d020405088	0.05	40	1	177.0	201	7080		88.15	**avg** 90.48
E01-1245-36159	02/13/2002	ms-200ppb-2, 02-13-02 (8032 ng/mL diluted sera)	d020405089	0.05	40	1	186.2	201	7447		92.72	**std dev** 2.285
E01-1245-36160	02/13/2002	ms-200ppb-3, 02-13-02 (8032 ng/mL diluted sera)	d020405090	0.05	40	1	181.9	201	7275		90.58	**%RSD** 2.525
E01-1245-36155	02/13/2002	ms-5ppb-1, 02-13-02 (201 ng/mL diluted sera)	d020410019	0.05	40	1	5.19	100	208		103.3	**avg** 95.85
E01-1245-36156	02/13/2002	ms-5ppb-2, 02-13-02 (201 ng/mL diluted sera)	d020410020	0.05	40	1	4.64	100	186		92.34	**std dev** 6.437
E01-1245-36157	02/13/2002	ms-5ppb-3, 02-13-02 (201 ng/mL diluted sera)	d020410021	0.05	40	1	4.62	100	185		91.94	**%RSD** 6.716

A = Not within +/-25% criteria

for all in rerun analysis (recovery)

avg %rec 95.57
std dev 14.18

Conc of PFOS in Sample (ng/ml) = (Inst conc(ng/ml)) x (additional dilution) x (dilution(diluted sera))

dilution(diluted sera) = final volume(mL)/amount of sera in diluted sample(mL) = 2.0 mL/0.05 mL = 40
amount of sera in diluted sample = amount of sera used.

Study number E01-1245 - QUAIL SERA

	3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/mL of Sera)	Conc. of PFOS in Sample (ng/mL of Sera)		average of dup. samples		
*	E01-1245-28444	06/15/2001	454-108-330, 0ppm female, adult, 01-31-02	d020131055	0.4	40	1	10.0	400	400	+	400		
				d020207059	0.4	40	1	10.0	400	400	+			
*	E01-1245-28446	06/15/2001	454-108-332, 0ppm female, adult, 01-31-02	d020131057	0.05	40	1	10.0	400	400	+	400		
				d020207061	0.05	40	1	10.0	400	400	+			
*	E01-1245-28449	06/15/2001	454-108-336, 0ppm female, adult, 01-31-02	d020131060	0.3	40	1	10.0	400	400	+	400		
				d020207064	0.3	40	1	10.0	400	400	+			
*	E01-1245-28451	06/15/2001	454-108-338, 0ppm female, adult, 01-31-02	d020131062	0.3	40	1	10.0	400	400	+	400		
				d020207066	0.3	40	1	10.0	400	400	+			
*	E01-1245-28453	06/15/2001	454-108-340, 0ppm female, adult, 01-31-02	d020131069	0.2	40	1	10.0	400	400	+	400		
				d020207073	0.2	40	1	10.0	400	400	+			
*	E01-1245-28456	06/15/2001	454-108-344, 0ppm female, adult, 01-31-02	d020131072	0.2	40	1	10.0	400	400	+	400		
				d020207076	0.2	40	1	10.0	400	400	+			
*	E01-1245-28458	06/15/2001	454-108-346, 0ppm female, adult, 02-01-02	d020201043	0.1	40	1	10.0	400	400	+	400		
				d020207087	0.1	40	1	10.0	400	400	+			
*	E01-1245-28460	06/15/2001	454-108-348, 0ppm female, adult, 02-01-02	d020201045	0.3	40	1	10.0	400	400	+	400		
				d020207089	0.3	40	1	10.0	400	400	+			
*	E01-1245-28462	06/15/2001	454-108-350, 0ppm female, adult, 02-01-02	d020201047	0.4	40	1	10.0	400	400	+	400		
				d020207091	0.4	40	1	10.0	400	400	+			
*	E01-1245-28464	06/15/2001	454-108-352, 0ppm female, adult, 02-01-02	d020201048	0.4	40	1	10.0	400	400	+	400		
				d020207092	0.4	40	1	10.0	400	400	+			
*	E01-1245-28466	06/15/2001	454-108-354, 0ppm female, adult, 02-01-02	d020201050	0.3	40	1	10.0	400	400	+	400		
				d020207094	0.3	40	1	10.0	400	400	+			
*	E01-1245-28468	06/15/2001	454-108-356, 0ppm female, adult, 02-01-02	d020201052	0.3	40	1	10.0	400	400	+	400		
				d020207096	0.3	40	1	10.0	400	400	+			
*	E01-1245-28470	06/15/2001	454-108-358, 0ppm female, adult, 02-01-02	d020201059	0.3	40	1	10.0	400	400	+	400		
				d020207103	0.3	40	1	10.0	400	400	+			**With outliers**
*	E01-1245-28472	06/15/2001	454-108-360, 0ppm female, adult, 02-01-02	d020201061	0.3	40	1	51.27	400	2051		2114	*rejected*	**avg 500.8**
				d020207105	0.3	40	1	54.43	400	2177			*rejected*	**std dev 409.7**
*	E01-1245-28474	06/15/2001	454-108-362, 0ppm female, adult, 02-01-02	d020201063	0.2	40	1	10.0	400	400	+	400		**%RSD 81.80**
				d020207107	0.2	40	1	10.0	400	400	+			
*	E01-1245-28477	06/15/2001	454-108-366, 0ppm female, adult, 02-01-02	d020201066	0.2	40	1	10.0	400	400	+	400		**Without outliers**
				d020207110	0.2	40	1	10.0	400	400	+			**avg 400**
*	E01-1245-28479	06/15/2001	454-108-368, 0ppm female, adult, 02-01-02	d020201073	0.3	40	1	10.0	400	400	+	400		**std dev 0**
				d020207117	0.3	40	1	10.0	400	400	+			**%RSD -**

+ Concentration <LOQ

* There are two results reported for these samples. Both values are included in the average

Conc of PFOS in Sample (ng/ml) = (Inst conc(ng/ml)) x (additional dilution) x (dilution(diluted sera))

dilution(diluted sera) = final volume(mL)/amount of sera in diluted sample(mL) = 2.0 mL/0.05 mL = 40
amount of sera in diluted sample = amount of sera used/total amount of sera + water. For all samples >0.05 mL this value is 0.05 mL. For samples <0.05 mL this value is = to the amount of sera used.
For example, sample E01-1245-28519- dilution(diluted sera)= 2.0 mL final volume/0.02 mL amount of sera used = 100

Study number E01-1245 - QUAIL SERA

	3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/mL of Sera)	Conc. of PFOS in Sample (ng/mL of Sera)		average of dup. samples		
	E01-1245-28481	06/15/2001	454-108-370, 10ppm female, adult, 02-01-02	d020203026	0.3	40	1	229.24	400	9170				
	E01-1245-28483	06/15/2001	454-108-372, 10ppm female, adult, 02-01-02	d020203027	0.4	40	1	228.41	400	9136				
	E01-1245-28485	06/15/2001	454-108-374, 10ppm female, adult, 02-01-02	d020203028	0.2	40	1	210.00	400	8400				
	E01-1245-28487	06/15/2001	454-108-376, 10ppm female, adult, 02-01-02	d020203029	0.3	40	1	231.92	400	9277				
	E01-1245-28489	06/15/2001	454-108-378, 10ppm female, adult, 02-01-02	d020203030	0.1	40	1	124.46	400	4978				
	E01-1245-28491	06/15/2001	454-108-380, 10ppm female, adult, 02-01-02	d020203031	0.1	40	1	144.86	400	5794				
	E01-1245-28493	06/15/2001	454-108-382, 10ppm female, adult, 02-01-02	d020416019	0.3	40	10	79.81	201	31924			*rejected*	
	E01-1245-28496	06/15/2001	454-108-386, 10ppm female, adult, 02-01-02	d020203033	0.4	40	1	367.26	400	14690				
	E01-1245-28498	06/15/2001	454-108-388, 10ppm female, adult, 02-01-02	d020203034	0.3	40	1	289.04	400	11562				
	E01-1245-28500	06/15/2001	454-108-390, 10ppm female, adult, 02-01-02	d020203038	0.2	40	1	157.64	400	6306				**With outliers**
	E01-1245-28502	06/15/2001	454-108-392, 10ppm female, adult, 02-01-02	d020203039	0.3	40	1	235.08	400	9403				**avg 9990**
	E01-1245-28504	06/15/2001	454-108-394, 10ppm female, adult, 02-01-02	d020203040	0.3	40	1	284.92	400	11397				**std dev 6010**
	E01-1245-28506	06/15/2001	454-108-396, 10ppm female, adult, 02-01-02	d020203041	0.09	40	1	117.84	400	4714				**%RSD 60.2**
	E01-1245-28508	06/15/2001	454-108-398, 10ppm female, adult, 02-01-02	d020203042	0.1	40	1	261.76	400	10470				
	E01-1245-28511	06/15/2001	454-108-402, 10ppm female, adult, 02-01-02	d020203043	0.4	40	1	167.91	400	6716				**Without outliers**
	E01-1245-28513	06/15/2001	454-108-404, 10ppm female, adult, 02-01-02	d020203044	0.1	40	1	226.40	400	9056				**avg 8699**
	E01-1245-28515	06/15/2001	454-108-406, 10ppm female, adult, 02-01-02	d020203045	0.2	40	1	224.49	400	8980				**std dev 2557**
	E01-1245-28517	06/15/2001	454-108-408, 10ppm female, adult, 02-01-02	d020203046	0.2	40	1	196.03	400	7841				**%RSD 29.4**
*	E01-1245-28518	07/23/2001	454-108-246, 0ppm female, offspring, 02-01-02	d020201132	0.1	40	1	10.0	400	400	+	400		
				d020207153	0.1	40	1	10.0	400	400	+			
*	E01-1245-28521	07/23/2001	454-108-259, 0ppm female, offspring, 02-01-02	d020201135	0.1	40	1	10.0	400	400	+	400		
				d020207156	0.1	40	1	10.0	400	400	+			
*	E01-1245-28524	07/23/2001	454-108-273, 0ppm female, offspring, 02-01-02	d020201138	0.08	40	1	10.0	400	400	+	400		
				d020207159	0.08	40	1	10.0	400	400	+			**avg 400**
*	E01-1245-28525	07/23/2001	454-108-277, 0ppm female, offspring, 02-01-02	d020201139	0.2	40	1	10.0	400	400	+	400		**std dev 0**
				d020207160	0.2	40	1	10.0	400	400	+			**%RSD -**
*	E01-1245-28528	07/23/2001	454-108-517, 10ppm female, offspring, 02-01-02	d020201147	0.02	100	1	185.25	400	18525		17566		
				d020207168	0.02	100	1	166.06	400	16606				
*	E01-1245-28530	07/23/2001	454-108-525, 10ppm female, offspring, 02-01-02	d020201149	0.03	66.67	1	192.74	400	12849		12452		
				d020207170	0.03	66.67	1	180.81	400	12054				
*	E01-1245-28532	07/23/2001	454-108-538, 10ppm female, offspring, 02-01-02	d020201151	0.08	40	1	365.44	400	14618		13405		
				d020207172	0.08	40	1	304.83	400	12193				
*	E01-1245-28535	07/23/2001	454-108-557, 10ppm female, offspring, 02-01-02	d020201154	0.06	40	1	208.24	400	8330		7897		
				d020207175	0.06	40	1	186.59	400	7464				**avg of dups 12423**
*	E01-1245-28536	07/23/2001	454-108-561, 10ppm female, offspring, 02-01-02	d020201155	0.05	40	1	287.26	400	11490		10793		**std dev 3457**
				d020207176	0.05	40	1	252.41	400	10096				**%RSD 27.8**

+ Concentration <LOQ

* There are two results reported for these samples. Both values are included in the average

Conc of PFOS in Sample (ng/ml) = (Inst conc(ng/ml)) x (additional dilution) x (dilution(diluted sera))

dilution(diluted sera) = final volume(mL)/amount of sera in diluted sample(mL) = 2.0 mL/0.05 mL = 40

amount of sera in diluted sample = amount of sera used/total amount of sera + water. For all samples >0.05 mL this value is 0.05 mL. For samples <0.05 mL this value is = to the amount of sera used.

For example, sample E01-1245-28519- dilution(diluted sera)= 2.0 mL final volume/0.02 mL amount of sera used = 100

Study number E01-1245 - QUAIL SERA

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/mL of Sera)	Conc. of PFOS in Sample (ng/mL of Sera)	average of dup. samples
* E01-1245-28443	06/15/2001	454-108-329, 0ppm male, adult, 01-31-02	d020131054	0.2	40	1	10.0	400	400 +	400
			d020207058	0.2	40	1	10.0	400	400 +	
* E01-1245-28445	06/15/2001	454-108-331, 0ppm male, adult, 01-31-02	d020131056	0.4	40	1	10.0	400	400 +	400
			d020207060	0.4	40	1	10.0	400	400 +	
* E01-1245-28447	06/15/2001	454-108-333, 0ppm male, adult, 01-31-02	d020131058	0.4	40	1	10.0	400	400 +	400
			d020207062	0.4	40	1	10.0	400	400 +	
* E01-1245-28448	06/15/2001	454-108-335, 0ppm male, adult, 01-31-02	d020131059	0.2	40	1	10.0	400	400 +	400
			d020207063	0.2	40	1	10.0	400	400 +	
* E01-1245-28450	06/15/2001	454-108-337, 0ppm male, adult, 01-31-02	d020131061	0.1	40	1	10.0	400	400 +	400
			d020207065	0.1	40	1	10.0	400	400 +	
* E01-1245-28452	06/15/2001	454-108-339, 0ppm male, adult, 01-31-02	d020131063	0.2	40	1	10.0	400	400 +	400
			d020207067	0.2	40	1	10.0	400	400 +	
* E01-1245-28454	06/15/2001	454-108-341, 0ppm male, adult, 01-31-02	d020131070	0.2	40	1	10.0	400	400 +	400
			d020207074	0.2	40	1	10.0	400	400 +	
* E01-1245-28455	06/15/2001	454-108-343, 0ppm male, adult, 01-31-02	d020131071	0.1	40	1	10.0	400	400 +	400
			d020207075	0.1	40	1	10.0	400	400 +	
* E01-1245-28457	06/15/2001	454-108-345, 0ppm male, adult, 01-31-02	d020131073	0.2	40	1	10.0	400	400 +	400
			d020207077	0.2	40	1	10.0	400	400 +	
* E01-1245-28459	06/15/2001	454-108-347, 0ppm male, adult, 02-01-02	d020201044	0.2	40	1	10.0	400	400 +	400
			d020207088	0.2	40	1	10.0	400	400 +	
* E01-1245-28461	06/15/2001	454-108-349, 0ppm male, adult, 02-01-02	d020201046	0.02	100	1	10.0	400	400 +	400
			d020207090	0.02	100	1	10.0	400	400 +	
* E01-1245-28465	06/15/2001	454-108-353, 0ppm male, adult, 02-01-02	d020201049	0.3	40	1	10.0	400	400 +	400
			d020207093	0.3	40	1	10.0	400	400 +	
* E01-1245-28467	06/15/2001	454-108-355, 0ppm male, adult, 02-01-02	d020201051	0.2	40	1	10.0	400	400 +	400
			d020207095	0.2	40	1	10.0	400	400 +	
* E01-1245-28469	06/15/2001	454-108-357, 0ppm male, adult, 02-01-02	d020201058	0.07	40	1	10.0	400	400 +	400
			d020207102	0.07	40	1	10.0	400	400 +	
* E01-1245-28471	06/15/2001	454-108-359, 0ppm male, adult, 02-01-02	d020201060	0.2	40	1	10.0	400	400 +	400
			d020207104	0.2	40	1	10.0	400	400 +	
* E01-1245-28473	06/15/2001	454-108-361, 0ppm male, adult, 02-01-02	d020201062	0.2	40	1	10.0	400	400 +	400
			d020207106	0.2	40	1	10.0	400	400 +	
* E01-1245-28475	06/15/2001	454-108-363, 0ppm male, adult, 02-01-02	d020201064	0.1	40	1	10.0	400	400 +	400
			d020207108	0.1	40	1	10.0	400	400 +	
* E01-1245-28476	06/15/2001	454-108-365, 0ppm male, adult, 02-01-02	d020201006	0.4	40	1	10.0	400	400 +	400
			d020207109	0.4	40	1	10.0	400	400 +	
* E01-1245-28478	06/15/2001	454-108-367, 0ppm male, adult, 02-01-02	d020201067	0.1	40	1	10.0	400	400 +	400
			d020207111	0.1	40	1	10.0	400	400 +	

avg 400
std dev 0
%RSD -

+ Concentration <LOQ

* There are two results reported for these samples. Both values are included in the average

Conc of PFOS in Sample (ng/ml) = (Inst conc(ng/ml)) x (additional dilution) x (dilution(diluted sera))

dilution(diluted sera) = final volume(mL)/amount of sera in diluted sample(mL) = 2.0 mL/0.05 mL = 40
amount of sera in diluted sample = amount of sera used/total amount of sera + water. For all samples >0.05 mL this value is 0.05 mL. For samples <0.05 mL this value is = to the amount of sera used.
For example, sample E01-1245-28519- dilution(diluted sera)= 2.0 mL final volume/0.02 mL amount of sera used = 100

Study number E01-1245 - QUAIL SERA

	3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/mL of Sera)	Conc. of PFOS in Sample (ng/mL of Sera)	average of dup. samples	
*	E01-1245-28480	06/15/2001	454-108-369, 10ppm male, adult, d100,02-01-02	d020201074	0.2	40	100	26.73	400	106920	111860	
				d020207118	0.2	40	100	29.20	400	116800		
*	E01-1245-28482	06/15/2001	454-108-371, 10ppm male, adult, d100,02-01-02	d020201076	0.2	40	100	45.31	400	181240	180340	
				d020207119	0.2	40	100	44.86	400	179440		
*	E01-1245-28484	06/15/2001	454-108-373, 10ppm male, adult, d100,02-01-02	d020201078	0.1	40	100	35.90	400	143600	150060	
				d020207120	0.1	40	100	39.13	400	156520		
*	E01-1245-28486	06/15/2001	454-108-375, 10ppm male, adult, d100,02-01-02	d020201080	0.3	40	100	34.52	400	138080	138000	
				d020207121	0.3	40	100	34.48	400	137920		
*	E01-1245-28488	06/15/2001	454-108-377, 10ppm male, adult, d100,02-01-02	d020201082	0.07	40	100	23.60	400	94400	95260	
				d020207122	0.07	40	100	24.03	400	96120		
*	E01-1245-28490	06/15/2001	454-108-379, 10ppm male, adult, d100,02-01-02	d020201089	0.1	40	100	37.59	400	150360	158420	
				d020207128	0.1	40	100	41.62	400	166480		
•	E01-1245-28492	06/15/2001	454-108-381, 10ppm male, adult, d100,02-01-02	d020201091	0.1	40	100	35.26	400	141040	144780	
				d020207129	0.1	40	100	37.13	400	148520		
*	E01-1245-28494	06/15/2001	454-108-383, 10ppm male, adult, d100,02-01-02	d020201093	0.3	40	100	36.65	400	146600	151420	
				d020207130	0.3	40	100	39.06	400	156240		
*	E01-1245-28495	06/15/2001	454-108-385, 10ppm male, adult, d100,02-01-02	d020201094	0.1	40	100	29.64	400	118560	120640	
				d020207131	0.1	40	100	30.68	400	122720		
*	E01-1245-28497	06/15/2001	454-108-387, 10ppm male, adult, d100,02-01-02	d020201096	0.3	40	100	43.62	400	174480	185280	
				d020207132	0.3	40	100	49.02	400	196080		
*	E01-1245-28499	06/15/2001	454-108-389, 10ppm male, adult, d100,02-01-02	d020201103	0.1	40	100	32.26	400	129040	132340	
				d020207133	0.1	40	100	33.91	400	135640		
•	E01-1245-28501	06/15/2001	454-108-391, 10ppm male, adult, d100,02-01-02	d020201105	0.06	40	100	35.28	400	141120	144000	
				d020207134	0.06	40	100	36.72	400	146880		
*	E01-1245-28503	06/15/2001	454-108-393, 10ppm male, adult, d100,02-01-02	d020201107	0.2	40	100	40.06	400	160240	162440	
				d020207135	0.2	40	100	41.16	400	164640		
*	E01-1245-28505	06/15/2001	454-108-395, 10ppm male, adult, d100,02-01-02	d020201109	0.2	40	100	31.80	400	127200	131460	
				d020207136	0.2	40	100	33.93	400	135720		
*	E01-1245-28507	06/15/2001	454-108-397, 10ppm male, adult, d100,02-01-02	d020201111	0.3	40	100	24.90	400	99600	103060	
				d020207137	0.3	40	100	26.63	400	106520		
•	E01-1245-28509	06/15/2001	454-108-399, 10ppm male, adult, d100,02-01-02	d020201118	0.1	40	100	34.45	400	137800	148200	
				d020207143	0.1	40	100	39.65	400	158600		
*	E01-1245-28510	06/15/2001	454-108-401, 10ppm male, adult, d100,02-01-02	d020201119	0.1	40	100	50.85	400	203400	214900	
				d020207144	0.1	40	100	56.60	400	226400		
*	E01-1245-28512	06/15/2001	454-108-403, 10ppm male, adult, d100,02-01-02	d020201121	0.2	40	100	31.64	400	126560	132060	
				d020207145	0.2	40	100	34.39	400	137560		
*	E01-1245-28514	06/15/2001	454-108-405, 10ppm male, adult, d100,02-01-02	d020201123	0.1	40	100	26.94	400	107760	112340	
				d020207146	0.1	40	100	29.23	400	116920		**avg of dups 141112**
•	E01-1245-28516	06/15/2001	454-108-407, 10ppm male, adult, d100,02-01-02	d020201125	0.1	40	100	24.79	400	99160	105380	**std dev 30225**
				d020207147	0.1	40	100	27.90	400	111600		**%RSD 21.4**

* There are two results reported for these samples. Both values are included in the average

Conc of PFOS in Sample (ng/ml) = (Inst conc(ng/ml)) x (additional dilution) x (dilution(diluted sera))

dilution(diluted sera) = final volume(mL)/amount of sera in diluted sample(mL) = 2.0 mL/0.05 mL = 40

amount of sera in diluted sample = amount of sera used/total amount of sera + water. For all samples >0.05 mL this value is 0.05 mL. For samples <0.05 mL this value is = to the amount of sera used.

For example, sample E01-1245-28519- dilution(diluted sera)= 2.0 mL final volume/0.02 mL amount of sera used = 100

Study number E01-1245 - QUAIL SERA

	3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/mL of Sera)	Conc. of PFOS in Sample (ng/mL of Sera)		average of dup. samples	
*	E01-1245-28519	07/23/2001	454-108-253, 0ppm male, offspring, 02-01-02	d020201133	0.02	100	1	10.0	400	400	+	400	
				d020207154	0.02	100	1	10.0	400	400	+		
*	E01-1245-28520	07/23/2001	454-108-256, 0ppm male, offspring, 02-01-02	d020201134	0.02	100	1	10.0	400	400	+	400	
				d020207155	0.02	100	1	10.0	400	400	+		
*	E01-1245-28522	07/23/2001	454-108-266, 0ppm male, offspring, 02-01-02	d020201136	0.07	40	1	10.0	400	400	+	400	
				d020207157	0.07	40	1	10.0	400	400	+		
*	E01-1245-28523	07/23/2001	454-108-270, 0ppm male, offspring, 02-01-02	d020201137	0.2	40	1	10.0	400	400	+	400	
				d020207158	0.2	40	1	10.0	400	400	+		
•	E01-1245-28526	07/23/2001	454-108-283, 0ppm male, offspring, 02-01-02	d020201140	0.02	100	1	10.0	400	400	+	400	
				d020207161	0.02	100	1	10.0	400	400	+		**avg 400**
*	E01-1245-28527	07/23/2001	454-108-291, 0ppm male, offspring, 02-01-02	d020201141	0.05	40	1	10.0	400	400	+	400	**std dev 0**
				d020207162	0.05	40	1	10.0	400	400	+		**%RSD -**
	E01-1245-28529	07/23/2001	454-108-522, 10ppm male, offspring, 02-01-02	d020405091	0.07	40	10	48.13	201	19252		19252	
*	E01-1245-28531	07/23/2001	454-108-533, 10ppm male, offspring, 02-01-02	d020201150	0.07	40	1	232.67	400	9307		8761	
				d020207171	0.07	40	1	205.40	400	8216			
*	E01-1245-28533	07/23/2001	454-108-545, 10ppm male, offspring, 02-01-02	d020201152	0.04	50	1	309.79	400	15490		14558	
				d020207173	0.04	50	1	272.51	400	13626			
*	E01-1245-28534	07/23/2001	454-108-552, 10ppm male, offspring, 02-01-02	d020201153	0.07	40	1	339.60	400	13584		12521	
				d020207174	0.07	40	1	286.47	400	11459			**avg 12601**
*	E01-1245-28537	07/23/2001	454-108-568, 10ppm male, offspring, 02-01-02	d020201156	0.1	40	1	301.89	400	12076		11239	**std dev 3374**
				d020207177	0.1	40	1	260.08	400	10403			**%RSD 26.8**

+ Concentration <LOQ

* There are two results reported for these samples. Both values are included in the average

Conc of PFOS in Sample (ng/ml) = (Inst conc(ng/ml)) x (additional dilution) x (dilution(diluted sera))

dilution(diluted sera) = final volume(mL)/amount of sera in diluted sample(mL) = 2.0 mL/0.05 mL = 40

amount of sera in diluted sample = amount of sera used/total amount of sera + water. For all samples >0.05 mL this value is 0.05 mL. For samples <0.05 mL this value is = to the amount of sera used.

For example, sample E01-1245-28519- dilution(diluted sera)= 2.0 mL final volume/0.02 mL amount of sera used = 100

Study number E01-1245 - QUAIL LIVER DATA SUMMARY, BLANKS AND SPIKES

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of liver weighed (g) or water aliquoted (mL)	Water Added for Homgenizing (mL)	Final Volume (mL)	Amount of liver in diluted sample (g) or water (mL)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/g)	Conc. of PFOS in Sample (ng/g of Liver)	
E01-1342-36000	02/11/2002	Blank H2O-day 1	d020211037	1.0000	9.00	2.00	0.100000	1	2.51	50.2	50.2	*
E01-1342-36026	02/12/2002	Blank H2O-day 2	d020212025	1.0000	9.00	2.00	0.100000	1	2.51	50.2	50.2	*
E01-1342-36031	02/13/2002	Blank H2O-day 3	d020301024	1.0000	9.00	2.00	0.100000	1	0.502	10.0	10.0	*
E01-1342-36036	02/15/2002	Blank H2O-day 4	d020218025	1.0000	9.00	2.00	0.100000	1	1.00	20.0	20.0	*
E01-1342-36001	02/11/2002	Blank female liver-day 1	d020211038	5.0353	45.0	2.00	0.100635	1	2.51	50.2	50.2	*
E01-1342-36027	02/12/2002	Blank female liver-day 2	d020212026	5.0353	45.0	2.00	0.100635	1	2.51	50.2	50.2	*
E01-1342-36032	02/13/2002	Blank female liver-day 3	d020301025	5.0353	45.0	2.00	0.100635	1	0.502	10.0	10.0	*
E01-1342-36037	02/15/2002	Blank female liver-day 4	d020218026	5.0353	45.0	2.00	0.100635	1	1.00	20.0	20.0	*
E01-1342-36002	02/11/2002	Blank male liver-day 1	d020211039	1.0015	9.00	2.00	0.100135	1	2.51	50.2	50.2	*
E01-1342-36028	02/12/2002	Blank male liver-day 2	d020212027	1.0015	9.00	2.00	0.100135	1	2.51	50.2	50.2	*
E01-1342-36033	02/13/2002	Blank male liver-day 3	d020301026	1.0015	9.00	2.00	0.100135	1	0.502	10.0	10.0	*
E01-1342-36038	02/15/2002	Blank male liver-day 4	d020218027	1.0015	9.00	2.00	0.100135	1	1.00	20.0	20.0	*

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of liver weighed (g)	Water Added for Homgenizing (mL)	Final Volume (mL)	Amount of liver in diluted sample (g)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/g)	Conc. of PFOS in Sample (ng/g of Liver)	% Rec	
E01-1342-36003	02/11/2002	Female liver MS-day 1	d020211040	5.0353	45.0	2.00	0.100635	1	29.01	50.2	577	115.5	**true val. 499**
E01-1342-36029	02/12/2002	Female liver MS-day 2	d020212028	5.0353	45.0	2.00	0.100635	1	28.77	50.2	572	114.6	**avg %rec 109.7**
E01-1342-36034	02/13/2002	Female liver MS-day 3	d020301027	5.0353	45.0	2.00	0.100635	1	26.03	10.0	517	103.7	**avg conc 547.2**
E01-1342-36039	02/15/2002	Female liver MS-day 4	d020218028	5.0353	45.0	2.00	0.100635	1	26.33	20.0	523	104.9	**std dev of conc 31.25**
E01-1342-36004	02/11/2002	Female liver MSD-day 1	d020211041	5.0353	45.0	2.00	0.100635	1	29.78	50.2	592	118.6	**true val. 499**
E01-1342-36030	02/12/2002	Female liver MSD-day 2	d020212029	5.0353	45.0	2.00	0.100635	1	28.53	50.2	567	113.6	**avg %rec 111.8**
E01-1342-36035	02/13/2002	Female liver MSD-day 3	d020301028	5.0353	45.0	2.00	0.100635	1	26.99	10.0	536	107.5	**avg conc 558.1**
E01-1342-36040	02/15/2002	Female liver MSD-day 4	d020218029	5.0353	45.0	2.00	0.100635	1	27.02	20.0	537	107.6	**std dev of conc 26.67**

* Concentration <LOQ

Conc of PFOS in Sample (ng/g) = (Inst conc(ng/mL)) x (additional dilution) x (final volume (mL))/ amount of liver in diluted sample (g)

amount of liver in diluted sample (g) = amount of liver weighed (g)/ (water added for homogenizing (mL=g)) + amount of liver weighed(g))

for both ms and msd

avg % rec 110.7
std dev of % rec 5.514
avg conc. 552.6
std dev of conc. 27.51

Study number E01-1245 - QUAIL LIVER

Absolute Recoveries

versus unextracted curve original

3M Sample Number	Sample Description	Inst. File Name	amount of liver weighed (g)	Water Added for Homgenizing (mL)	Final Volume (mL)	Amount of liver in diluted sample (g)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/g)	Conc. of PFOS in Sample (ng/g of Liver)		% Rec.	
E01-1342-36017	qc-10ppb-1 (200ng/g)	d020211047	5.0353	45.0	2.00	0.100635	1	13.59	50.2	270	A	135.0	**avg** 131.2
E01-1342-36018	qc-10ppb-2 (200ng/g)	d020211048	5.0353	45.0	2.00	0.100635	1	12.74	50.2	253	A	126.6	**std dev** 4.274
E01-1342-36019	qc-10ppb-3 (200ng/g)	d020211049	5.0353	45.0	2.00	0.100635	1	13.28	50.2	264	A	132.0	**%RSD** 3.258
E01-1342-36020	qc-75ppb-1 (1496 ng/g)	d020211050	5.0353	45.0	2.00	0.100635	1	95.85	50.2	1905	A	127.3	**avg** 123.4
E01-1342-36021	qc-75ppb-2 (1496 ng/g)	d020211051	5.0353	45.0	2.00	0.100635	1	90.27	50.2	1794		119.9	**std dev** 3.725
E01-1342-36022	qc-75ppb-3 (1496 ng/g)	d020211052	5.0353	45.0	2.00	0.100635	1	92.58	50.2	1840		123.0	**%RSD** 3.018
E01-1342-36023	qc-150ppb-1 (2993 ng/g)	d020211053	5.0353	45.0	2.00	0.100635	1	169.63	50.2	3371		112.6	**avg** 113.1
E01-1342-36024	qc-150ppb-2 (2993 ng/g)	d020211054	5.0353	45.0	2.00	0.100635	1	167.02	50.2	3319		110.9	**std dev** 2.395
E01-1342-36025	qc-150ppb-3 (2993 ng/g)	d020211055	5.0353	45.0	2.00	0.100635	1	174.15	50.2	3461		115.6	**%RSD** 2.119

for all QC in original analysis (absolute recovery)

avg %rec 122.6
std dev 8.461

Study number E01-1245 - QUAIL LIVER

versus unextracted curve rerun

3M Sample Number	Sample Description	Inst. File Name	amount of liver weighed (g)	Water Added for Homgenizing (mL)	Final Volume (mL)	Amount of liver in diluted sample (g)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/g)	Conc. of PFOS in Sample (ng/g of Liver)		% Rec.	
E01-1342-36017	qc-10ppb-1 (200ng/g)	d020405136	5.0353	45.0	2.00	0.100635	1	14.86	100	295	A	147.7	**avg** 145.3
E01-1342-36018	qc-10ppb-2 (200ng/g)	d020405137	5.0353	45.0	2.00	0.100635	1	14.24	100	283	A	141.5	**std dev** 3.329
E01-1342-36019	qc-10ppb-3 (200ng/g)	d020405138	5.0353	45.0	2.00	0.100635	1	14.77	100	294	A	146.8	**%RSD** 2.291
E01-1342-36020	qc-75ppb-1 (1496 ng/g)	d020405139	5.0353	45.0	2.00	0.100635	1	100.51	100	1998	A	133.5	**avg** 131.3
E01-1342-36021	qc-75ppb-2 (1496 ng/g)	d020405140	5.0353	45.0	2.00	0.100635	1	94.89	100	1886	A	126.1	**std dev** 4.554
E01-1342-36022	qc-75ppb-3 (1496 ng/g)	d020405141	5.0353	45.0	2.00	0.100635	1	101.10	100	2009	A	134.3	**%RSD** 3.468
E01-1342-36023	qc-150ppb-1 (2993 ng/g)	d020405142	5.0353	45.0	2.00	0.100635	1	187.94	100	3735		124.8	**avg** 119.6
E01-1342-36024	qc-150ppb-2 (2993 ng/g)	d020405143	5.0353	45.0	2.00	0.100635	1	172.20	100	3422		114.3	**std dev** 5.226
E01-1342-36025	qc-150ppb-3 (2993 ng/g)	d020405144	5.0353	45.0	2.00	0.100635	1	180.27	100	3583		119.7	**%RSD** 4.369

A = Not within +/-25% criteria.

for all QC in rerun analysis (absolute recovery)

avg %rec 132.1
std dev 11.79

Conc of PFOS in Sample (ng/g) = (Inst conc(ng/mL)) x (additional dilution) x (final volume (mL))/ amount of liver in diluted sample (g)

amount of liver in diluted sample (g) = amount of liver weighed (g)/ (water added for homogenizing (mL=g)) + amount of liver weighed(g))

Study number E01-1245 - QUAIL LIVER

QC Sample Recoveries

versus extracted curve original

3M Sample Number	Sample Description	Inst. File Name	amount of liver weighed (g)	Water Added for Homgenizing (mL)	Final Volume (mL)	Amount of liver in diluted sample (g)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/g)	Conc. of PFOS in Sample (ng/g of Liver)		% Rec.	
E01-1342-36017	qc-10ppb-1 (200ng/g)	d020211047	5.0353	45.0	2.00	0.100635	1	14.20	50.2	282	A	141.1	**avg** 137.5
E01-1342-36018	qc-10ppb-2 (200ng/g)	d020211048	5.0353	45.0	2.00	0.100635	1	13.41	50.2	267	A	133.3	**std dev** 3.971
E01-1342-36019	qc-10ppb-3 (200ng/g)	d020211049	5.0353	45.0	2.00	0.100635	1	13.91	50.2	276	A	138.2	**%RSD** 2.887
E01-1342-36020	qc-75ppb-1 (1496 ng/g)	d020211050	5.0353	45.0	2.00	0.100635	1	89.60	50.2	1781		119.0	**avg** 115.5
E01-1342-36021	qc-75ppb-2 (1496 ng/g)	d020211051	5.0353	45.0	2.00	0.100635	1	84.54	50.2	1680		112.3	**std dev** 3.378
E01-1342-36022	qc-75ppb-3 (1496 ng/g)	d020211052	5.0353	45.0	2.00	0.100635	1	86.63	50.2	1722		115.1	**%RSD** 2.925
E01-1342-36023	qc-150ppb-1 (2993 ng/g)	d020211053	5.0353	45.0	2.00	0.100635	1	155.46	50.2	3090		103.2	**avg** 103.6
E01-1342-36024	qc-150ppb-2 (2993 ng/g)	d020211054	5.0353	45.0	2.00	0.100635	1	153.16	50.2	3044		101.7	**std dev** 2.106
E01-1342-36025	qc-150ppb-3 (2993 ng/g)	d020211055	5.0353	45.0	2.00	0.100635	1	159.43	50.2	3168		105.9	**%RSD** 2.033

for all QC in original analysis (recovery)

avg %rec 118.9
std dev 15.17

Study number E01-1245 - QUAIL LIVER

versus extracted curve rerun

3M Sample Number	Sample Description	Inst. File Name	amount of liver weighed (g)	Water Added for Homgenizing (mL)	Final Volume (mL)	Amount of liver in diluted sample (g)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/g)	Conc. of PFOS in Sample (ng/g of Liver)		% Rec.	
E01-1342-36017	qc-10ppb-1 (200ng/g)	d020405136	5.0353	45.0	2.00	0.100635	1	13.43	100	267	A	133.5	**avg** 131.5
E01-1342-36018	qc-10ppb-2 (200ng/g)	d020405137	5.0353	45.0	2.00	0.100635	1	12.91	100	257	A	128.3	**std dev** 2.782
E01-1342-36019	qc-10ppb-3 (200ng/g)	d020405138	5.0353	45.0	2.00	0.100635	1	13.35	100	265	A	132.7	**%RSD** 2.116
E01-1342-36020	qc-75ppb-1 (1496 ng/g)	d020405139	5.0353	45.0	2.00	0.100635	1	84.61	100	1682		112.4	**avg** 110.6
E01-1342-36021	qc-75ppb-2 (1496 ng/g)	d020405140	5.0353	45.0	2.00	0.100635	1	79.97	100	1589		106.2	**std dev** 3.756
E01-1342-36022	qc-75ppb-3 (1496 ng/g)	d020405141	5.0353	45.0	2.00	0.100635	1	85.09	100	1691		113.0	**%RSD** 3.398
E01-1342-36023	qc-150ppb-1 (2993 ng/g)	d020405142	5.0353	45.0	2.00	0.100635	1	155.84	100	3097		103.5	**avg** 99.30
E01-1342-36024	qc-150ppb-2 (2993 ng/g)	d020405143	5.0353	45.0	2.00	0.100635	1	143.14	100	2845		95.05	**std dev** 4.217
E01-1342-36025	qc-150ppb-3 (2993 ng/g)	d020405144	5.0353	45.0	2.00	0.100635	1	149.66	100	2974		99.38	**%RSD** 4.247

A = Not within +/-25% criteria.

for all QC in rerun analysis (recovery)

avg %rec 113.8
std dev 14.48

Conc of PFOS in Sample (ng/g) = (Inst conc(ng/mL)) x (additional dilution) x (final volume (mL))/ amount of liver in diluted sample (g)

amount of liver in diluted sample (g) = amount of liver weighed (g)/ (water added for homogenizing (mL=g)) + amount of liver weighed(g))

Study number E01-1245 - QUAIL LIVER DATA SUMMARY

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of liver weighed (g)	Water Added for Homgenizing (mL)	Final Volume (mL)	Amount of liver in diluted sample (g)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/g)	Conc. of PFOS in Sample (ng/g of Liver)				
E01-1342-30321	06/19/2001	0 ppm a.i. 330 F Liver	d020211062	1.0749	9.00	2.00	0.106691	1	2.51	50.2	50.2	*			
E01-1342-30339	06/19/2001	0 ppm a.i. 332 F Liver	d020211064	1.0469	9.00	2.00	0.104201	1	2.51	50.2	50.2	*			
E01-1342-30357	04/30/2001	0 ppm a.i. 334 F Liver	d020211066	1.0232	9.00	2.00	0.102083	1	2.51	50.2	50.2	*			
E01-1342-30375	06/19/2001	0 ppm a.i. 336 F Liver	d020211068	1.0188	9.00	2.00	0.101689	1	2.51	50.2	50.2	*			
E01-1342-30393	06/19/2001	0 ppm a.i. 338 F Liver	d020211070	1.0543	9.00	2.00	0.104861	1	2.51	50.2	50.2	*			
E01-1342-30411	06/19/2001	0 ppm a.i. 340 F Liver	d020212034	1.0182	9.00	2.00	0.101635	1	2.51	50.2	50.2	*			
E01-1342-30429	05/31/2001	0 ppm a.i. 342 F Liver	d020212036	1.0904	9.00	2.00	0.108063	1	2.51	50.2	50.2	*			
E01-1342-30447	06/19/2001	0 ppm a.i. 344 F Liver	d020212038	1.0087	9.00	2.00	0.100782	1	2.51	50.2	50.2	*			
E01-1342-30465	06/19/2001	0 ppm a.i. 346 F Liver	d020212040	1.0998	9.00	2.00	0.108893	1	2.51	50.2	50.2	*			
E01-1342-30483	06/19/2001	0 ppm a.i. 348 F Liver	d020212042	1.1036	9.00	2.00	0.109228	1	2.51	50.2	50.2	*			
E01-1342-30501	06/19/2001	0 ppm a.i. 350 F Liver	d020212047	1.0237	9.00	2.00	0.102128	1	2.51	50.2	50.2	*			
E01-1342-30519	06/19/2001	0 ppm a.i. 352 F Liver	d020212049	1.1509	9.00	2.00	0.113379	1	2.51	50.2	50.2	*			
E01-1342-30537	06/19/2001	0 ppm a.i. 354 F Liver	d020212051	1.0578	9.00	2.00	0.105172	1	2.51	50.2	50.2	*			
E01-1342-30555	06/19/2001	0 ppm a.i. 356 F Liver	d020212053	1.0312	9.00	2.00	0.102799	1	2.51	50.2	50.2	*			
E01-1342-30573	06/19/2001	0 ppm a.i. 358 F Liver	d020212055	1.1031	9.00	2.00	0.109184	1	2.51	50.2	50.2	*			
E01-1342-30591	06/19/2001	0 ppm a.i. 360 F Liver	d020212059	1.0586	9.00	2.00	0.105243	1	2.51	50.2	50.2	*			
E01-1342-30609	06/19/2001	0 ppm a.i. 362 F Liver	d020212061	1.0424	9.00	2.00	0.103800	1	2.51	50.2	50.2	*			
E01-1342-30627	06/19/2001	0 ppm a.i. 364 F Liver	d020212063	1.0364	9.00	2.00	0.103264	1	2.51	50.2	50.2	*	**avg 50.2**		
E01-1342-30645	06/19/2001	0 ppm a.i. 366 F Liver	d020212065	1.0577	9.00	2.00	0.105163	1	2.51	50.2	50.2	*	**std dev 0**		
E01-1342-30663	06/19/2001	0 ppm a.i. 368 F Liver	d020212067	1.0920	9.00	2.00	0.108205	1	2.51	50.2	50.2	*	**%RSD -**		
E01-1342-30681	06/20/2001	10 ppm a.i. 370 F Liver	d020301032	1.0868	9.00	2.00	0.107745	1	319.18	10.0	5925				
E01-1342-30699	06/20/2001	10 ppm a.i. 372 F Liver	d020301033	1.0748	9.00	2.00	0.106682	1	236.52	10.0	4434				
E01-1342-30717	06/20/2001	10 ppm a.i. 374 F Liver	d020301034	1.0790	9.00	2.00	0.107054	1	290.48	10.0	5427				
E01-1342-30735	06/20/2001	10 ppm a.i. 376 F Liver	d020301035	1.1036	9.00	2.00	0.109228	1	217.55	10.0	3983				
E01-1342-30753	06/20/2001	10 ppm a.i. 378 F Liver	d020301036	1.0021	9.00	2.00	0.100189	1	187.79	10.0	3749				
E01-1342-30771	06/20/2001	10 ppm a.i. 380 F Liver	d020301037	1.0825	9.00	2.00	0.107364	1	214.22	10.0	3991				
E01-1342-30789	06/20/2001	10 ppm a.i. 382 F Liver	d020301038	1.0122	9.00	2.00	0.101097	1	166.41	10.0	3292				
E01-1342-30820	04/11/2001	10 ppm a.i. 384 F Liver	d020301039	0.6864	6.30	2.00	0.0982480	1	206.04	10.0	4194				
E01-1342-30838	06/20/2001	10 ppm a.i. 386 F Liver	d020301040	1.1215	9.00	2.00	0.110804	1	418.20	10.0	7548				
E01-1342-30856	06/20/2001	10 ppm a.i. 388 F Liver	d020301041	1.0878	9.00	2.00	0.107833	1	187.91	10.0	3485				
E01-1342-30874	06/20/2001	10 ppm a.i. 390 F Liver	d020301045	1.0078	9.00	2.00	0.100701	1	245.00	10.0	4866				
E01-1342-30892	06/20/2001	10 ppm a.i. 392 F Liver	d020301046	1.0060	9.00	2.00	0.100540	1	298.53	10.0	5939				
E01-1342-30910	06/20/2001	10 ppm a.i. 394 F Liver	d020301047	1.0238	9.00	2.00	0.102137	1	256.81	10.0	5029				
E01-1342-30928	06/20/2001	10 ppm a.i. 396 F Liver	d020301048	1.0370	9.00	2.00	0.103318	1	253.83	10.0	4914				
E01-1342-30946	06/20/2001	10 ppm a.i. 398 F Liver	d020301049	1.0240	9.00	2.00	0.102155	1	224.76	10.0	4400				
E01-1342-30964	06/20/2001	10 ppm a.i. 400 F Liver	d020218033	1.0181	9.00	2.00	0.101626	1	255.94	20.0	5037				
E01-1342-30982	06/20/2001	10 ppm a.i. 402 F Liver	d020218034	1.0876	9.00	2.00	0.107816	1	318.13	20.0	5901				
E01-1342-31000	06/20/2001	10 ppm a.i. 404 F Liver	d020218035	1.0173	9.00	2.00	0.101554	1	275.17	20.0	5419		**avg 4898**		
E01-1342-31018	06/20/2001	10 ppm a.i. 406 F Liver	d020218036	1.0047	9.00	2.00	0.100423	1	294.79	20.0	5871		**std dev 1035**		
E01-1342-31036	06/20/2001	10 ppm a.i. 408 F Liver	d020218037	1.0044	9.00	2.00	0.100396	1	228.77	20.0	4557		**%RSD 21.1**		
E01-1365-31184	07/23/2001	0 ppm a.i. 246 F Liver, Offspring	d020218038	0.7302	6.30	2.00	0.103866	1	1.00	20.0	20.0	*			
E01-1365-31204	07/23/2001	0 ppm a.i. 259 F Liver, Offspring	d020218041	0.4434	3.60	2.00	0.109660	1	1.00	20.0	20.0	*	**avg 20.0**		
E01-1365-31219	07/23/2001	0 ppm a.i. 273 F Liver, Offspring	d020218047	0.5004	4.50	2.00	0.100072	1	1.00	20.0	20.0	*	**std dev 0**		
E01-1365-31224	07/23/2001	0 ppm a.i. 277 F Liver, Offspring	d020218048	0.4326	3.60	2.00	0.107276	1	1.00	20.0	20.0	*	**%RSD -**		
E01-1365-31240	07/23/2001	10 ppm a.i. 517 F Liver, Offspring	d020218051	0.6570	5.40	2.00	0.108470	1	308.44	20.0	5687				
E01-1365-31250	07/23/2001	10 ppm a.i. 525 F Liver, Offspring	d020218053	0.5720	5.40	2.00	0.0957803	1	336.48	20.0	7026		**Without outliers**	**With outliers**	
E01-1365-31260	07/23/2001	10 ppm a.i. 538 F Liver, Offspring	d020405158	0.5211	4.50	2.00	0.103782	10	72.30	100	13933	*rejected*	**avg 5494**	**avg 7182**	
E01-1365-31275	07/23/2001	10 ppm a.i. 557 F Liver, Offspring	d020218061	0.4654	4.50	2.00	0.0937286	1	219.12	20.0	4676		**std dev 1137**	**std dev 3900**	
E01-1365-31280	07/23/2001	10 ppm a.i. 561 F Liver, Offspring	d020218062	0.9417	8.10	2.00	0.104151	1	238.83	20.0	4586		**%RSD 20.7**	**%RSD 54.3**	

* Concentration <LOQ

Conc of PFOS in Sample (ng/g) = (Inst conc(ng/mL)) x (additional dilution) x (final volume (mL))/ amount of liver in diluted sample (g)

amount of liver in diluted sample (g) = amount of liver weighed (g)/ (water added for homogenizing (mL=g)) + amount of liver weighed(g))

Study number E01-1245 - QUAIL LIVER DATA SUMMARY

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of liver weighed (g)	Water Added for Homgenizing (mL)	Final Volume (mL)	Amount of liver in diluted sample (g)	additional dilution	Inst Conc. (ng/mL of Methanol)	loq for samples (ng/g)	Conc. of PFOS in Sample (ng/g of Liver)				
E01-1342-30312	06/19/2001	0 ppm a.i. 329 M Liver	d020211061	1.0050	9.00	2.00	0.100450	1	2.51	50.2	50.2	*			
E01-1342-30330	06/19/2001	0 ppm a.i. 331 M Liver	d020211063	1.0530	9.00	2.00	0.104745	1	2.51	50.2	50.2	*			
E01-1342-30348	06/19/2001	0 ppm a.i. 333 M Liver	d020211065	1.0338	9.00	2.00	0.103032	1	10.76	50.2	209		*rejected*		
E01-1342-30366	06/19/2001	0 ppm a.i. 335 M Liver	d020211067	1.0062	9.00	2.00	0.100558	1	2.51	50.2	50.2	*			
E01-1342-30384	06/19/2001	0 ppm a.i. 337 M Liver	d020211069	1.0269	9.00	2.00	0.102415	1	2.51	50.2	50.2	*			
E01-1342-30402	06/19/2001	0 ppm a.i. 339 M Liver	d020212033	1.0886	9.00	2.00	0.107904	1	2.51	50.2	50.2	*			
E01-1342-30420	06/19/2001	0 ppm a.i. 341 M Liver	d020212035	1.0235	9.00	2.00	0.102110	1	2.51	50.2	50.2	*			
E01-1342-30438	06/19/2001	0 ppm a.i. 343 M Liver	d020212037	1.0090	9.00	2.00	0.100809	1	2.51	50.2	50.2	*			
E01-1342-30456	06/19/2001	0 ppm a.i. 345 M Liver	d020212039	1.1046	9.00	2.00	0.109317	1	2.51	50.2	50.2	*			
E01-1342-30474	06/19/2001	0 ppm a.i. 347 M Liver	d020212041	1.0586	9.00	2.00	0.105243	1	2.51	50.2	50.2	*			
E01-1342-30492	06/19/2001	0 ppm a.i. 349 M Liver	d020212046	1.0358	9.00	2.00	0.103211	1	2.51	50.2	50.2	*			
E01-1342-30510	06/19/2001	0 ppm a.i. 351 M Liver	d020212048	1.0583	9.00	2.00	0.105217	1	2.51	50.2	50.2	*	**With outliers**		
E01-1342-30528	06/19/2001	0 ppm a.i. 353 M Liver	d020212050	1.0183	9.00	2.00	0.101644	1	2.51	50.2	50.2	*	**avg 58.1**		
E01-1342-30546	06/19/2001	0 ppm a.i. 355 M Liver	d020212052	1.0899	9.00	2.00	0.108019	1	2.51	50.2	50.2	*	**std dev 35.5**		
E01-1342-30564	06/19/2001	0 ppm a.i. 357 M Liver	d020212054	1.0382	9.00	2.00	0.103425	1	2.51	50.2	50.2	*	**%RSD 61.0**		
E01-1342-30582	06/19/2001	0 ppm a.i. 359 M Liver	d020212058	1.0941	9.00	2.00	0.108390	1	2.51	50.2	50.2	*			
E01-1342-30600	06/19/2001	0 ppm a.i. 361 M Liver	d020212060	1.0084	9.00	2.00	0.100755	1	2.51	50.2	50.2	*	**Without outliers**		
E01-1342-30618	06/19/2001	0 ppm a.i. 363 M Liver	d020212062	1.0818	9.00	2.00	0.107302	1	2.51	50.2	50.2	*	**avg 50.2**		
E01-1342-30636	06/19/2001	0 ppm a.i. 365 M Liver	d020212064	1.0916	9.00	2.00	0.108169	1	2.51	50.2	50.2	*	**std dev 0**		
E01-1342-30654	06/19/2001	0 ppm a.i. 367 M Liver	d020212066	1.0327	9.00	2.00	0.102933	1	2.51	50.2	50.2	*	**%RSD -**		
E01-1342-30672	06/20/2001	10 ppm a.i. 369 M Liver	d020301050	1.0010	9.00	2.00	0.100090	10	393.21	10.0	78571				
E01-1342-30690	06/20/2001	10 ppm a.i. 371 M Liver	d020405148	1.0856	9.00	2.00	0.107639	10	422.59	100	78520				
E01-1342-30708	06/20/2001	10 ppm a.i. 373 M Liver	d020405149	1.0276	9.00	2.00	0.102477	10	441.96	100	86255				
E01-1342-30726	06/20/2001	10 ppm a.i. 375 M Liver	d020301053	1.1008	9.00	2.00	0.108981	10	381.61	10.0	70032				
E01-1342-30744	06/20/2001	10 ppm a.i. 377 M Liver	d020301054	1.0236	9.00	2.00	0.102119	10	409.88	10.0	80275				
E01-1342-30762	06/20/2001	10 ppm a.i. 379 M Liver	d020405150	1.0312	9.00	2.00	0.102799	10	466.19	100	90699				
E01-1342-30780	06/20/2001	10 ppm a.i. 381 M Liver	d020405151	1.0501	9.00	2.00	0.104487	10	417.98	100	80006				
E01-1342-30798	06/20/2001	10 ppm a.i. 383 M Liver	d020301060	1.0110	9.00	2.00	0.100989	10	371.06	10.0	73485				
E01-1342-30829	06/20/2001	10 ppm a.i. 385 M Liver	d020405152	1.0678	9.00	2.00	0.106061	10	442.30	100	83405				
E01-1342-30847	06/20/2001	10 ppm a.i. 387 M Liver	d020301062	1.0315	9.00	2.00	0.102826	10	407.70	10.0	79299				
E01-1342-30865	06/20/2001	10 ppm a.i. 389 M Liver	d020301063	1.0310	9.00	2.00	0.102781	10	361.37	10.0	70318				
E01-1342-30883	06/20/2001	10 ppm a.i. 391 M Liver	d020301064	1.0947	9.00	2.00	0.108443	10	381.87	10.0	70428				
E01-1342-30901	06/20/2001	10 ppm a.i. 393 M Liver	d020405153	1.0834	9.00	2.00	0.107444	10	466.09	100	86760				
E01-1342-30919	06/20/2001	10 ppm a.i. 395 M Liver	d020301066	1.0489	9.00	2.00	0.104380	10	339.50	10.0	65051				
E01-1342-30937	06/20/2001	10 ppm a.i. 397 M Liver	d020301067	1.0309	9.00	2.00	0.102772	10	257.69	10.0	50148				
E01-1342-30955	06/20/2001	10 ppm a.i. 399 M Liver	d020218064	1.0276	9.00	2.00	0.102477	10	322.88	20.0	63015				
E01-1342-30973	06/20/2001	10 ppm a.i. 401 M Liver	d020405159	1.0361	9.00	2.00	0.103237	100	76.08	100	147389				
E01-1342-30991	06/20/2001	10 ppm a.i. 403 M Liver	d020405160	1.0019	9.00	2.00	0.100171	100	72.89	100	145531		**avg 88476**		
E01-1342-31009	06/20/2001	10 ppm a.i. 405 M Liver	d020405161	1.0336	9.00	2.00	0.103014	100	71.24	100	138311		**std dev 28517**		
E01-1342-31027	06/20/2001	10 ppm a.i. 407 M Liver	d020405162	1.0956	9.00	2.00	0.108523	100	71.64	100	132028		**%RSD 32.2**		
E01-1365-31194	07/23/2001	0 ppm a.i. 253 M Liver, Offspring	d020218039	0.3806	3.60	2.00	0.0956137	1	1.00	20.0	20.0	*			
E01-1365-31199	07/23/2001	0 ppm a.i. 256 M Liver, Offspring	d020218040	0.5947	5.40	2.00	0.0992043	1	1.00	20.0	20.0	*			
E01-1365-31209	07/23/2001	0 ppm a.i. 266 M Liver, Offspring	d020218042	0.2673	2.70	2.00	0.0900819	1	1.00	20.0	20.0	*			
E01-1365-31214	07/23/2001	0 ppm a.i. 270 M Liver, Offspring	d020218046	0.5900	5.40	2.00	0.0984975	1	1.00	20.0	20.0	*	**avg 20.0**		
E01-1365-31229	07/23/2001	0 ppm a.i. 283 M Liver, Offspring	d020218049	0.6666	6.30	2.00	0.0956851	1	1.00	20.0	20.0	*	**std dev 0**		
E01-1365-31234	07/23/2001	0 ppm a.i. 291 M Liver, Offspring	d020218050	0.6072	5.40	2.00	0.101079	1	1.00	20.0	20.0	*	**%RSD -**		
E01-1365-31245	07/23/2001	10 ppm a.i. 522 M Liver, Offspring	d020405157	0.7325	6.30	2.00	0.104159	10	71.33	100	13696		*rejected*		
E01-1365-31255	07/23/2001	10 ppm a.i. 533 M Liver, Offspring	d020218054	0.6903	6.30	2.00	0.0987511	1	244.12	20.0	4944		**Without outliers**	**With outliers**	
E01-1365-31265	07/23/2001	10 ppm a.i. 545 M Liver, Offspring	d020218059	0.4624	4.50	2.00	0.0931807	1	259.80	20.0	5576		**avg 5758**	**avg 7346**	
E01-1365-31270	07/23/2001	10 ppm a.i. 552 M Liver, Offspring	d020218060	0.5353	4.50	2.00	0.106309	1	318.62	20.0	5994		**std dev 666**	**std dev 3597**	
E01-1365-31285	07/23/2001	10 ppm a.i. 568 M Liver, Offspring	d020218063	0.7581	7.20	2.00	0.0952614	1	310.49	20.0	6519		**%RSD 11.6**	**%RSD 49.0**	

* Concentration <LOQ

Conc of PFOS in Sample (ng/g) = (Inst conc(ng/mL)) x (additional dilution) x (final volume (mL))/ amount of liver in diluted sample (g)

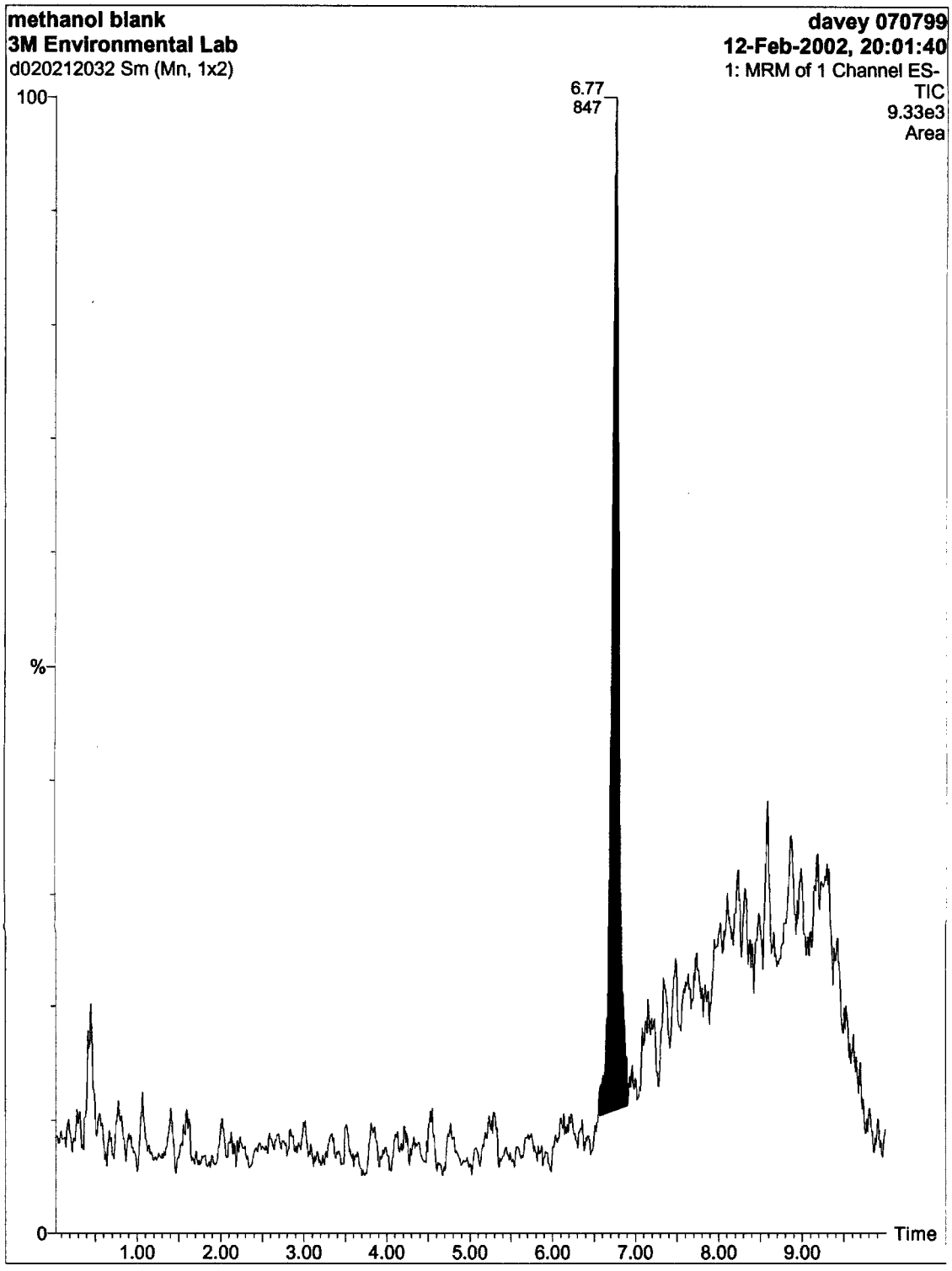
amount of liver in diluted sample (g) = amount of liver weighed (g)/ (water added for homogenizing (mL=g)) + amount of liver weighed(g))

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

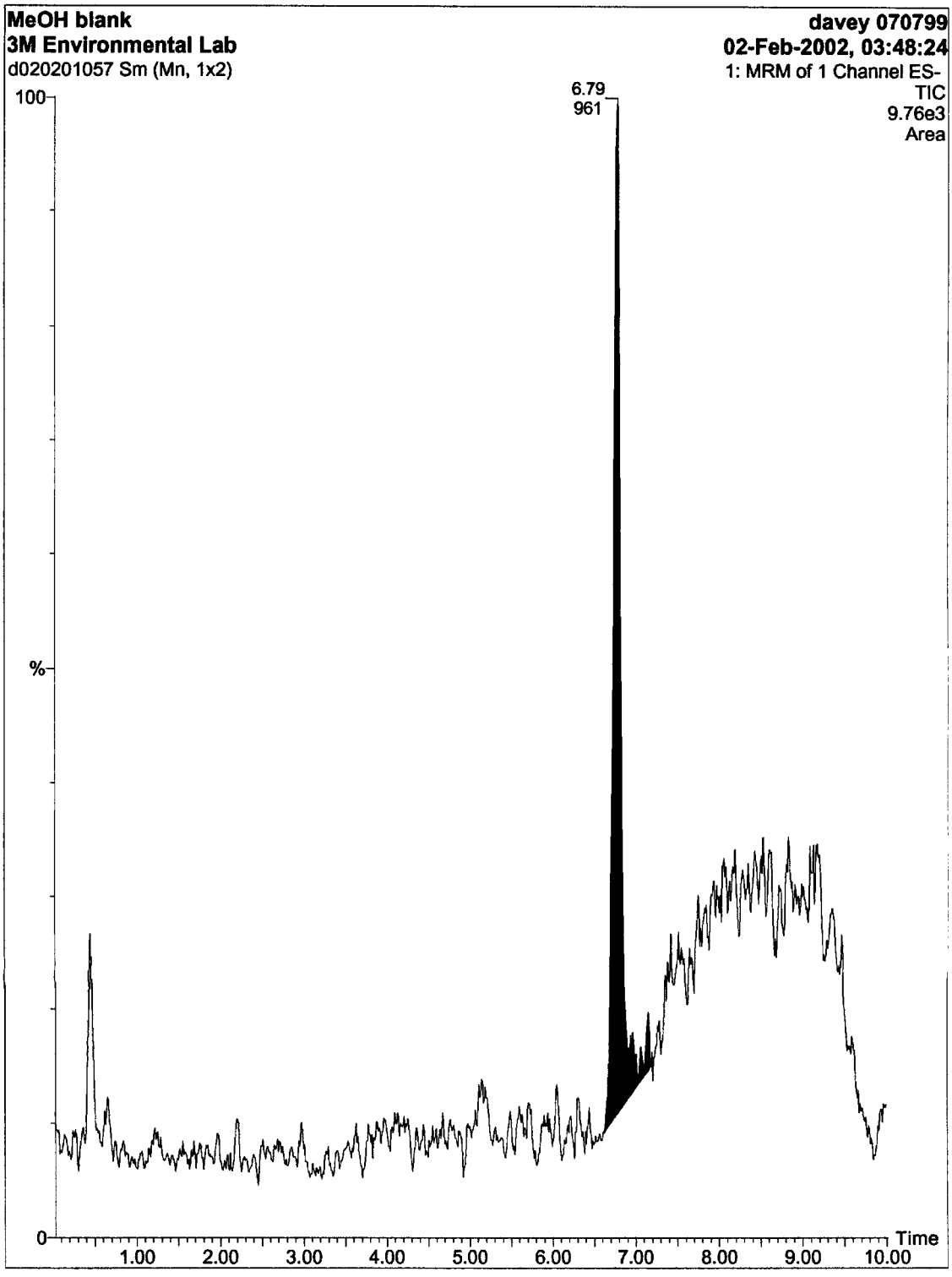
ATTACHMENT C: SAMPLE CHROMATOGRAMS, CALIBRATION INFORMATION, AND INSTRUMENT INFORMATION

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

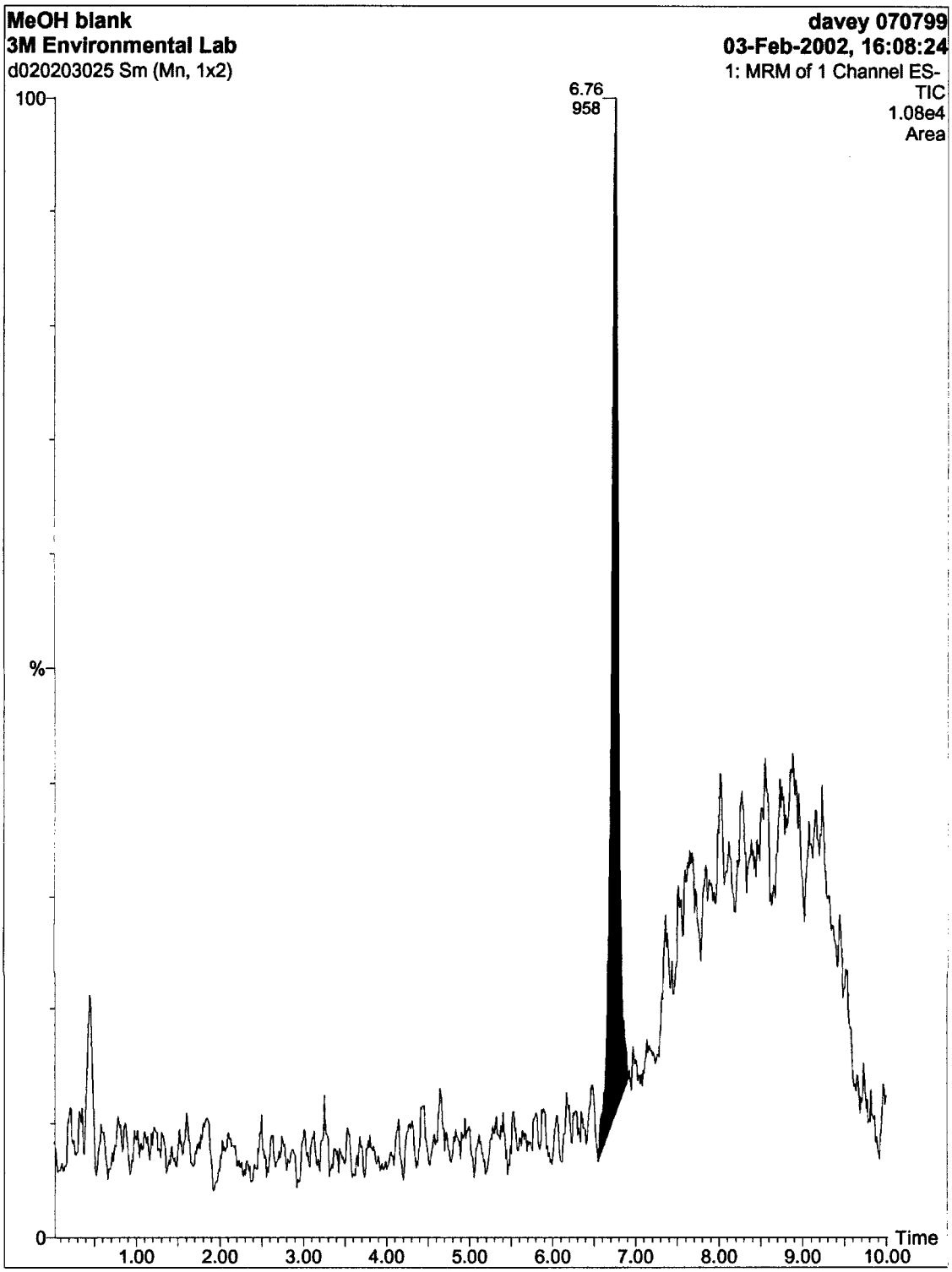
Methanol Solvent Blanks



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

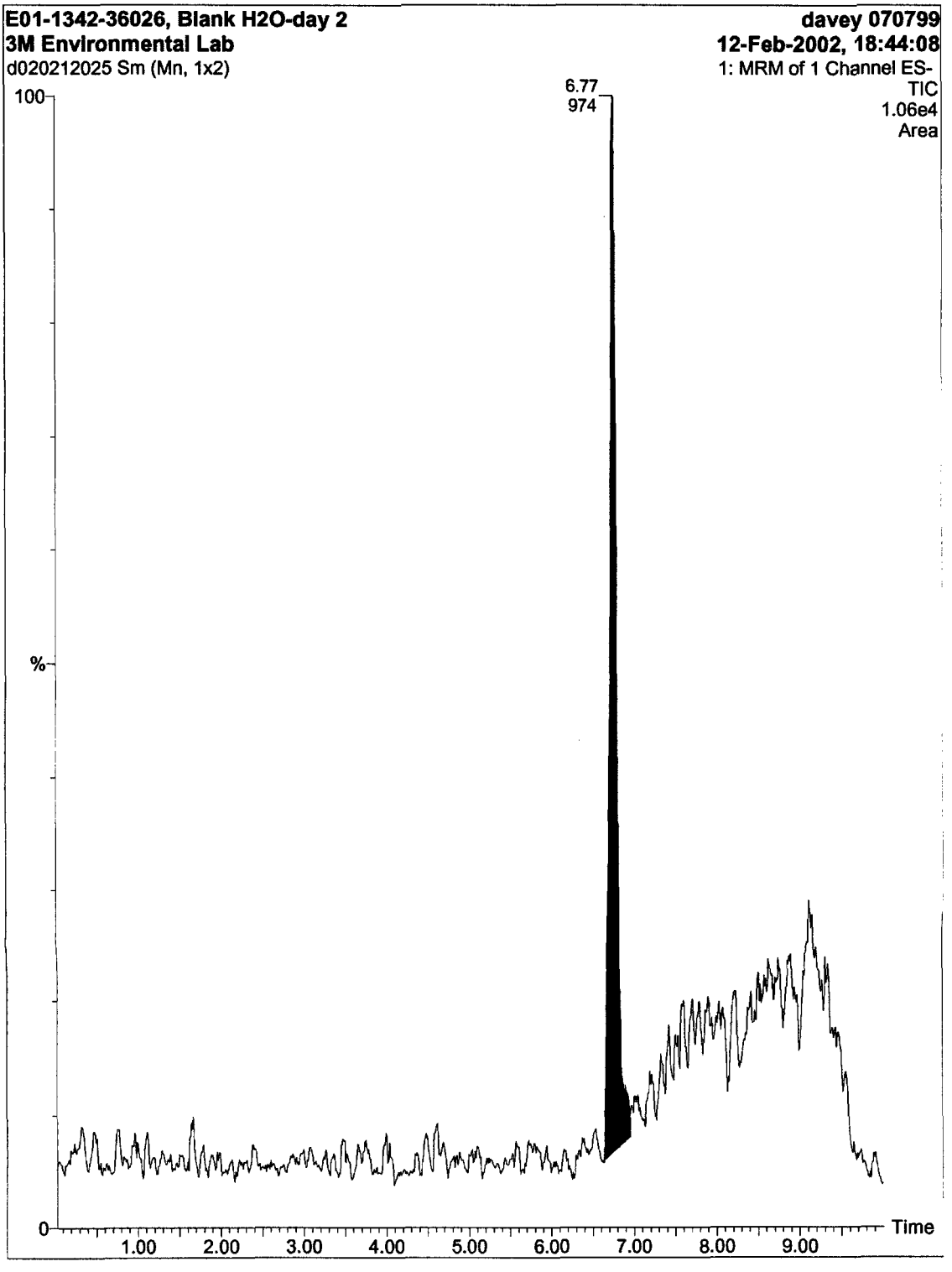


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

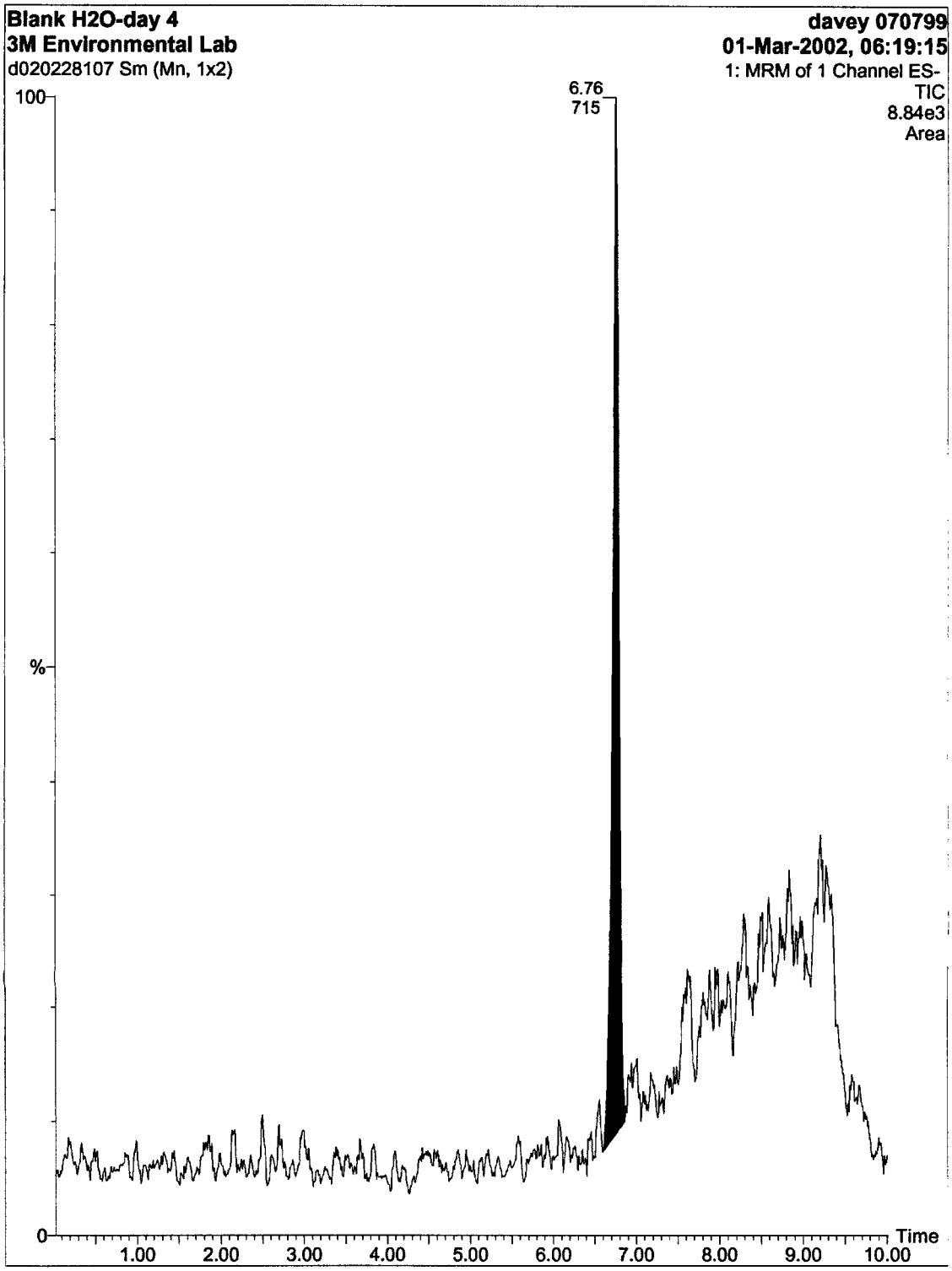


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

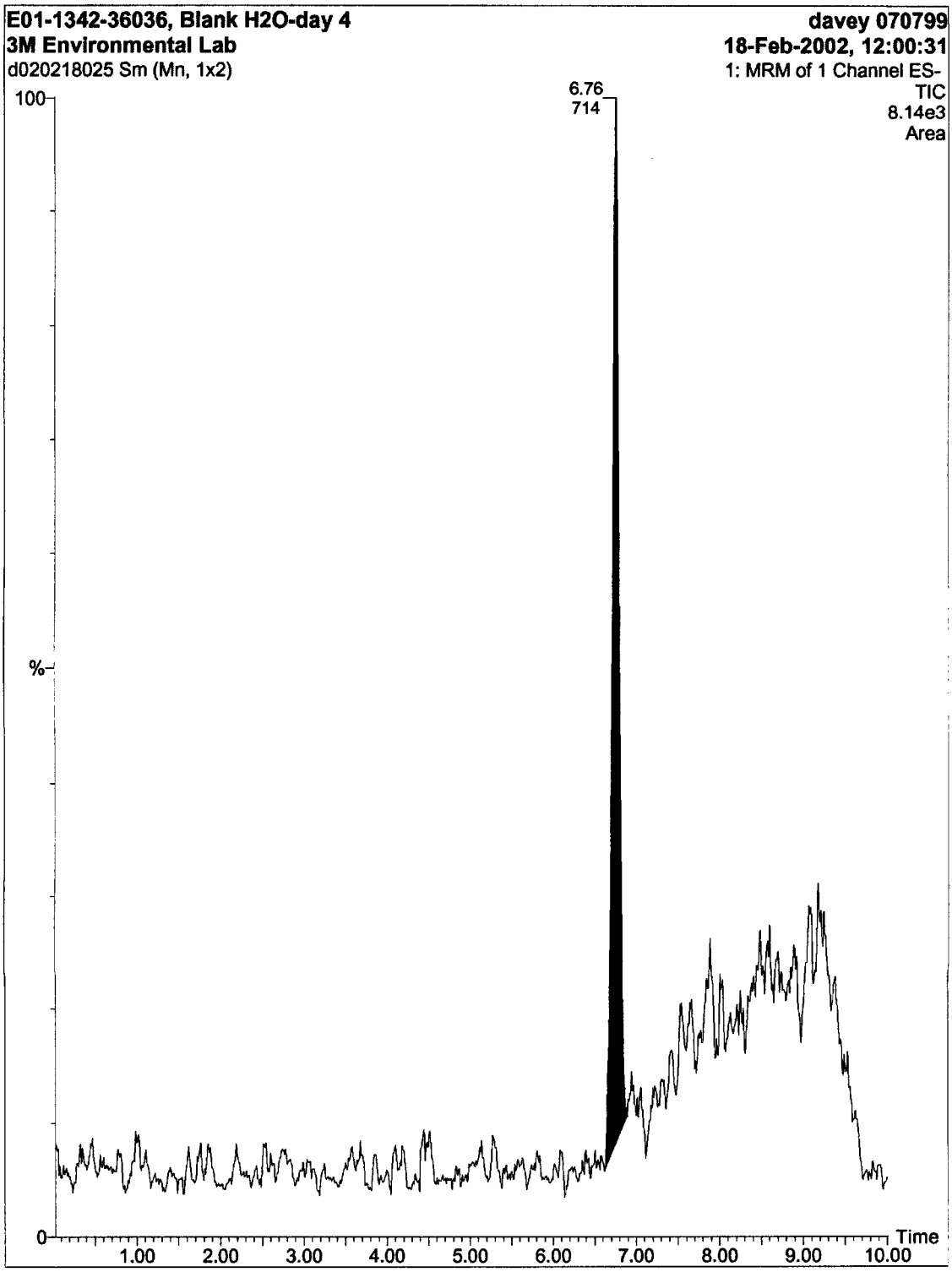
Water Blank Samples



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

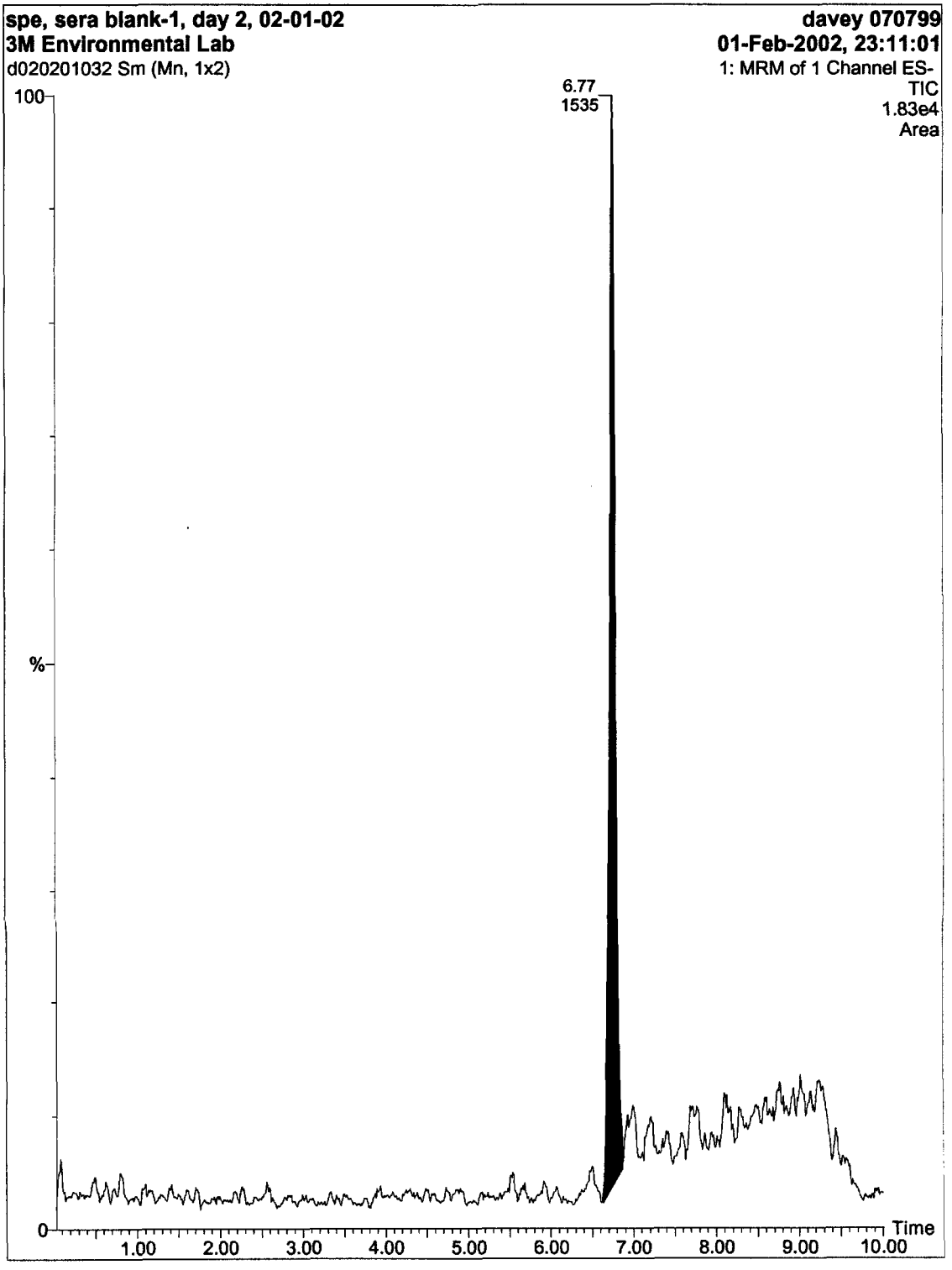


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

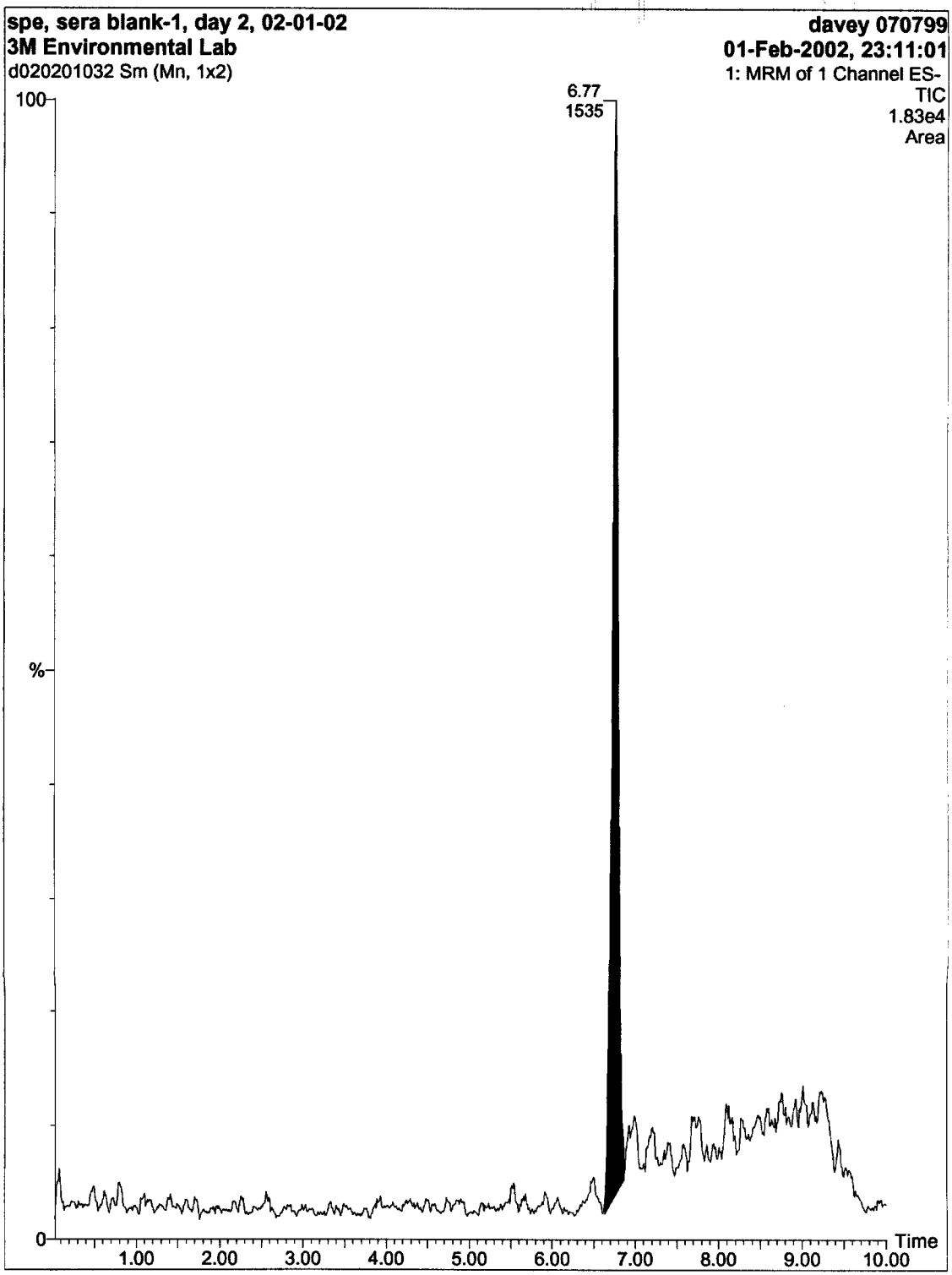


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

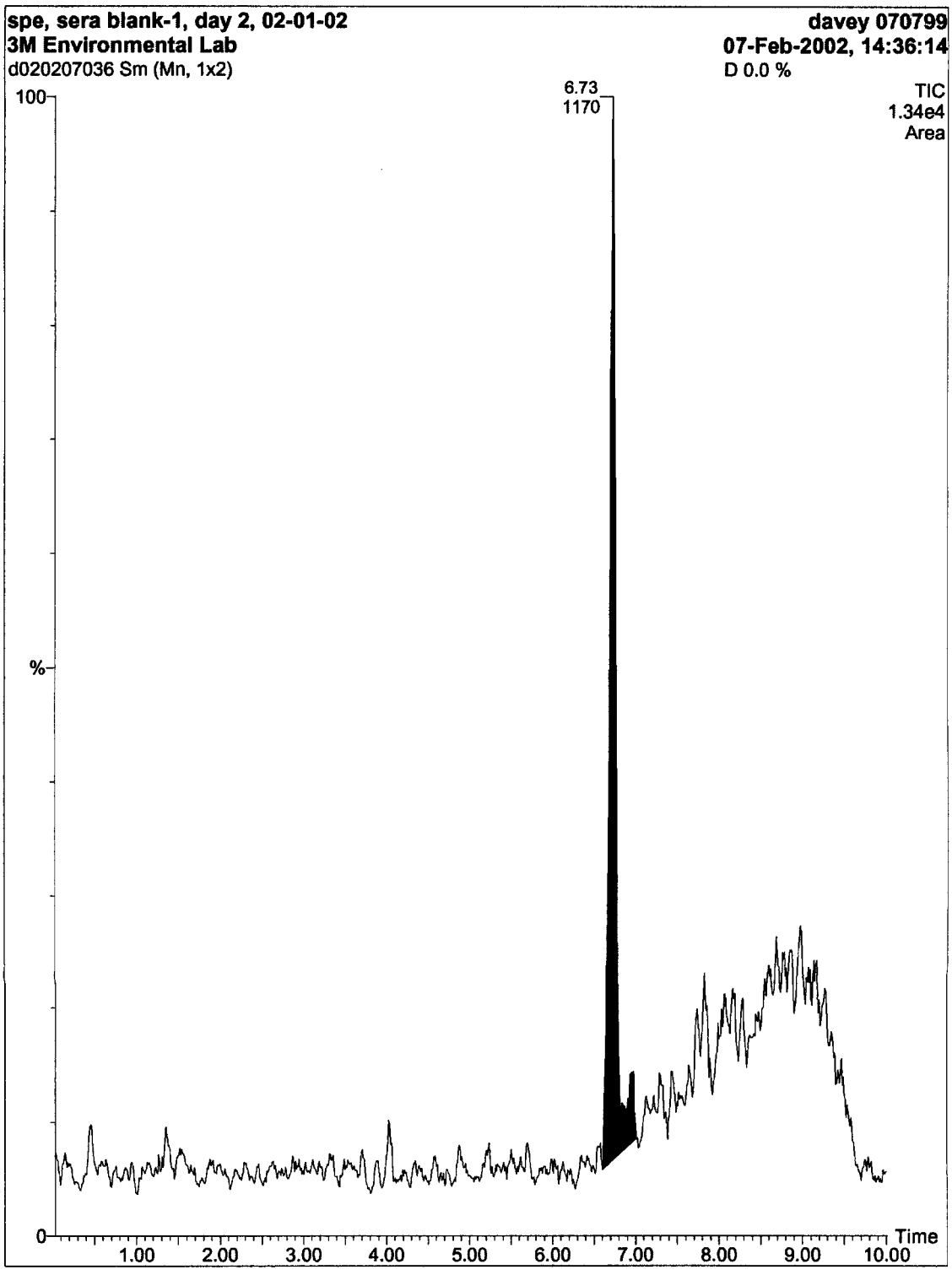
Sera Blank Samples



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

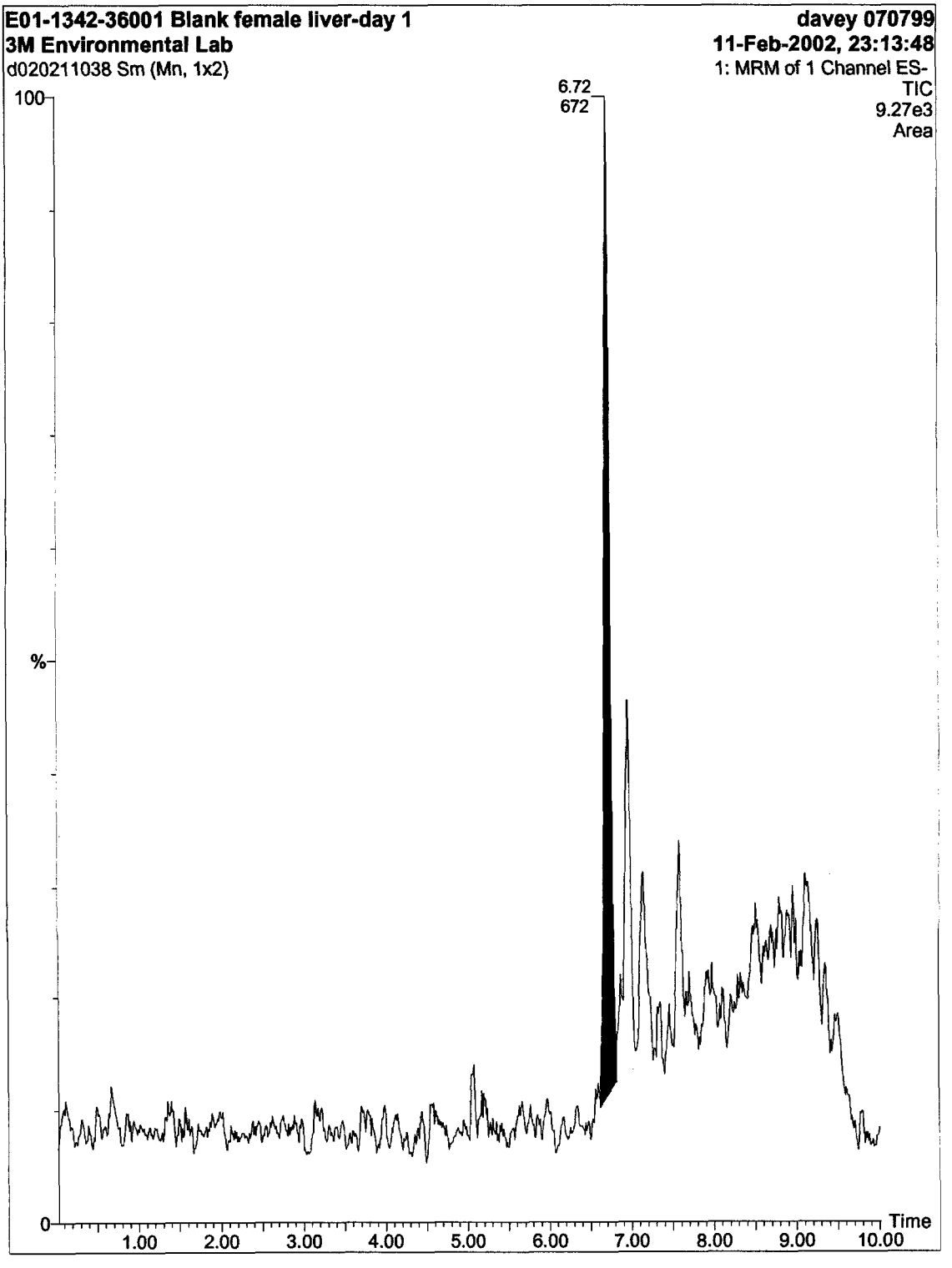


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

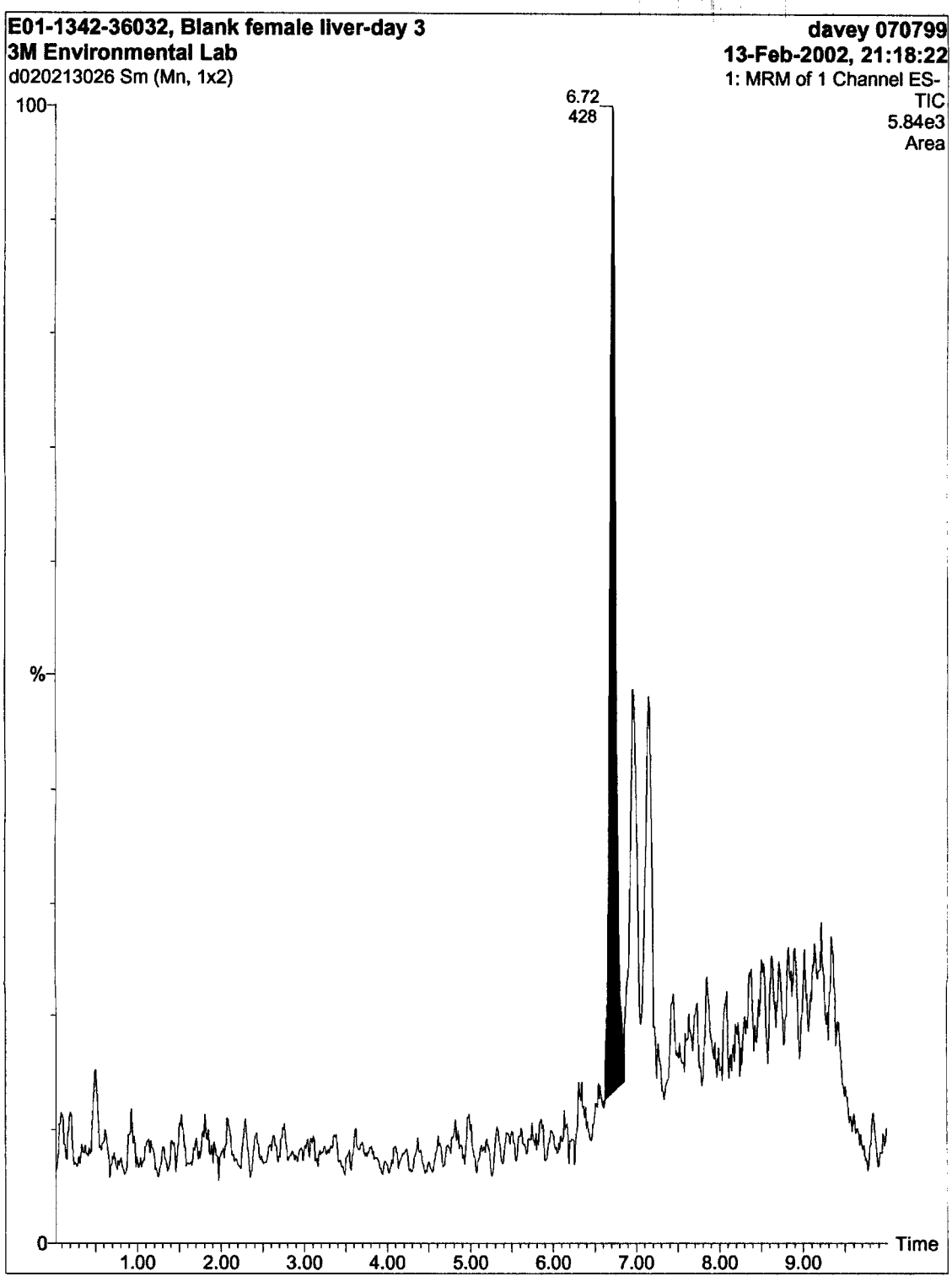


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

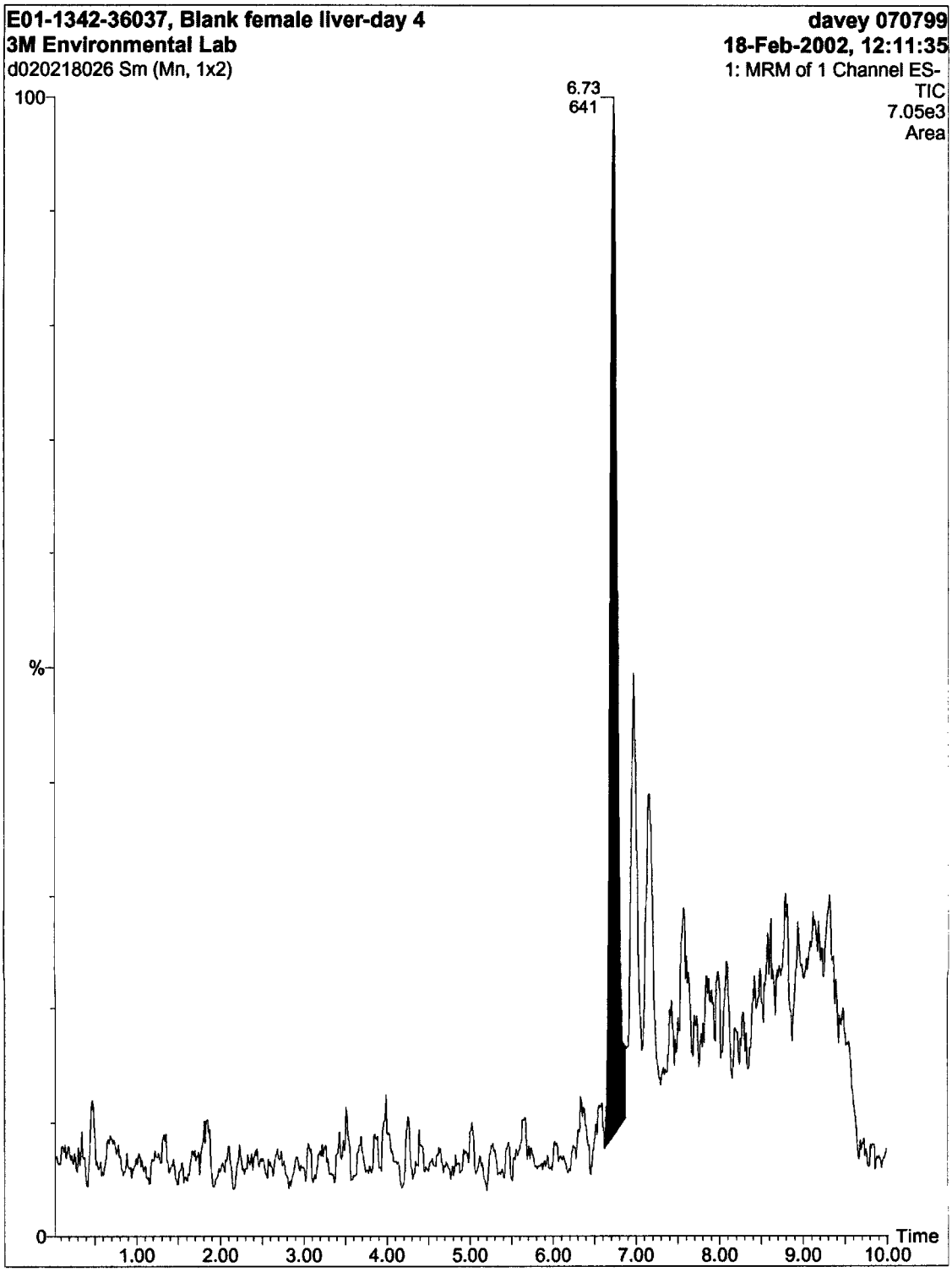
Female Liver Blank Samples



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

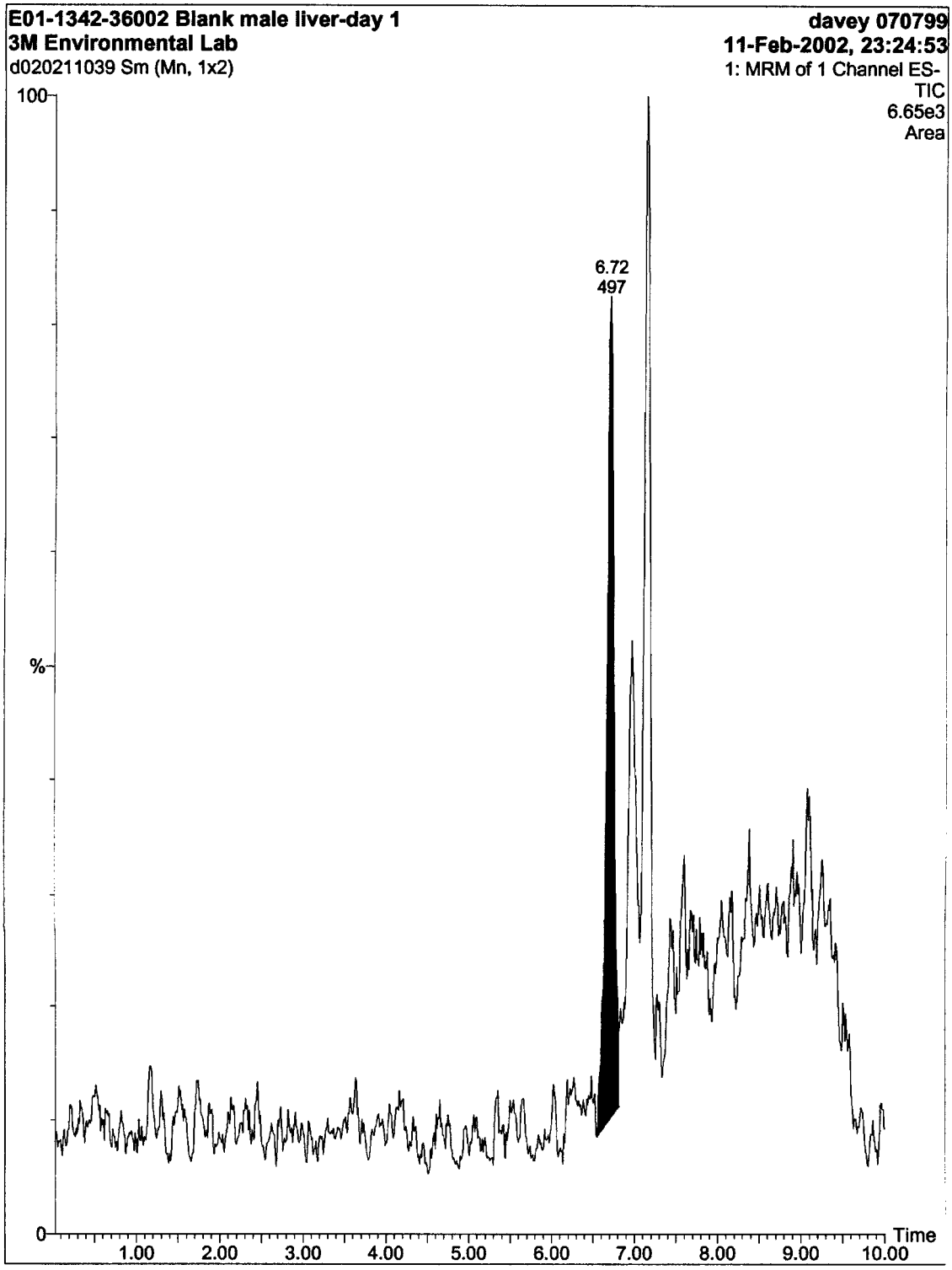


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

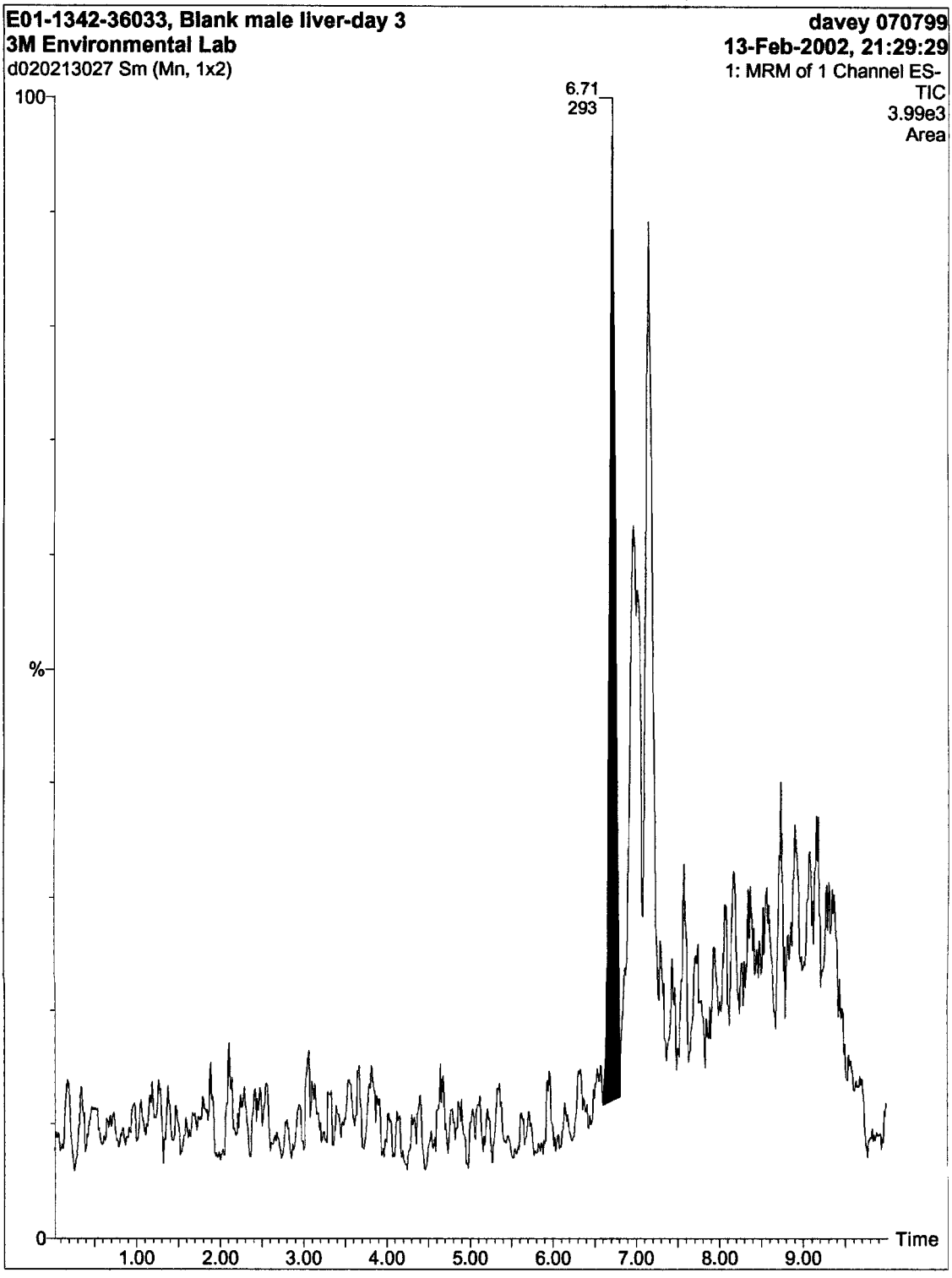


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

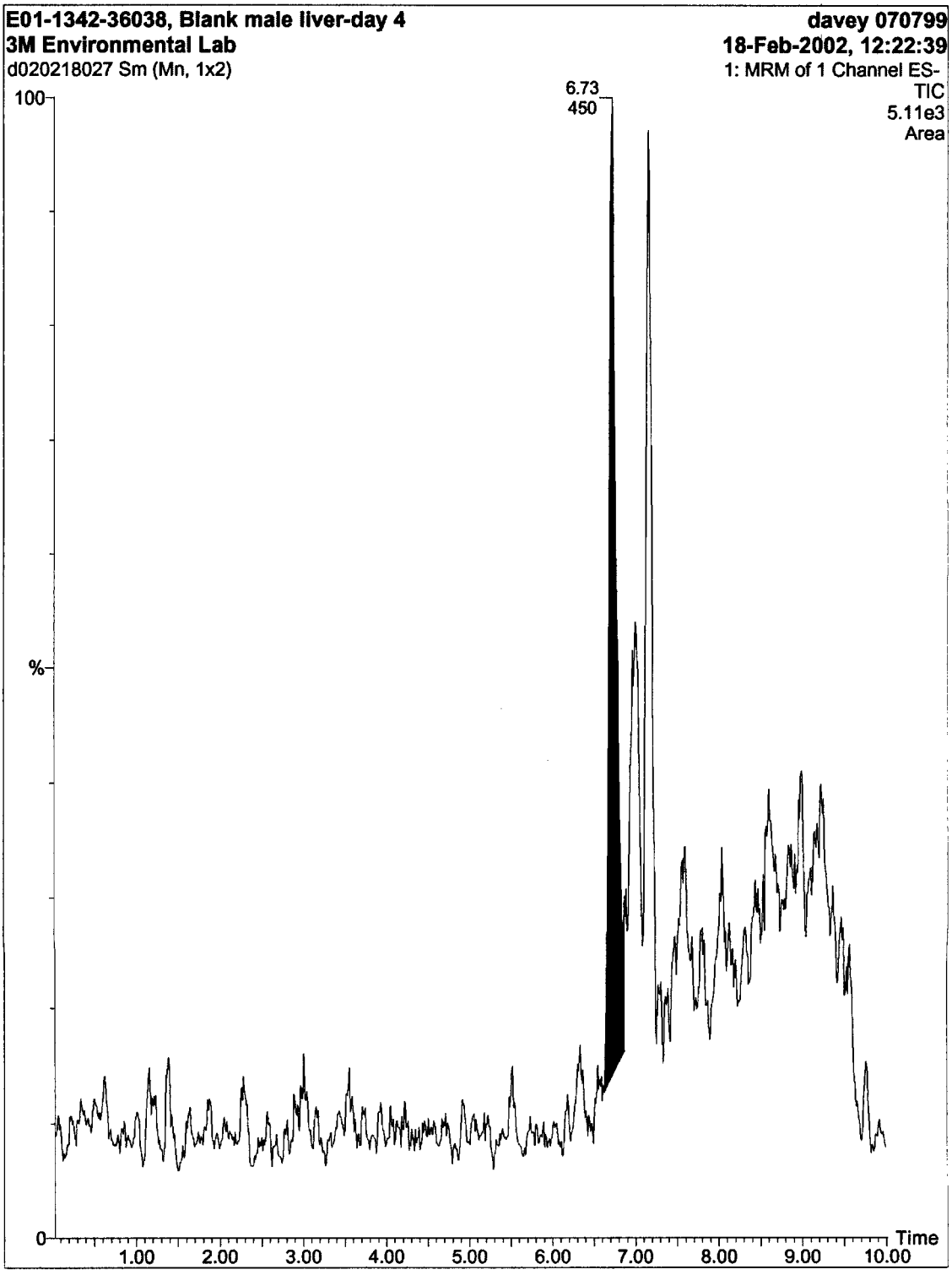
Male Liver Blank Samples



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

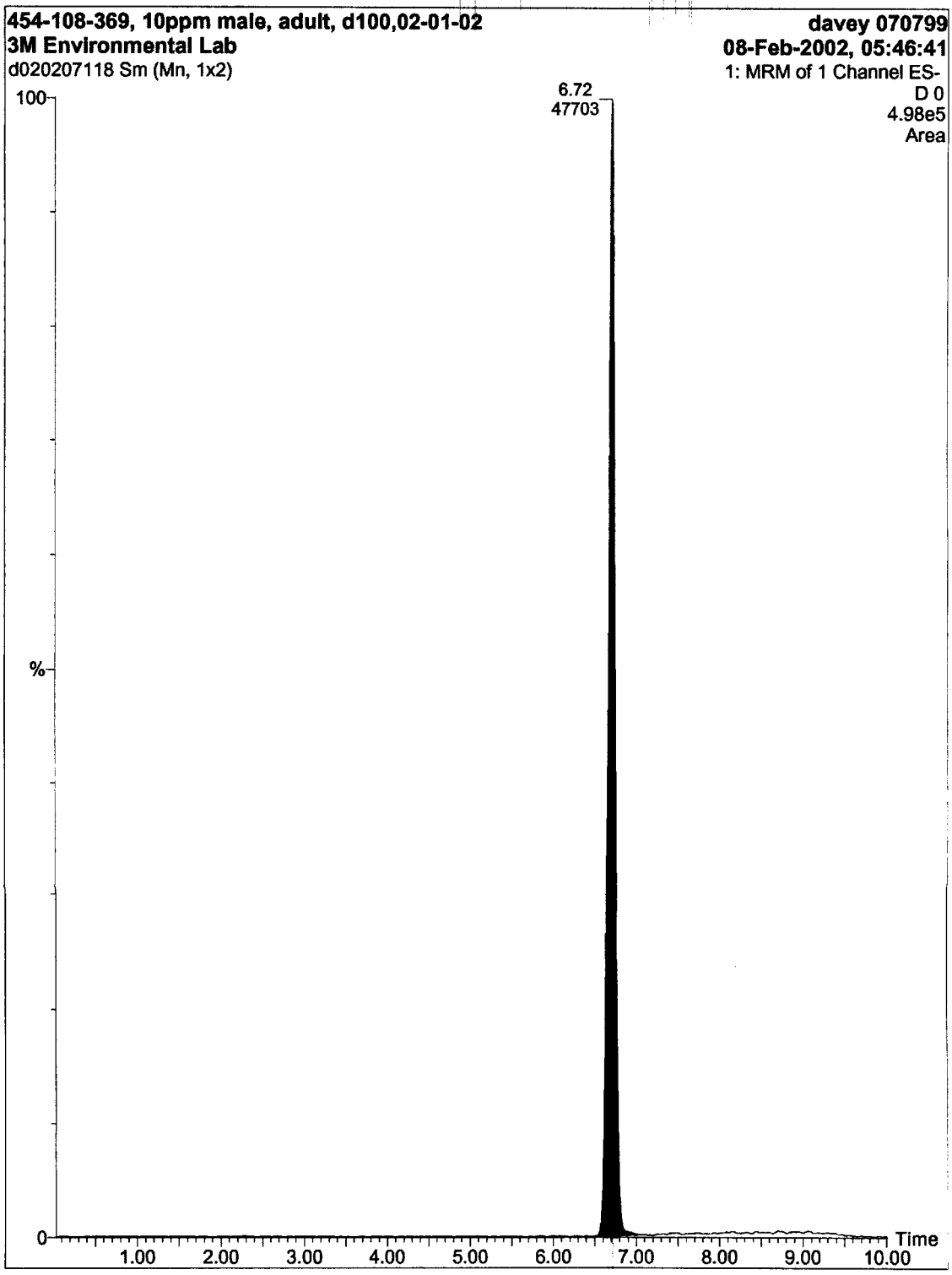


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

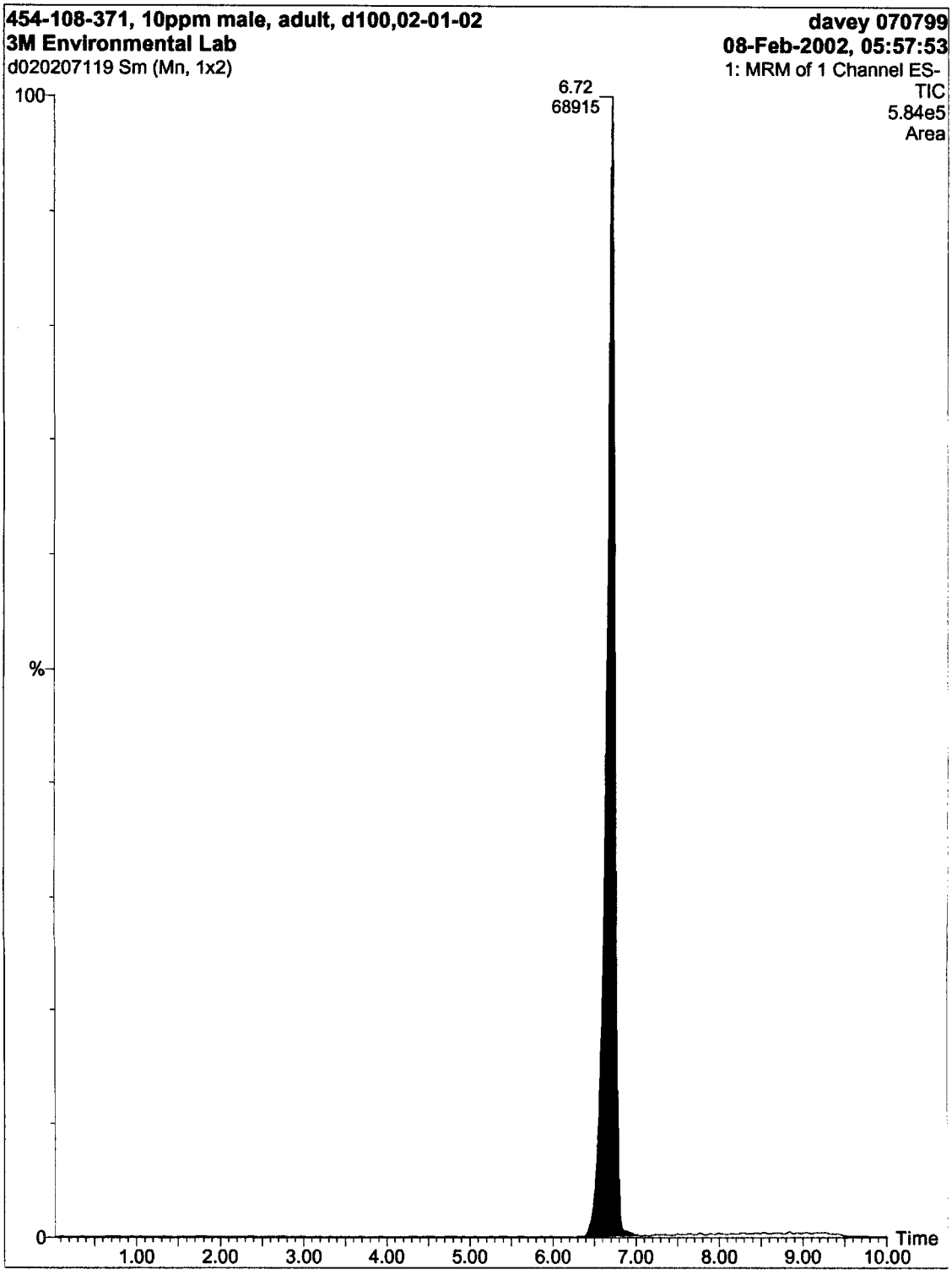


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

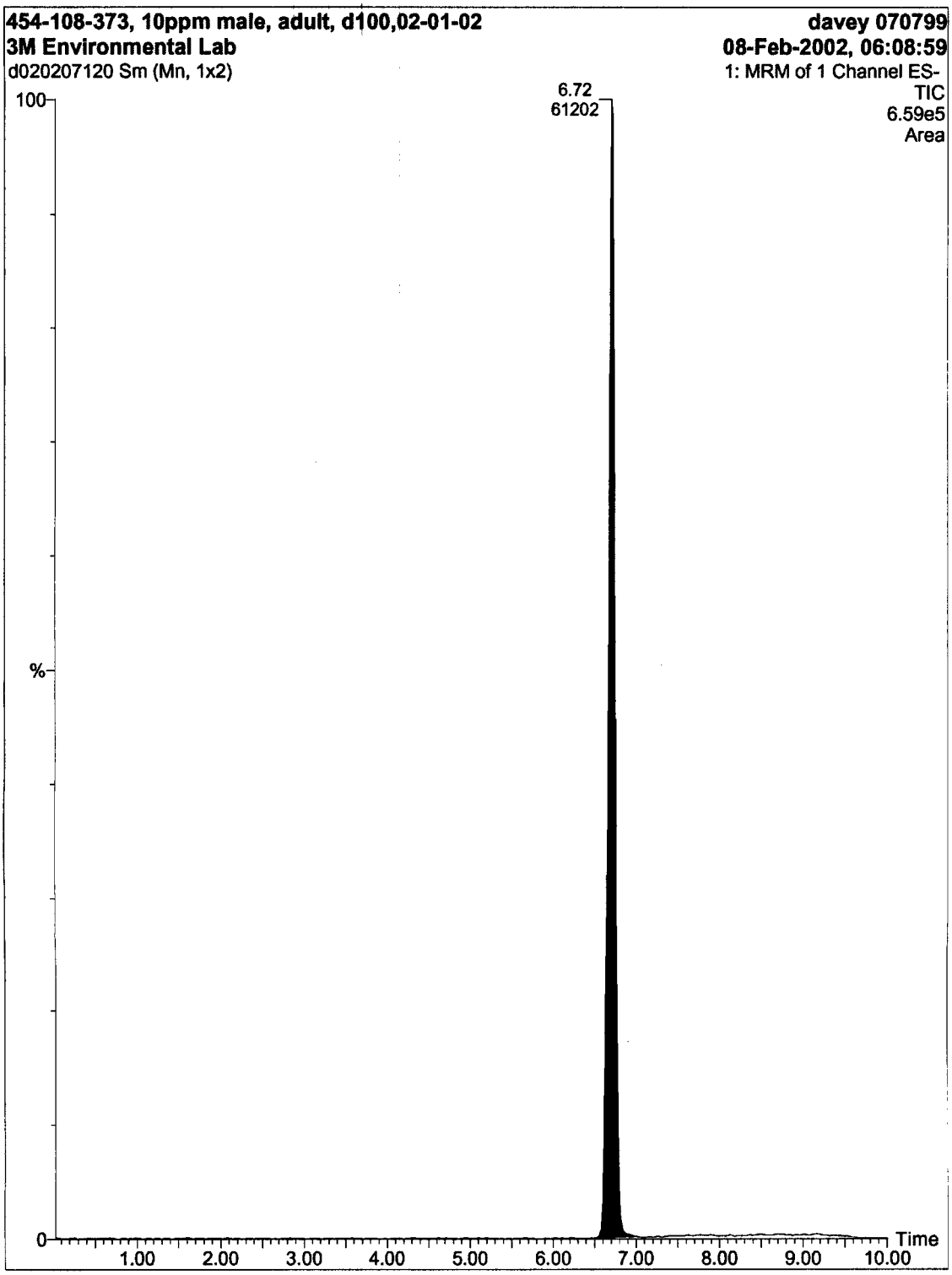
Dosed Group Adult Male Sera Samples, Diluted 1:10



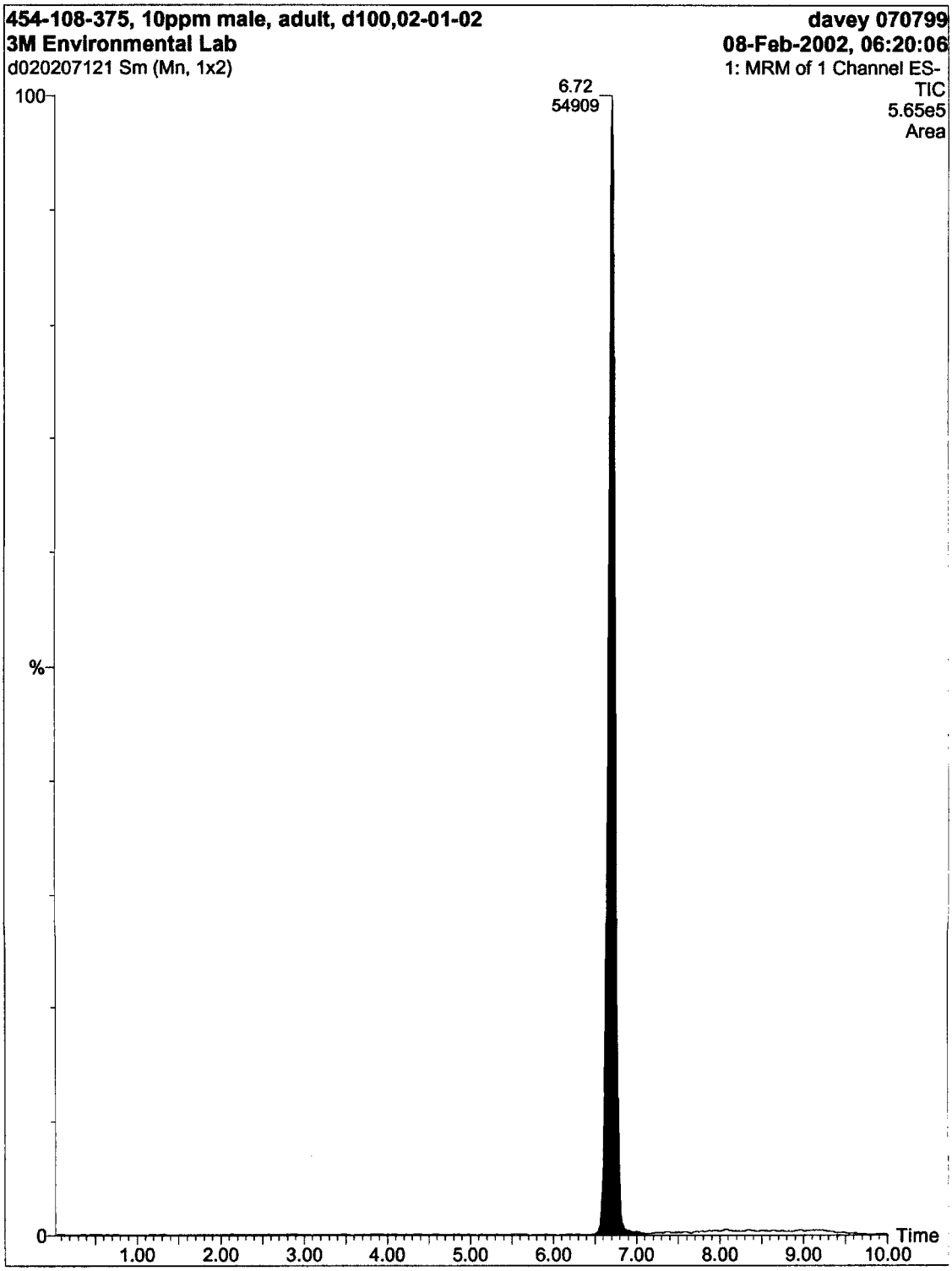
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



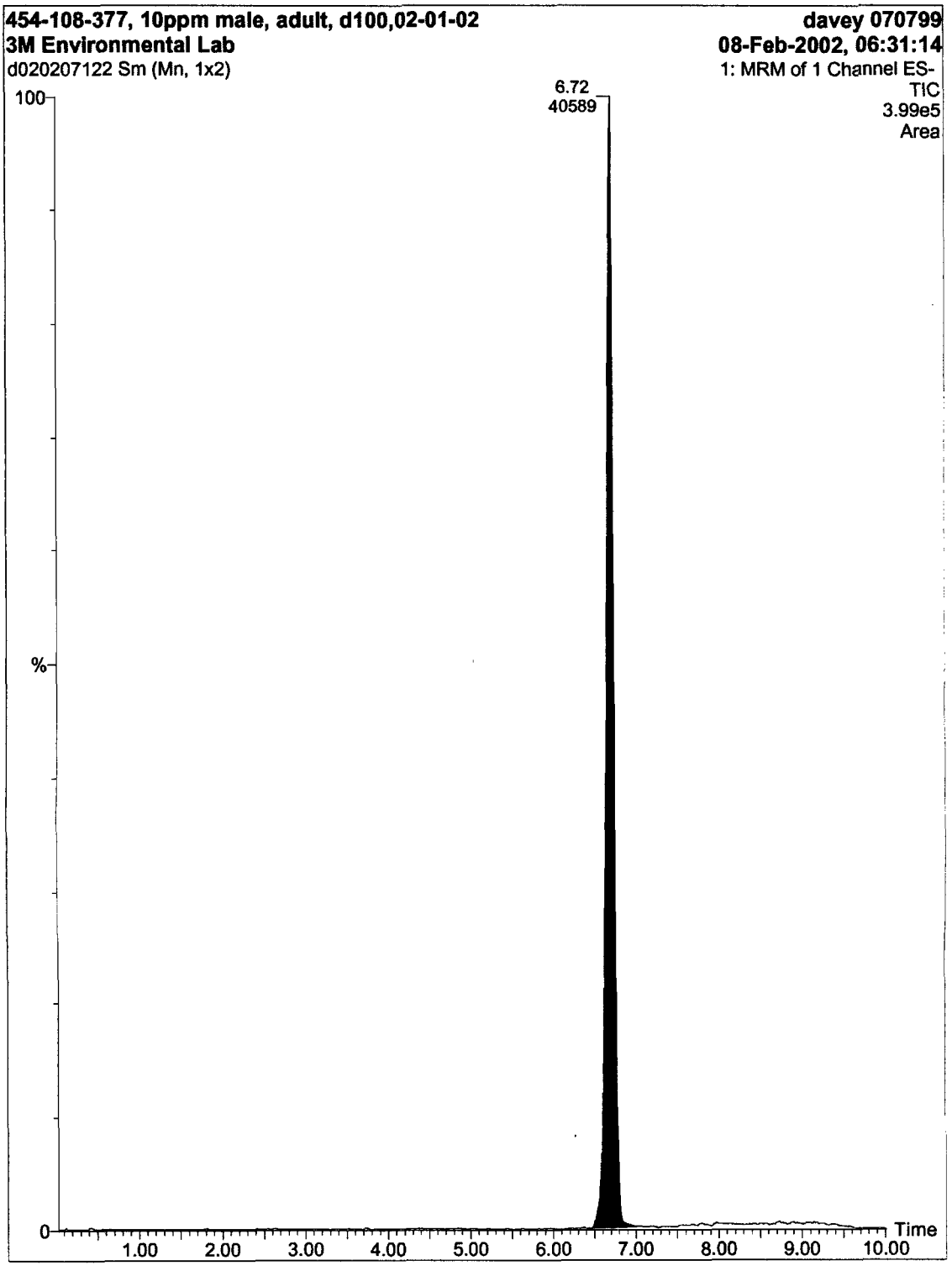
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



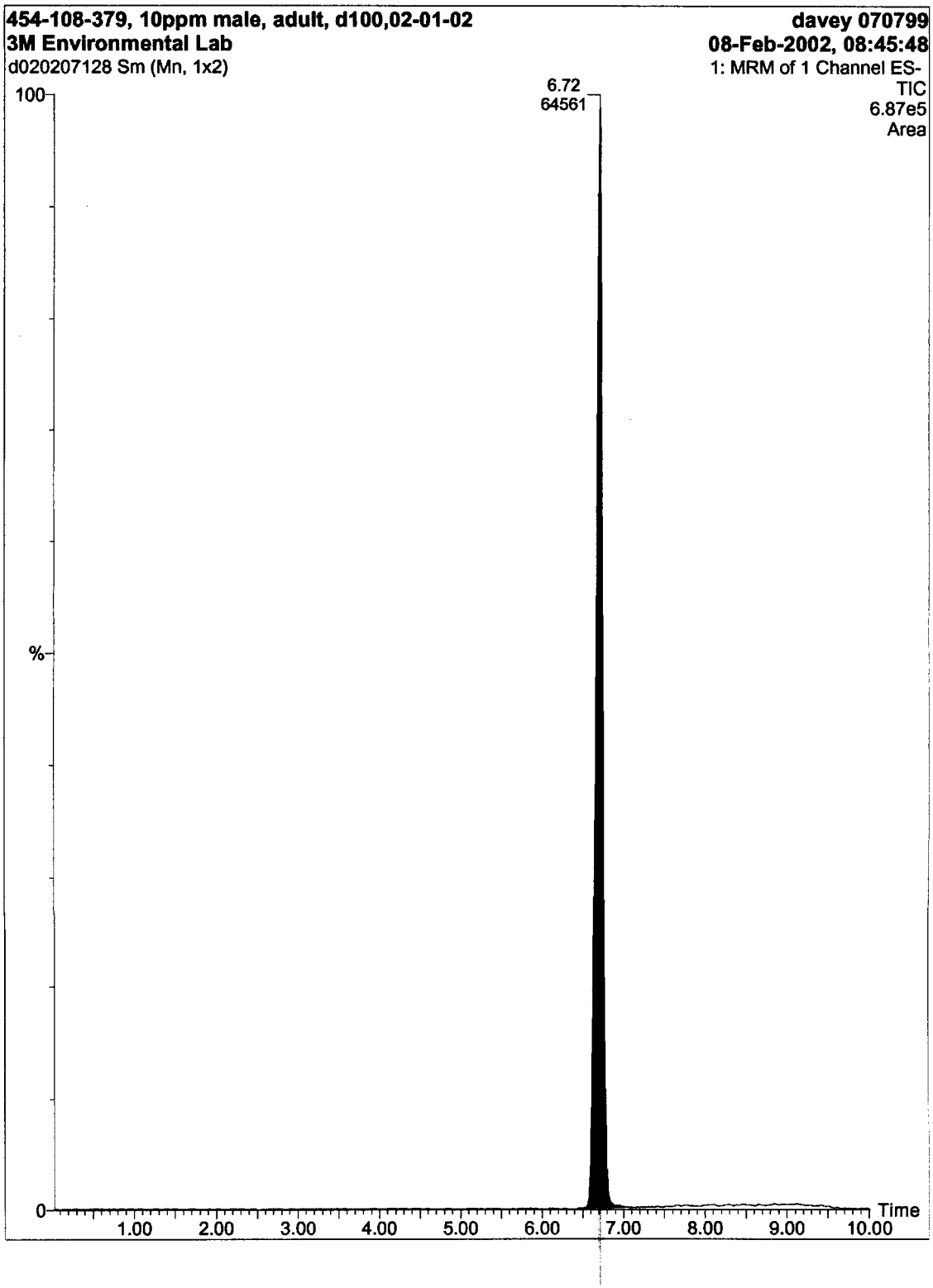
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



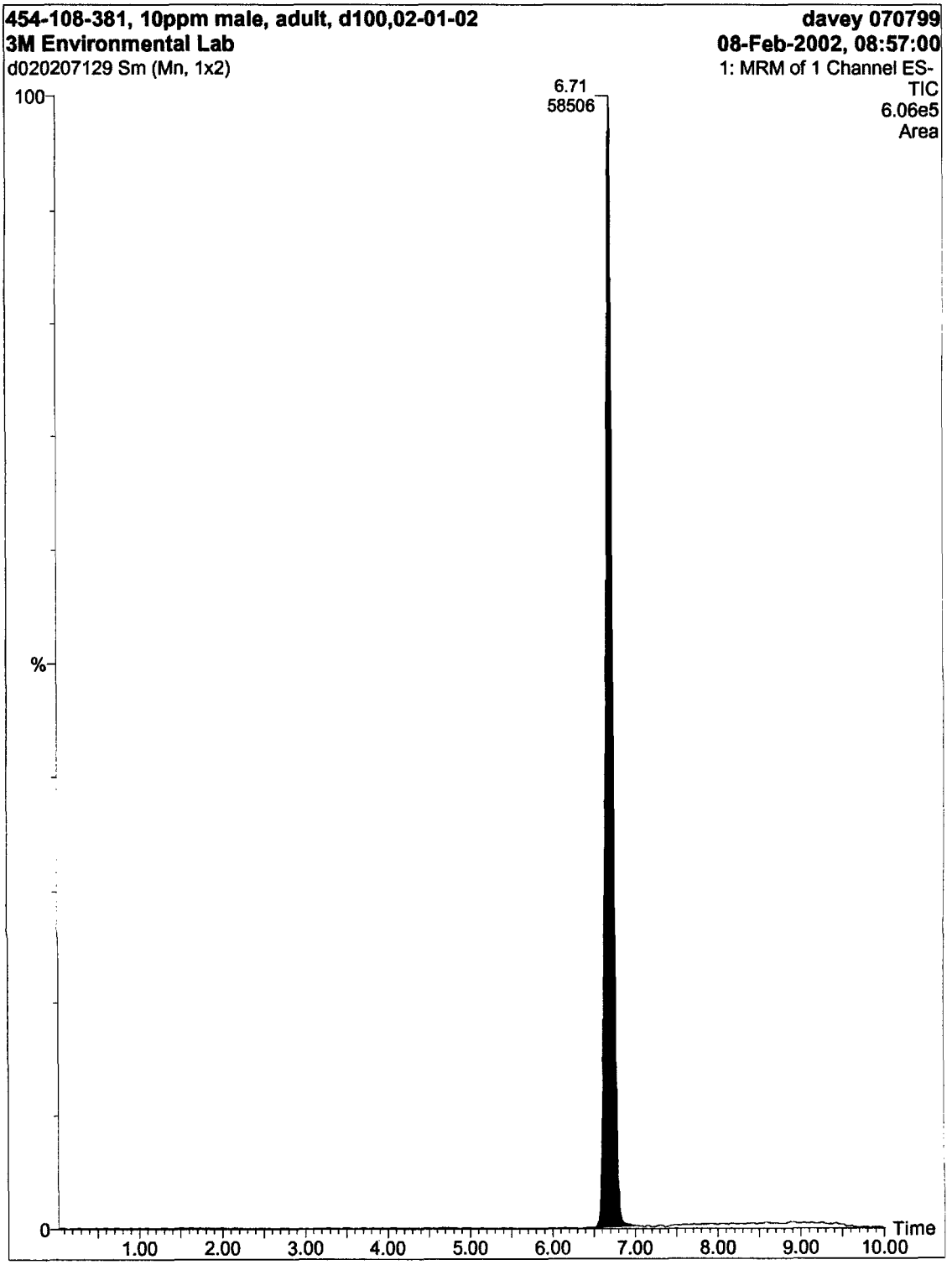
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



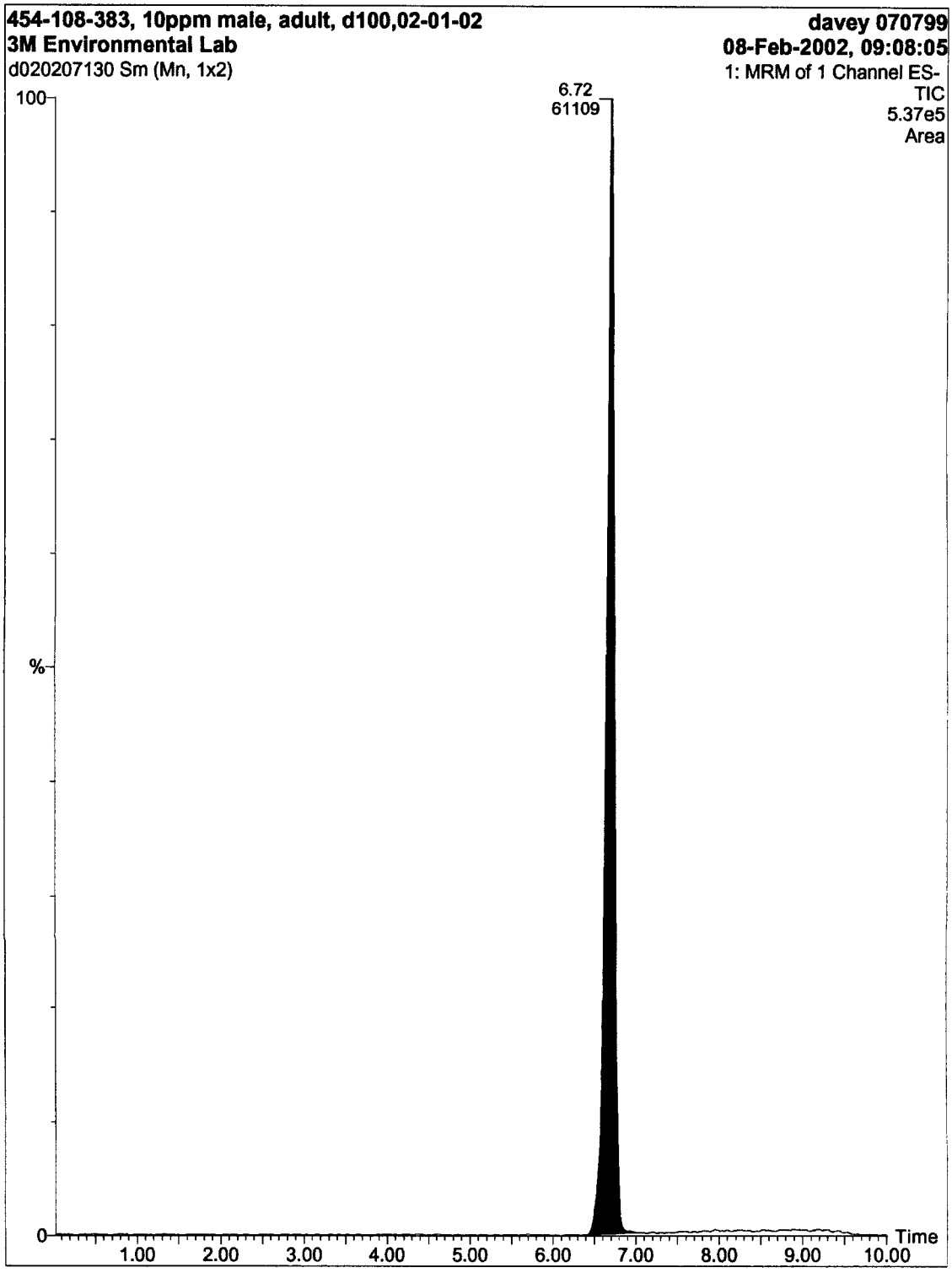
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



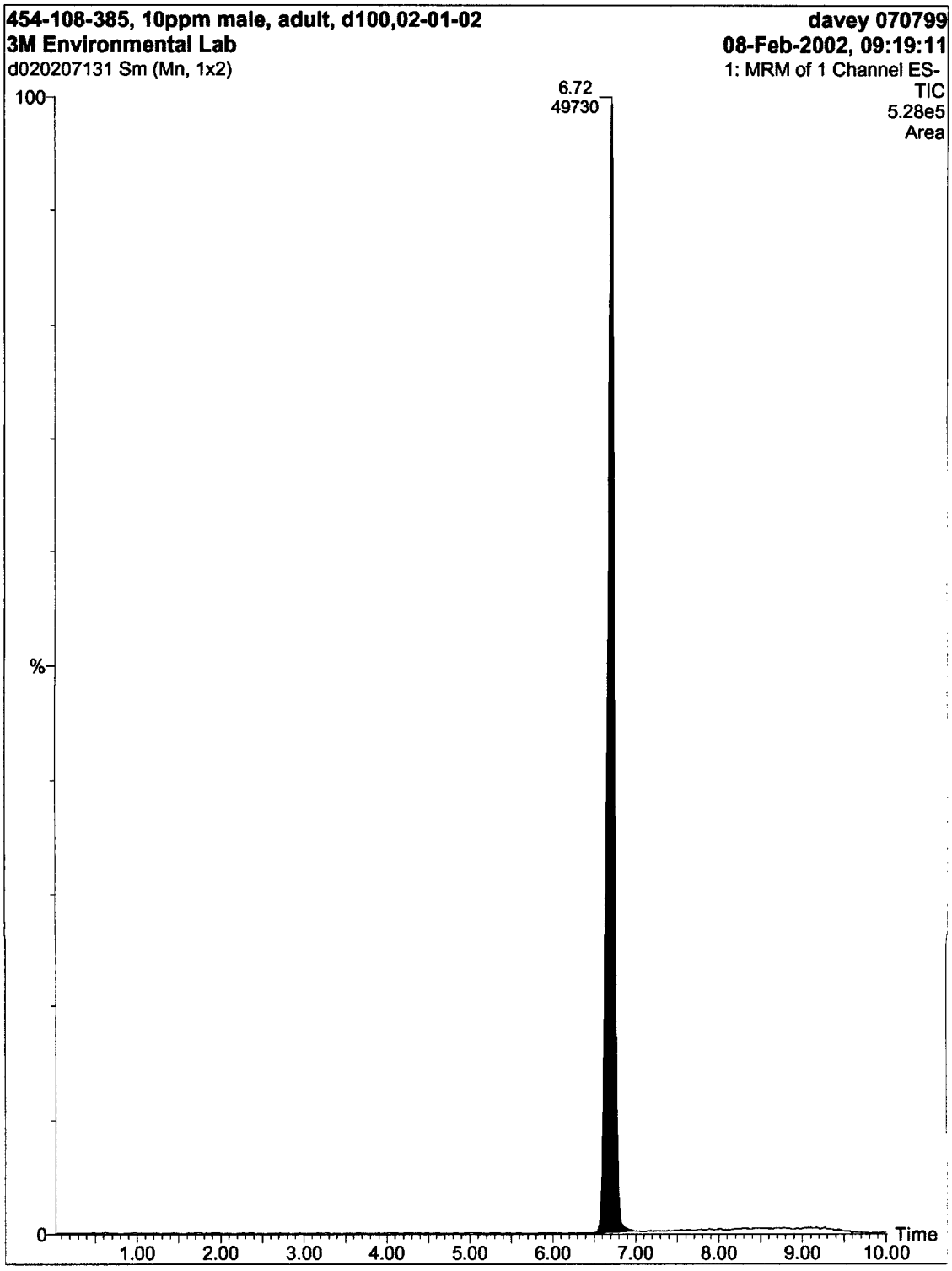
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



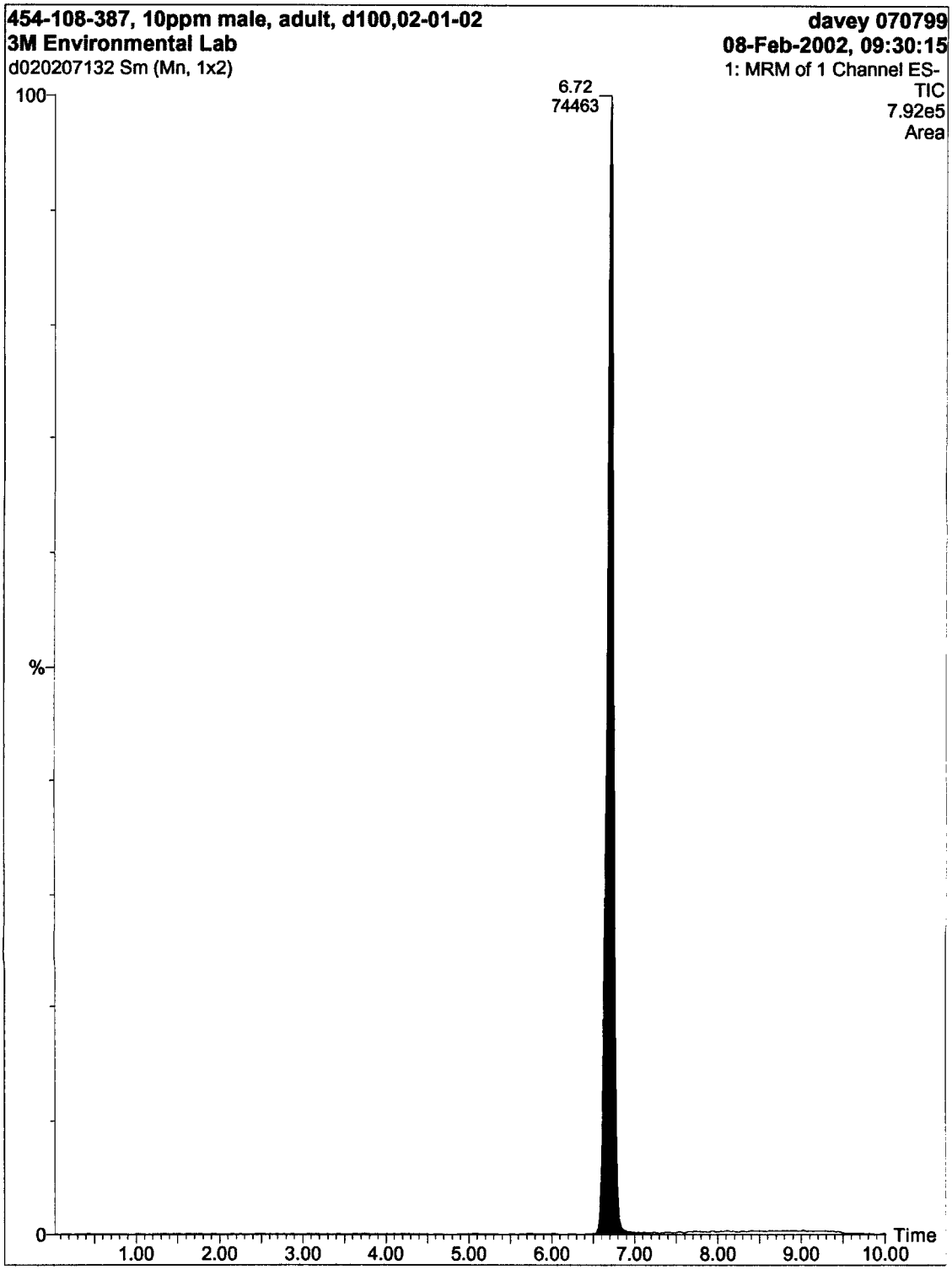
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

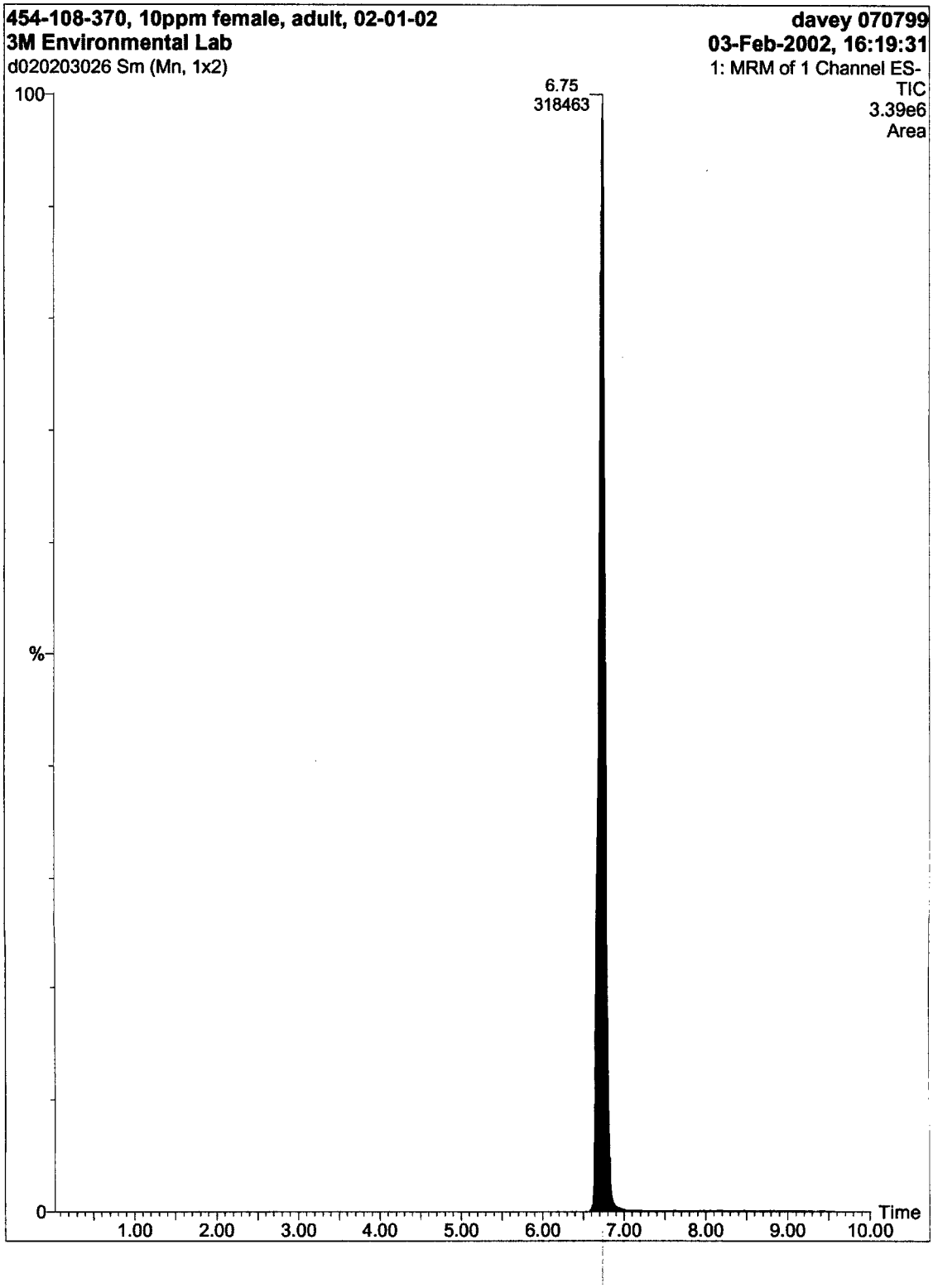


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

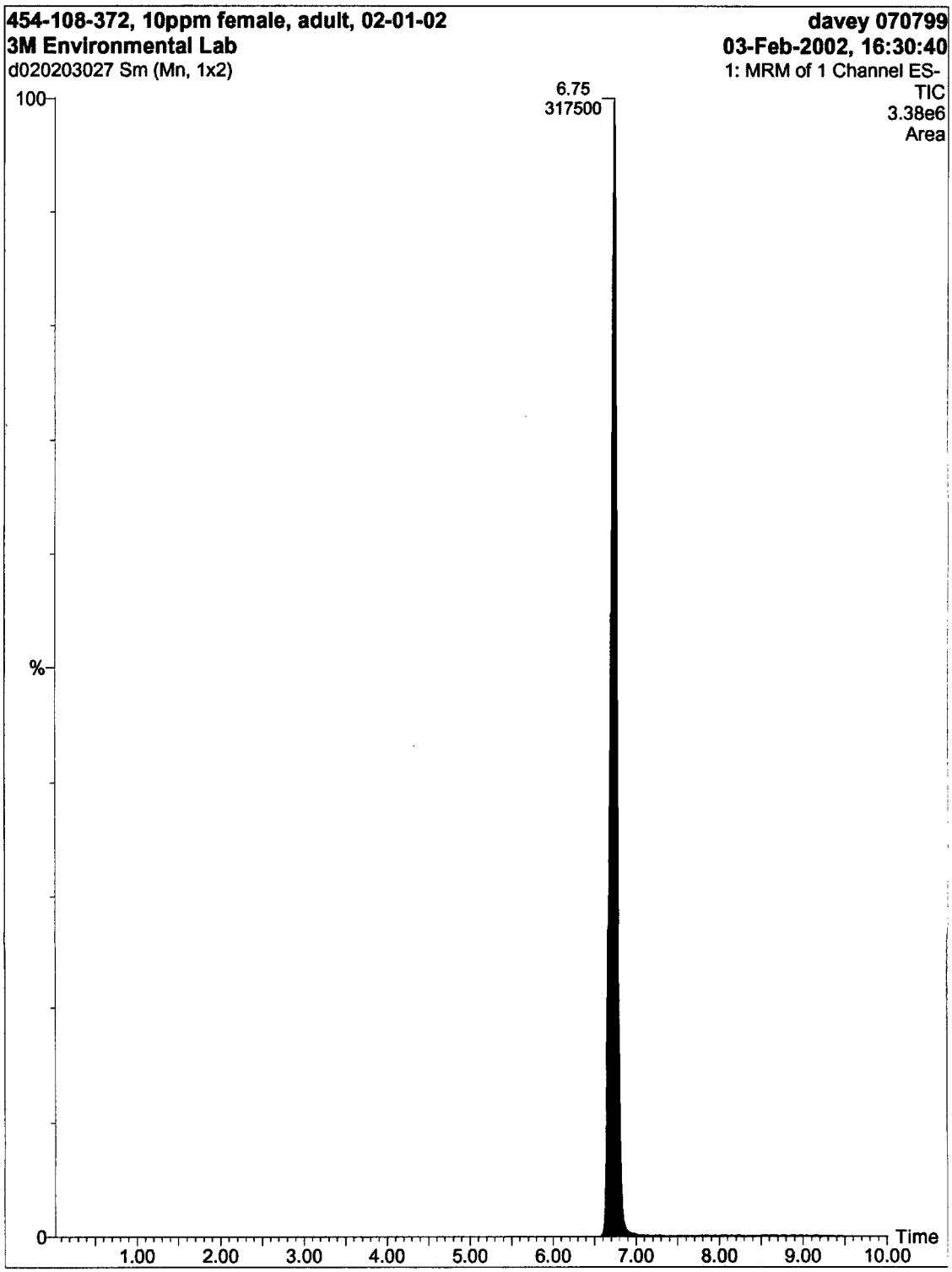


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

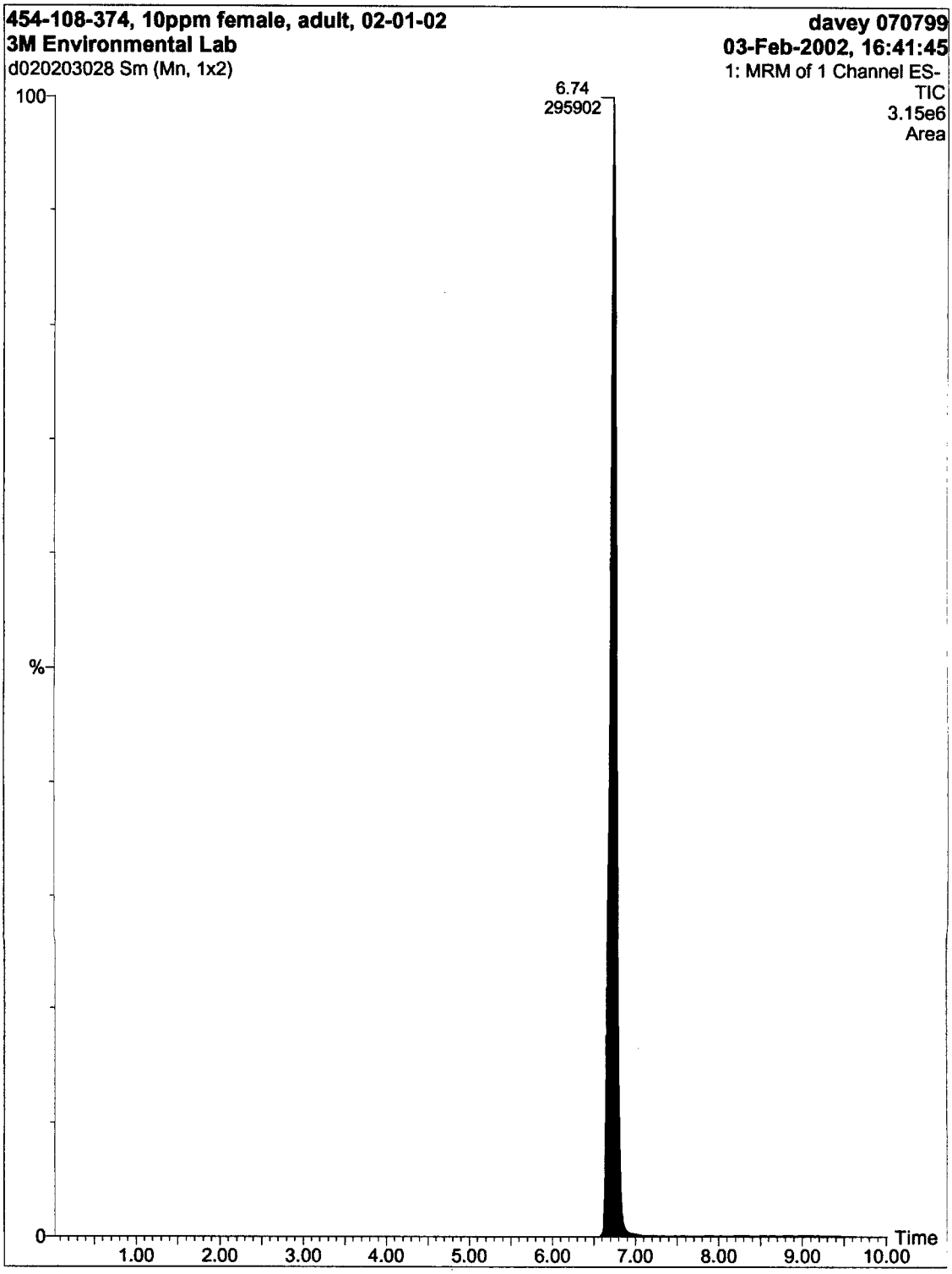
Dosed Group Adult Female Sera Samples



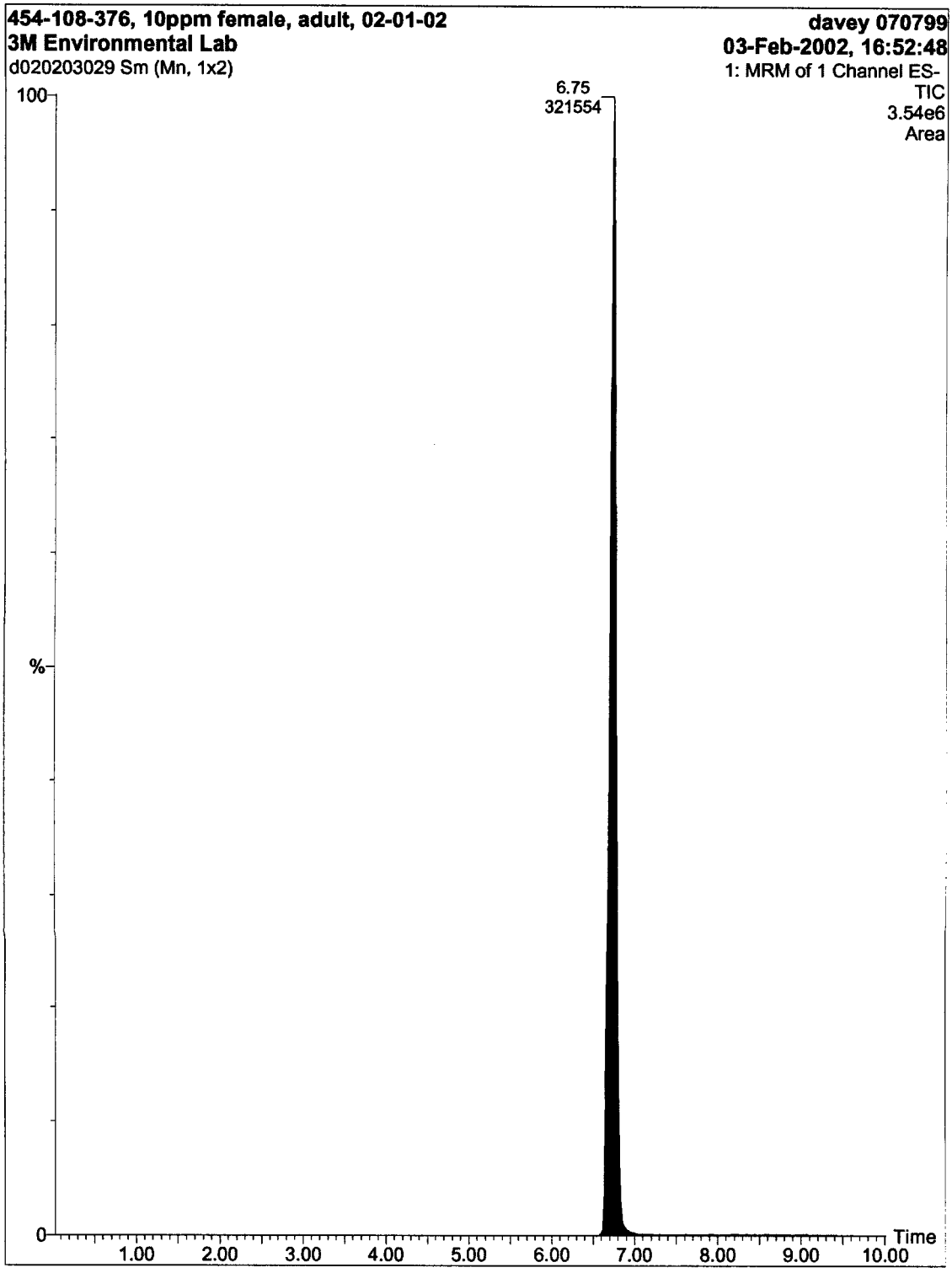
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



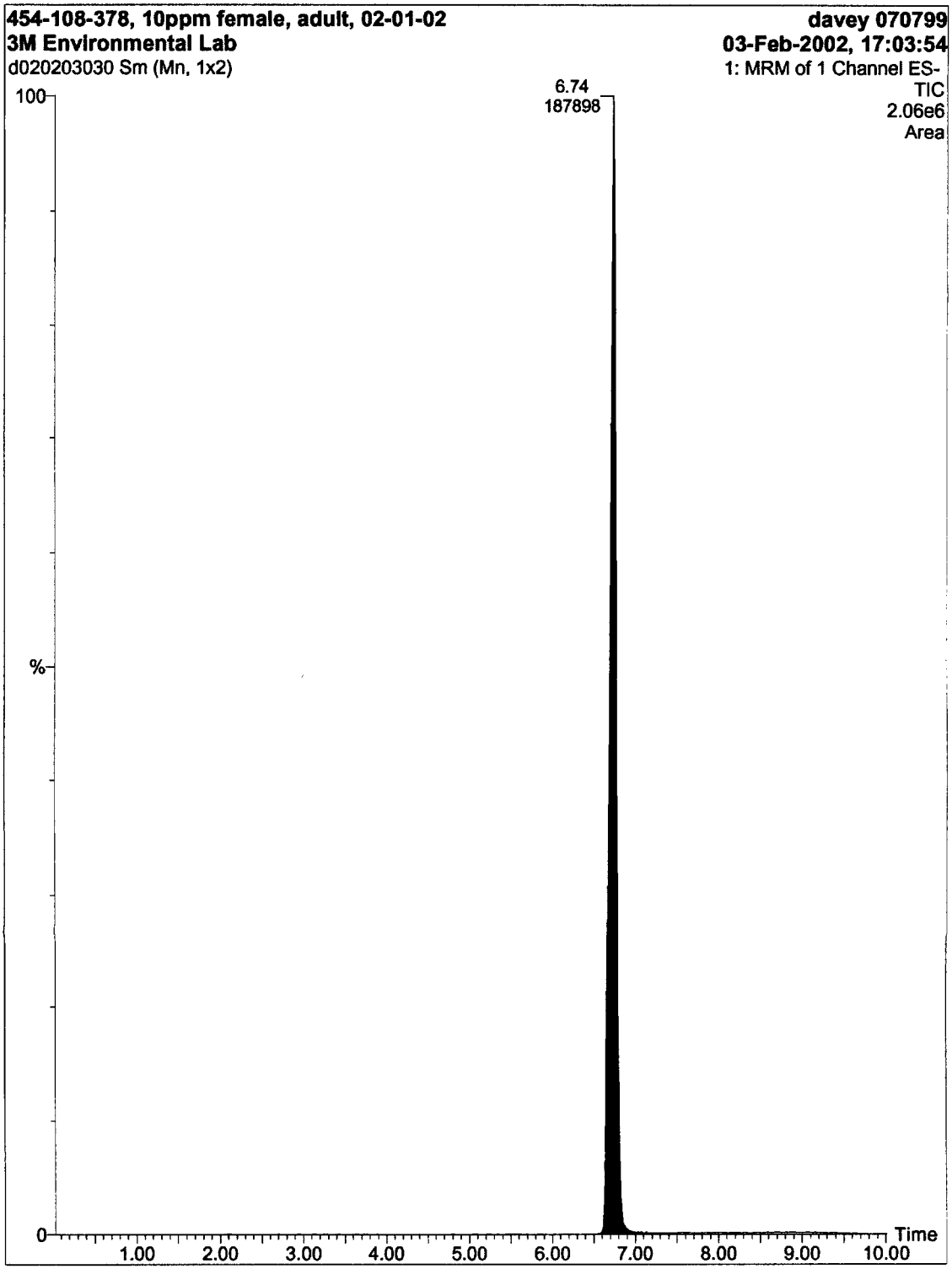
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



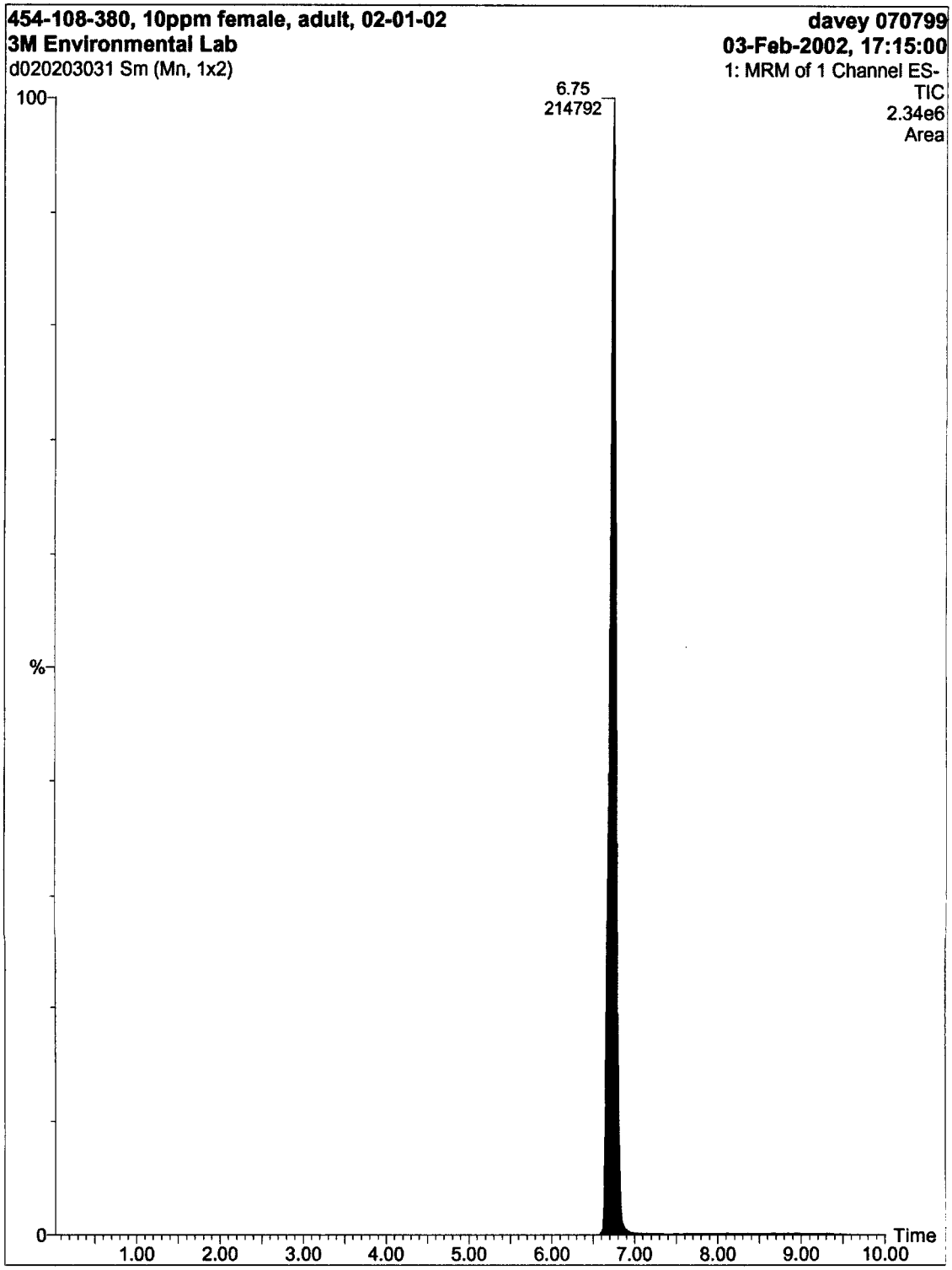
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



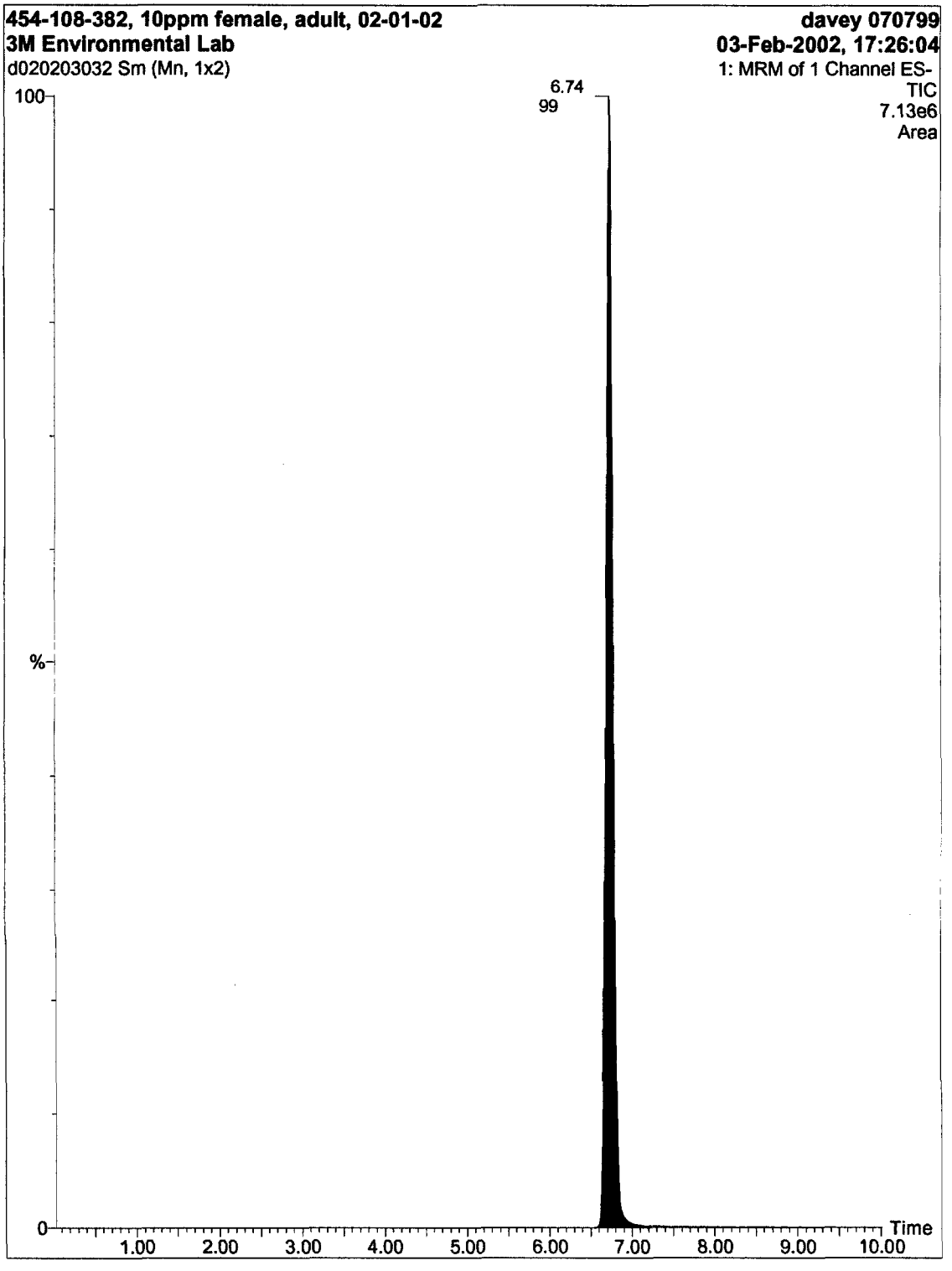
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



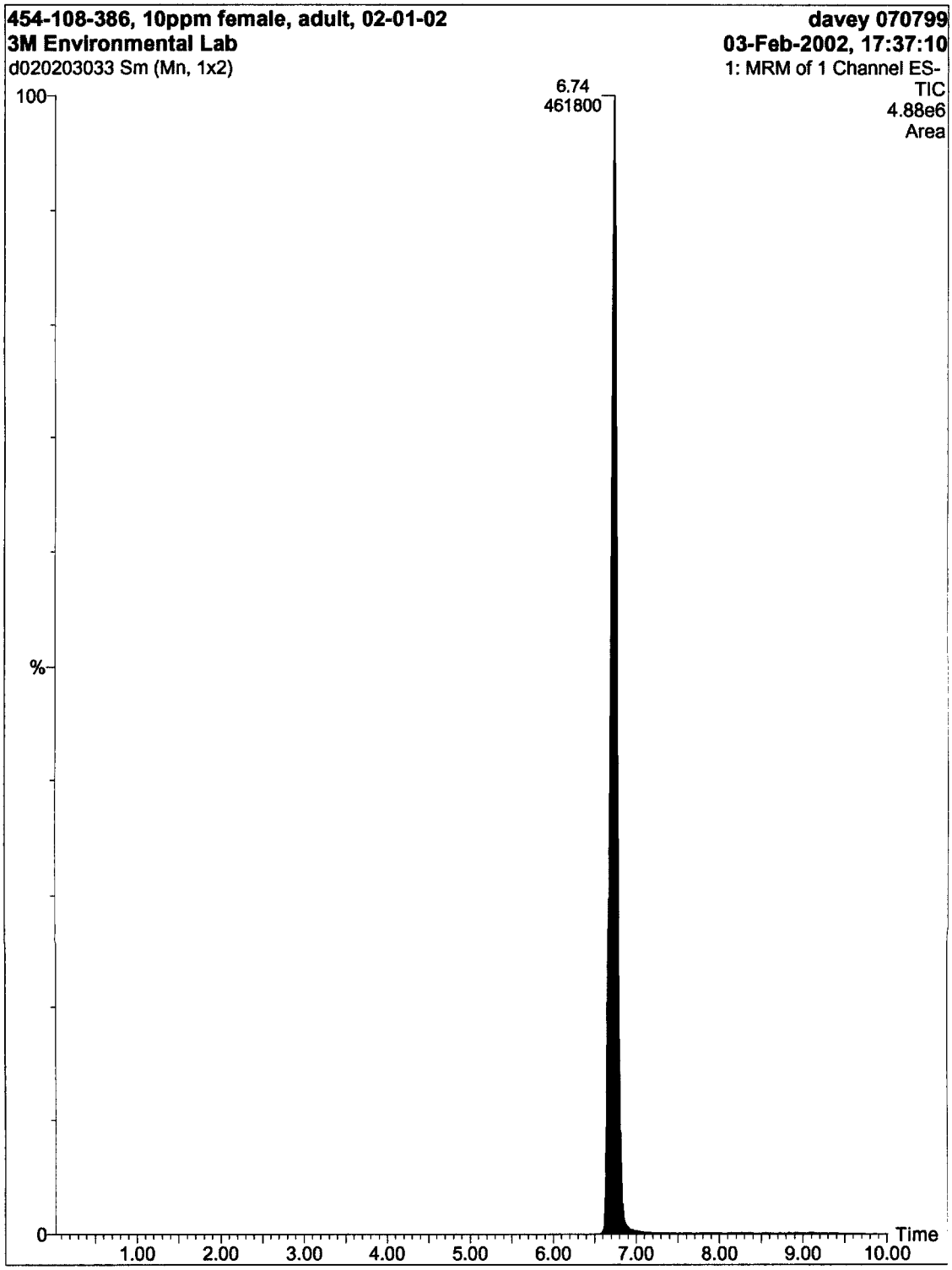
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



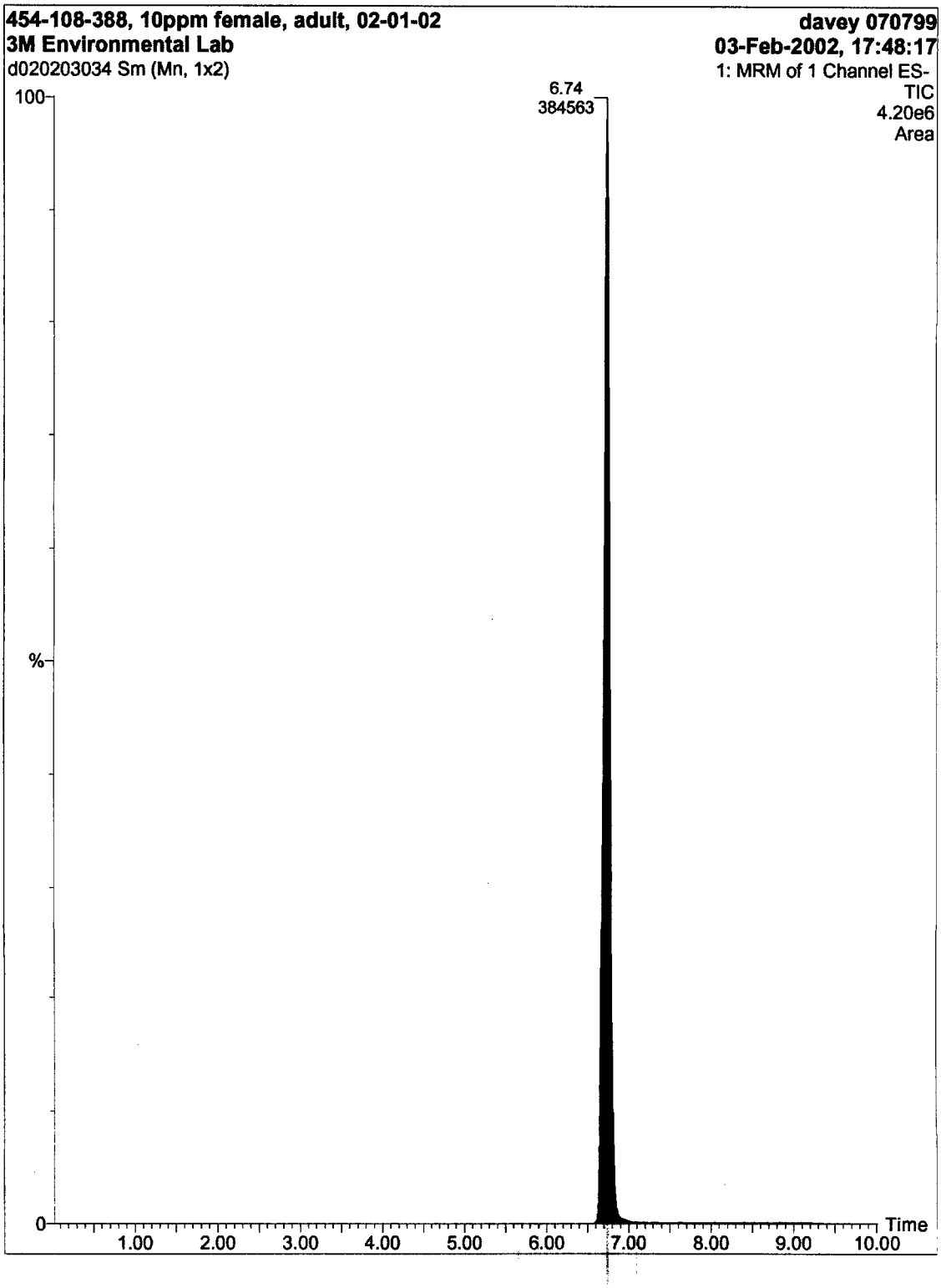
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



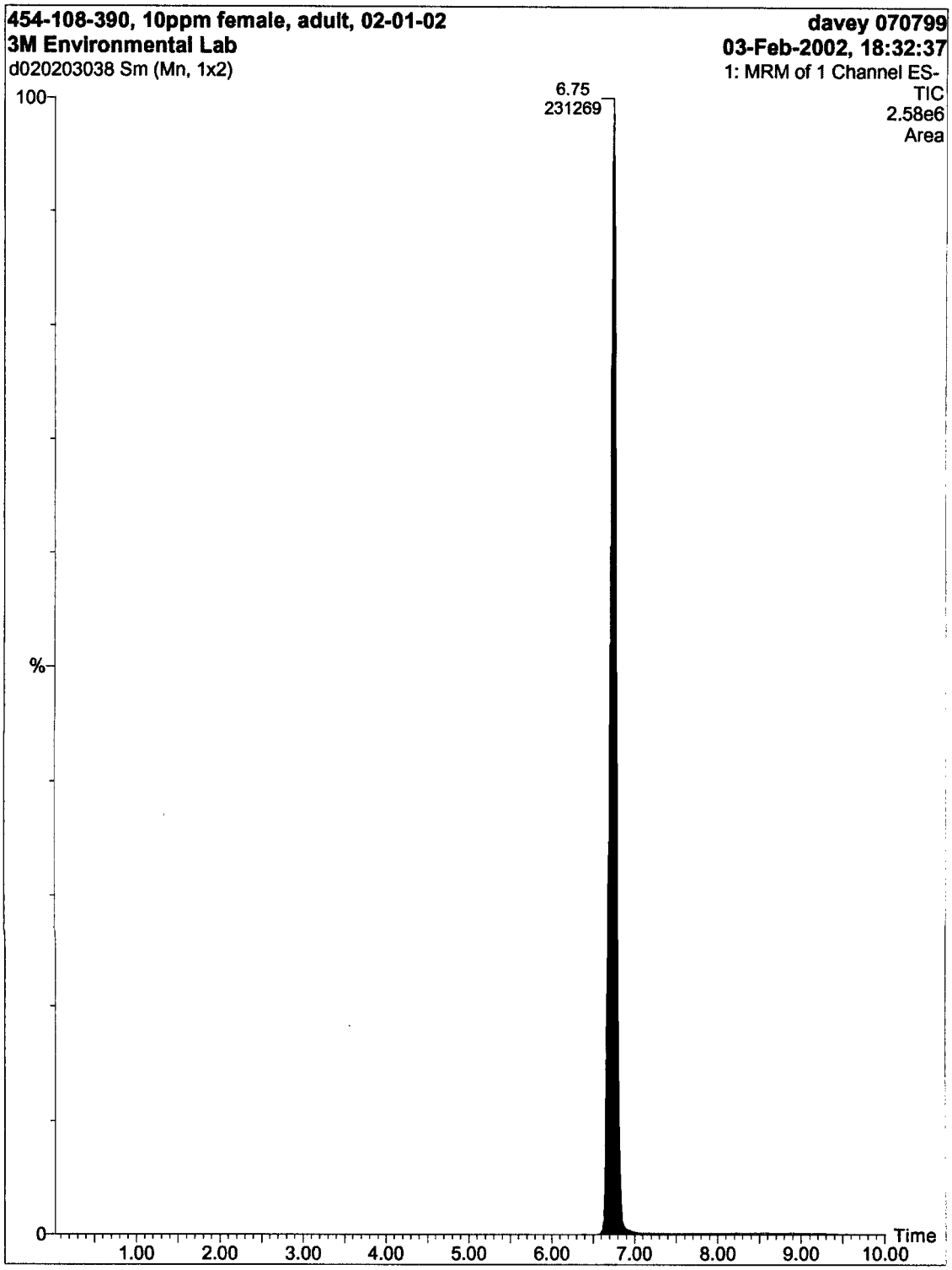
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

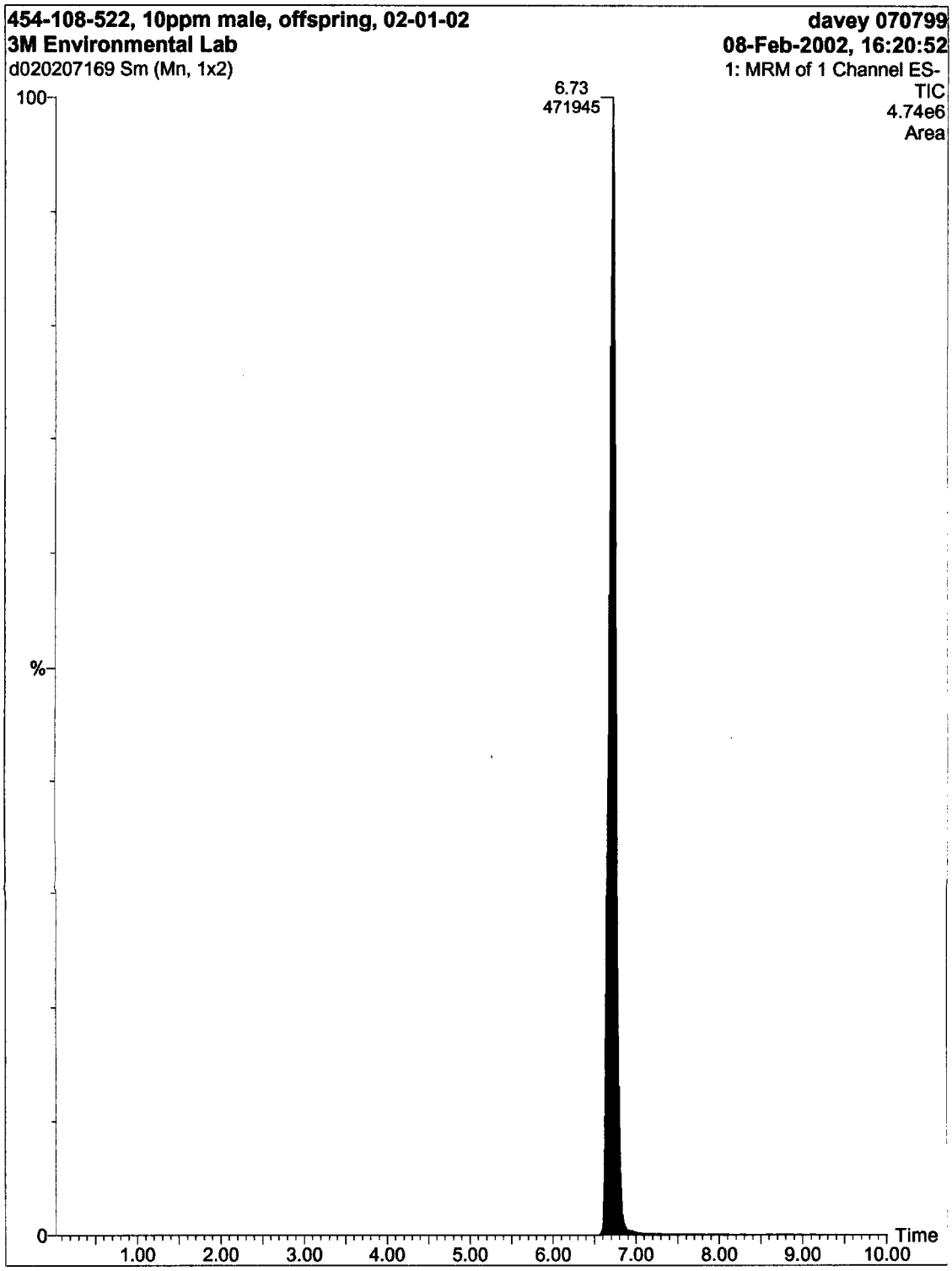


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

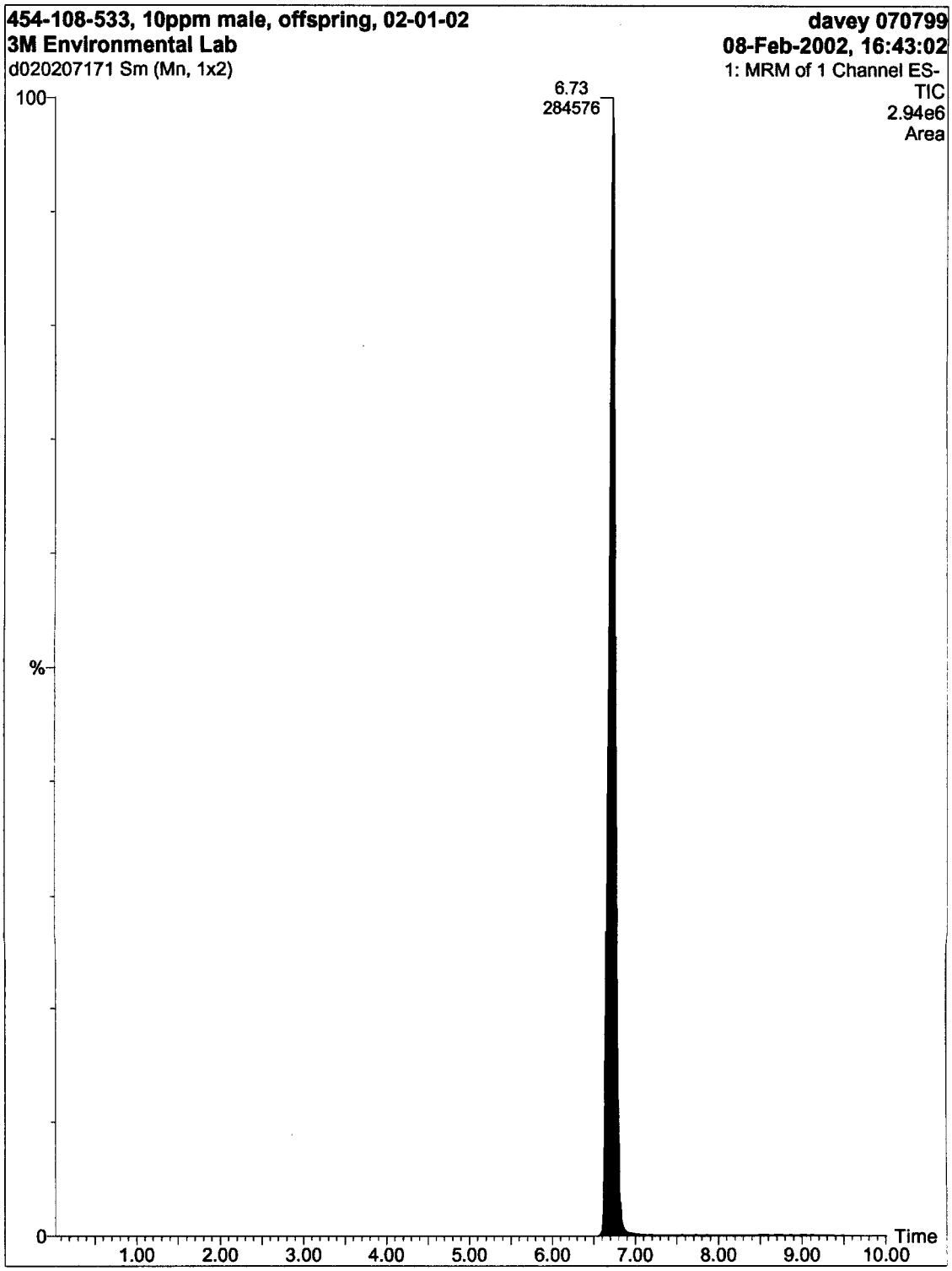


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

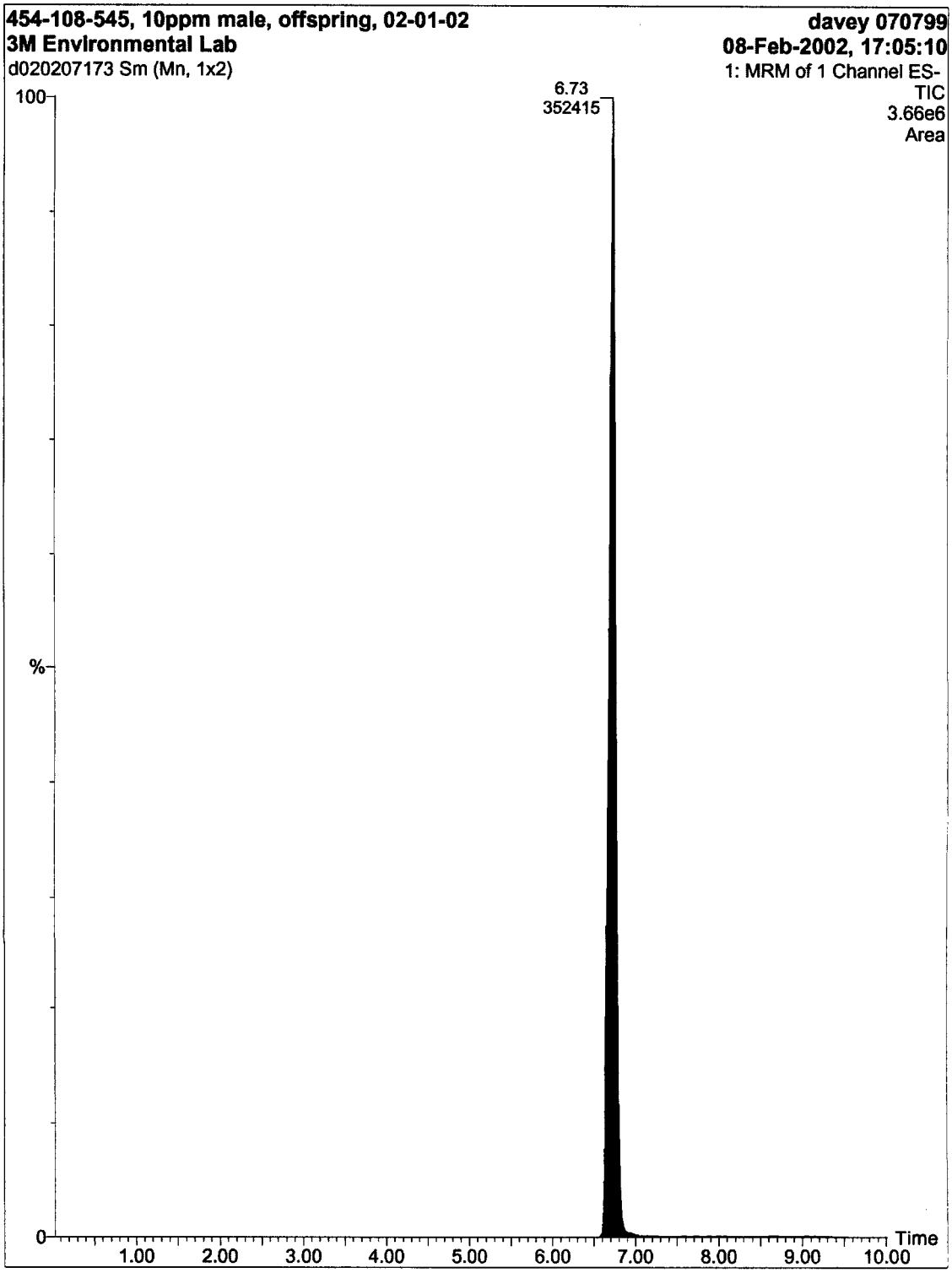
Dosed Group Male Offspring Sera Samples



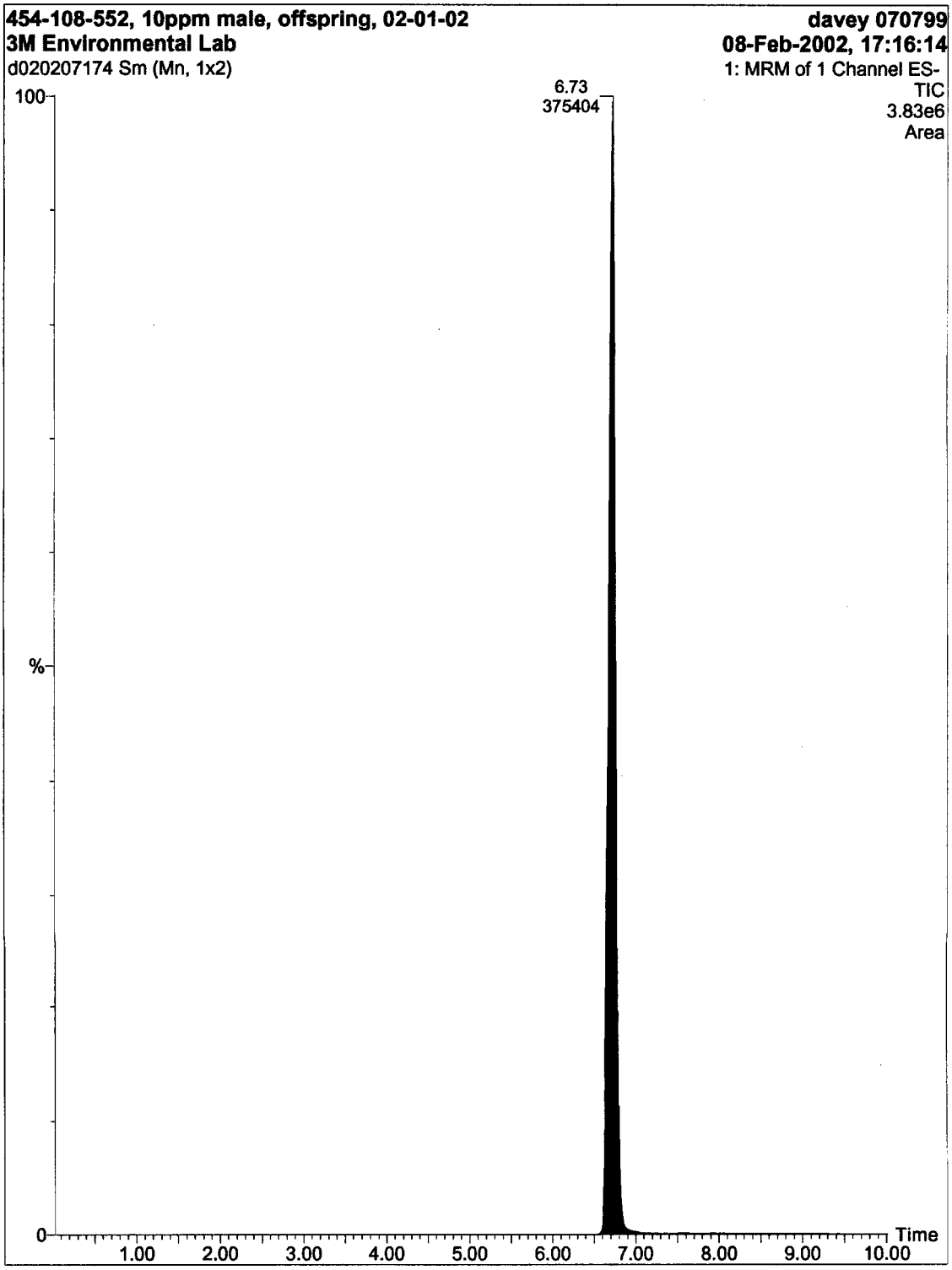
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



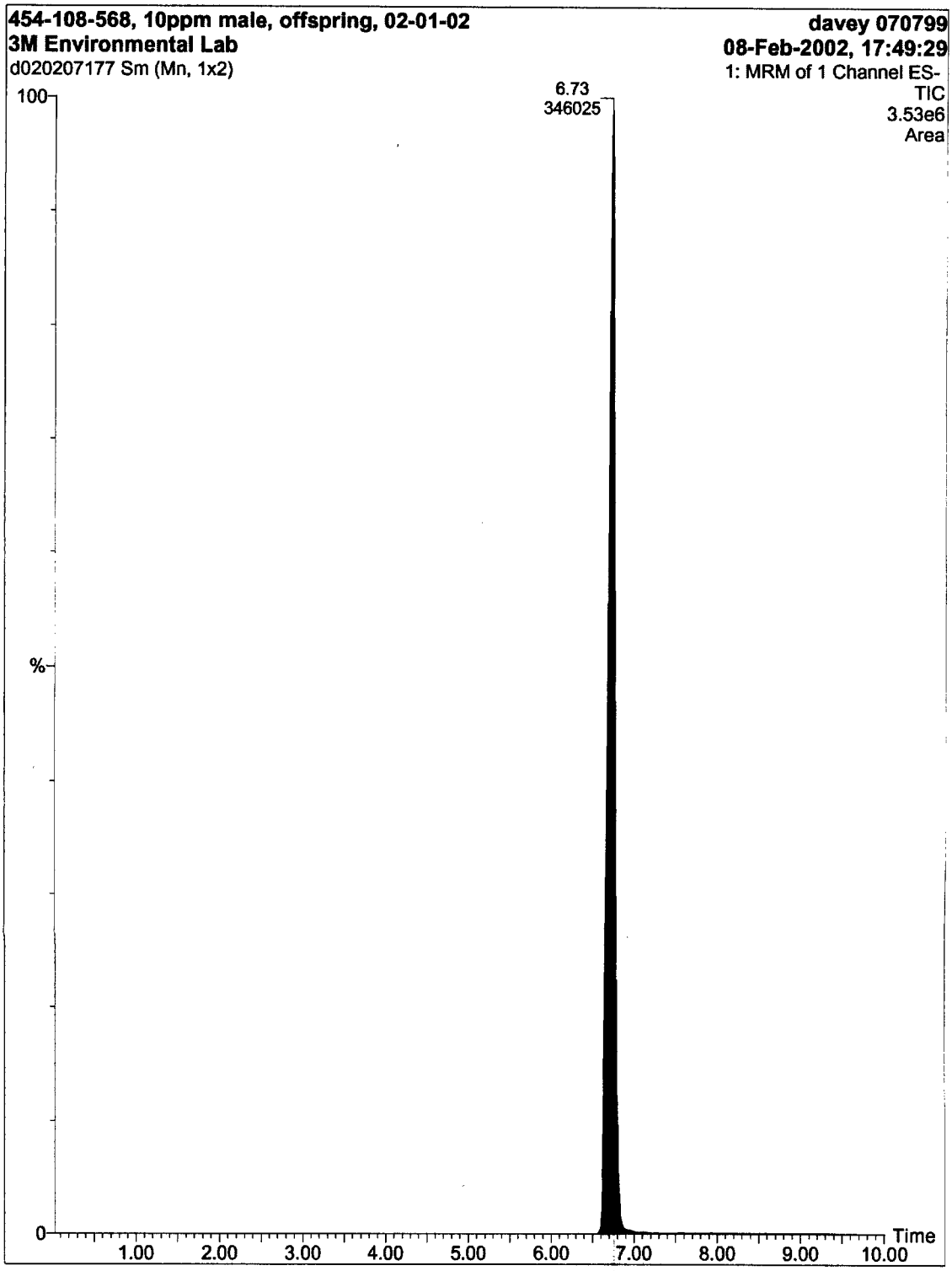
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

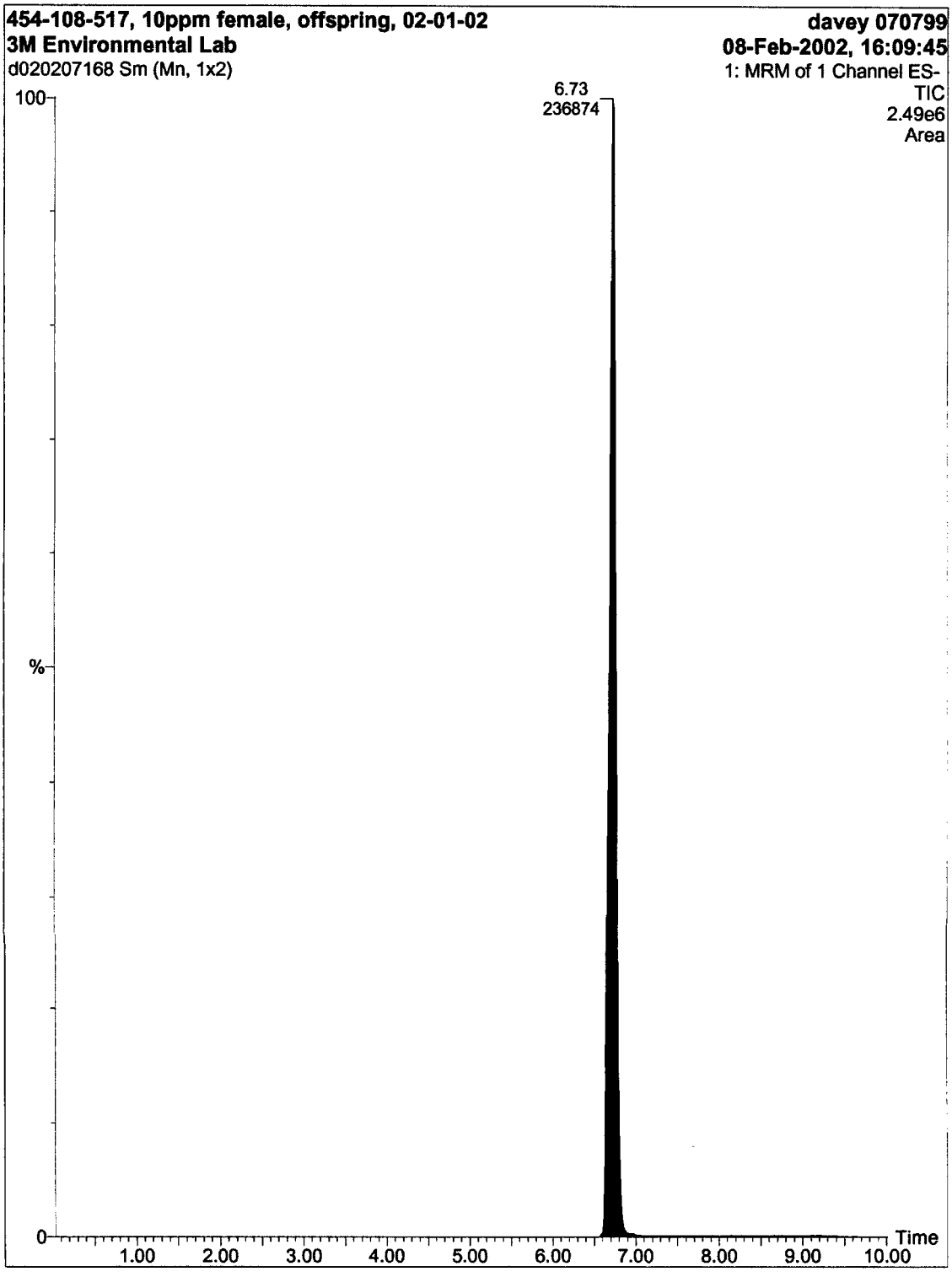


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

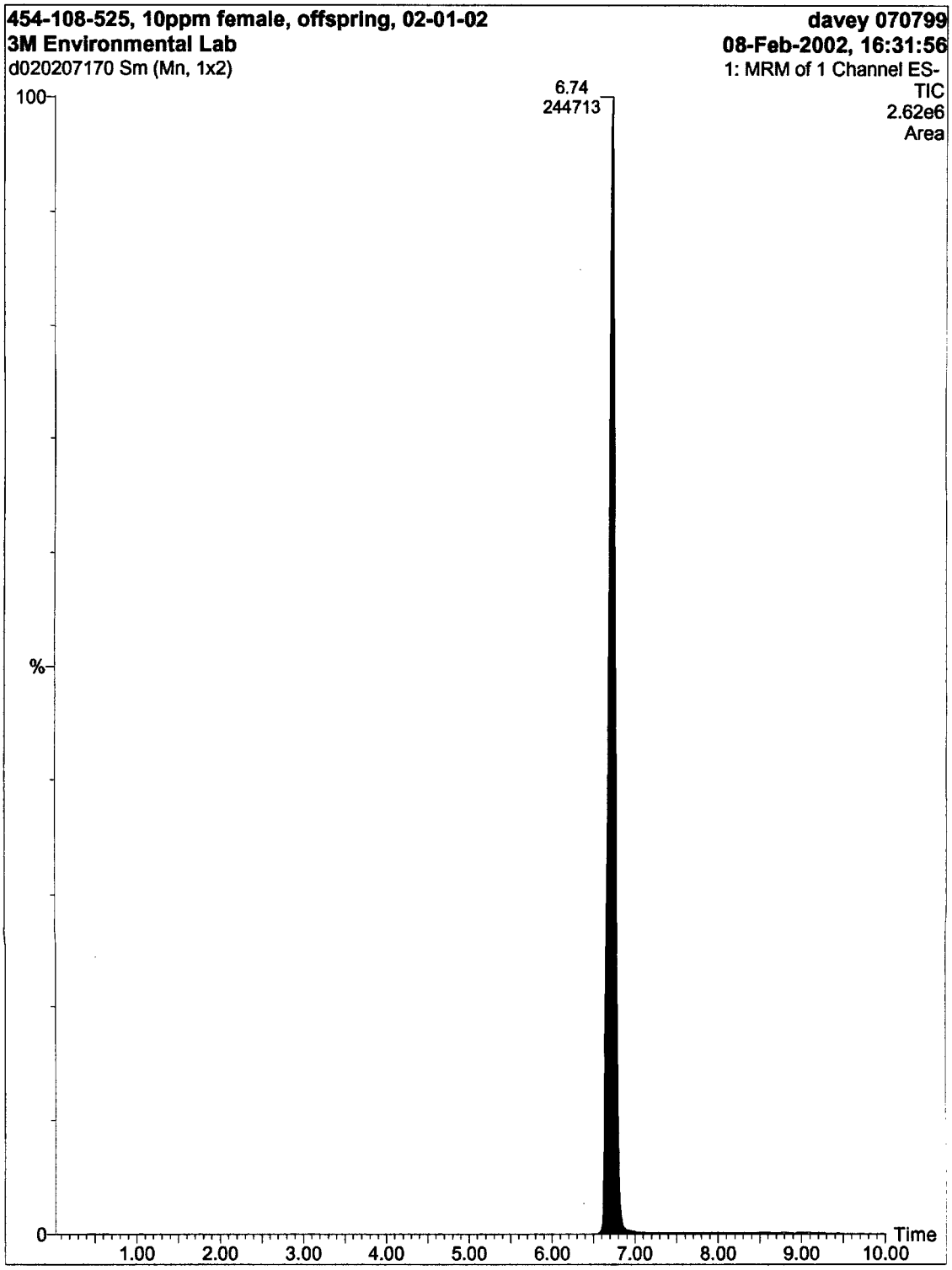


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

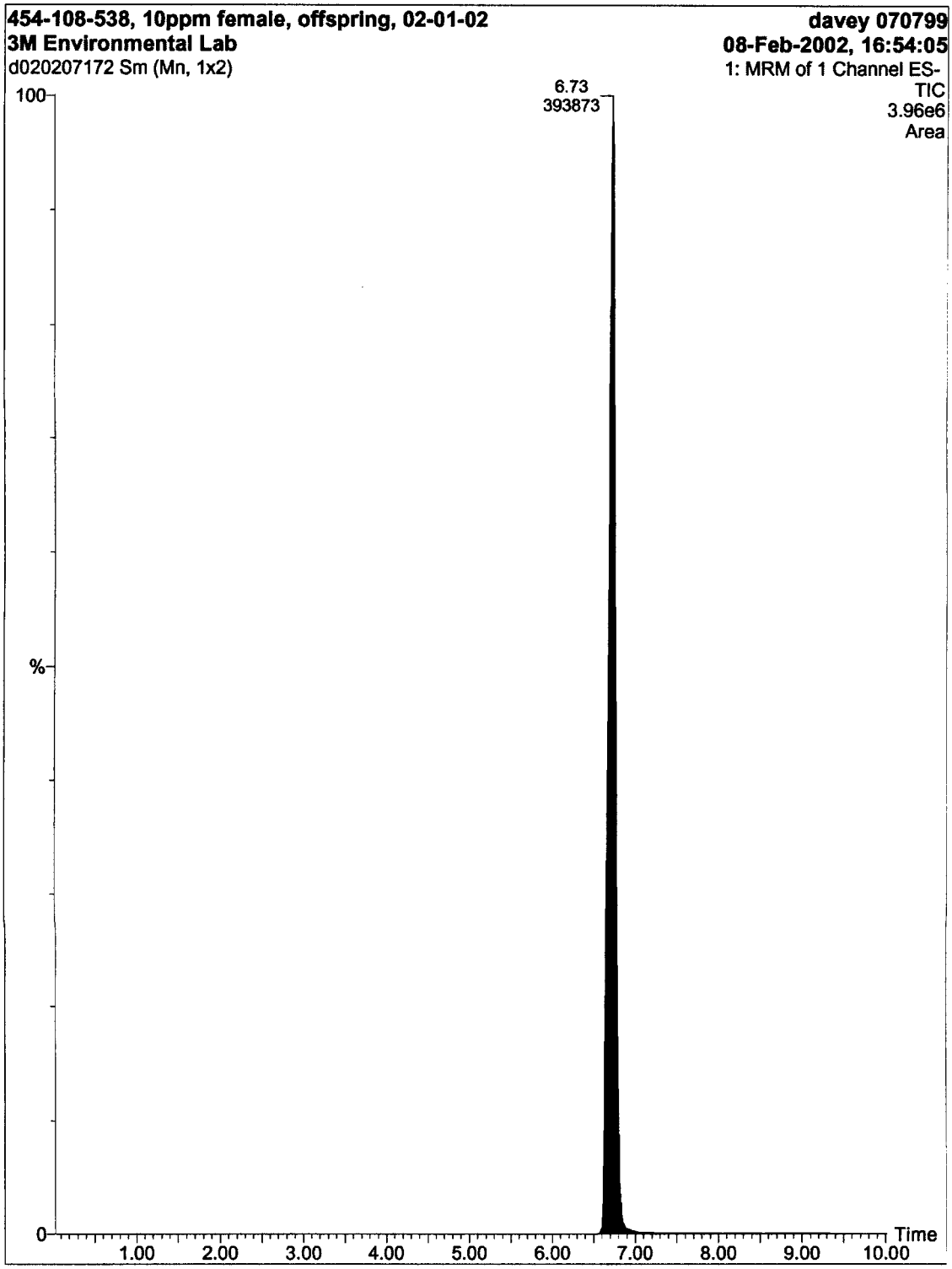
Dosed Group Female Offspring Sera Samples



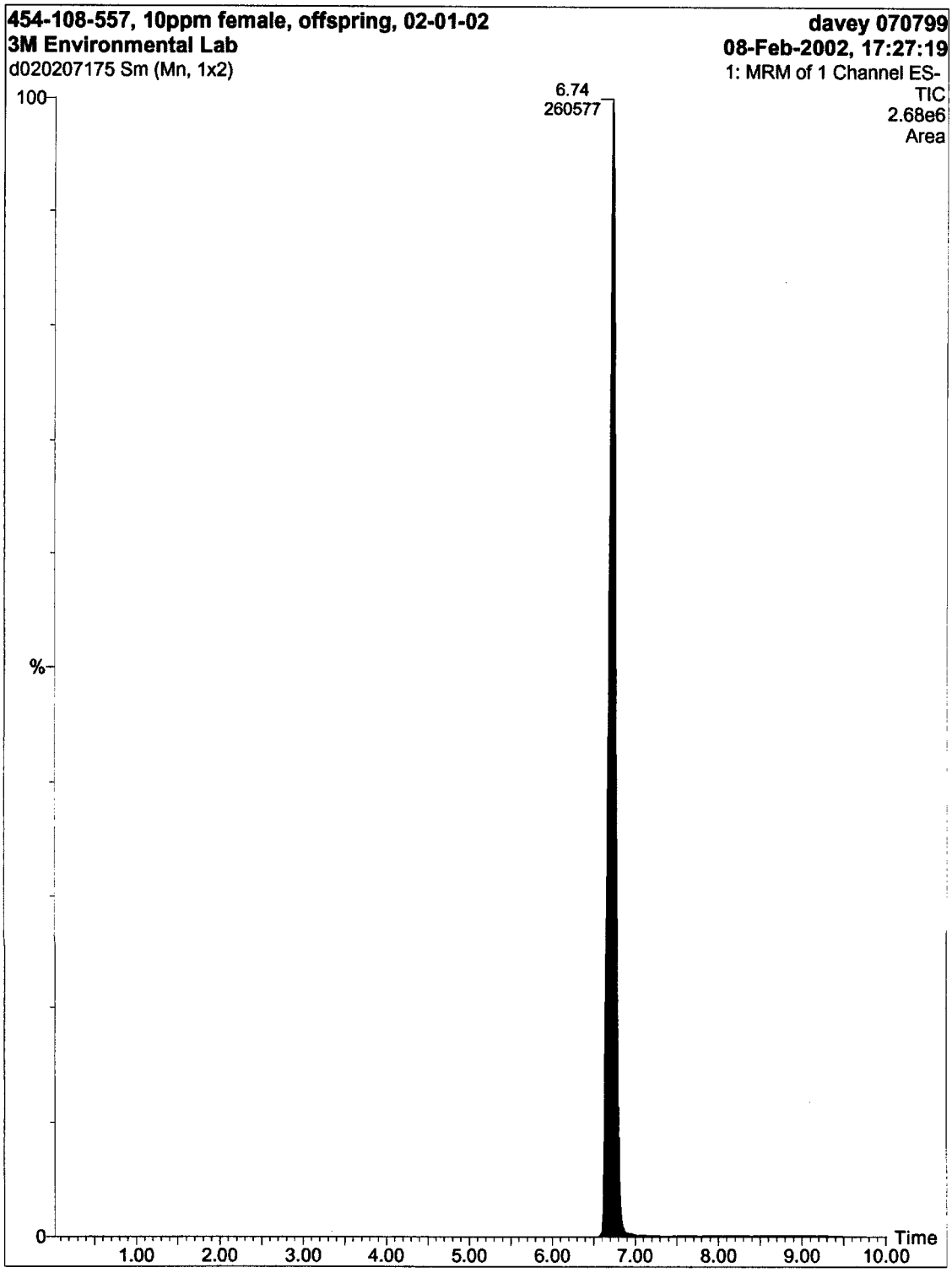
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



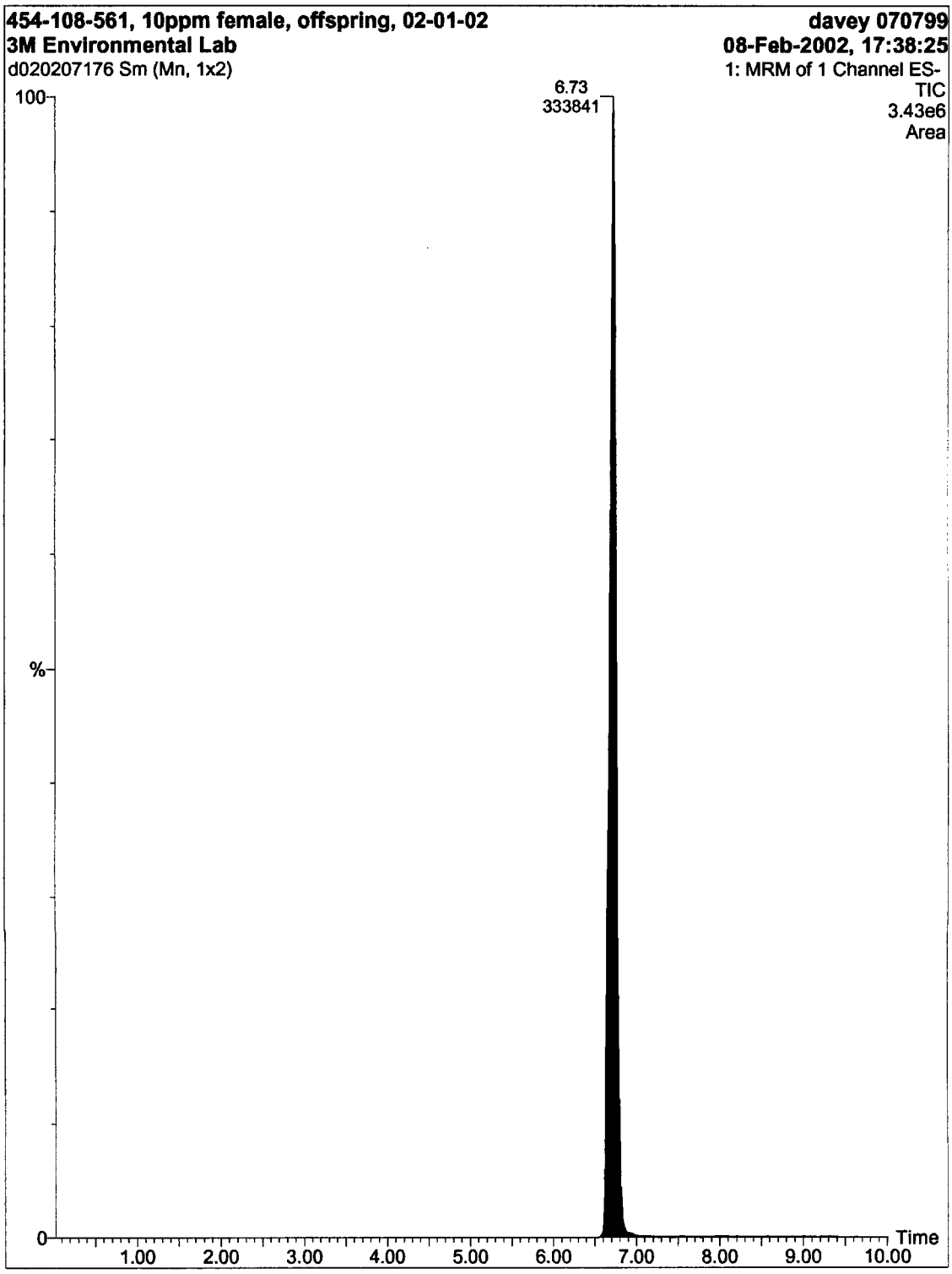
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

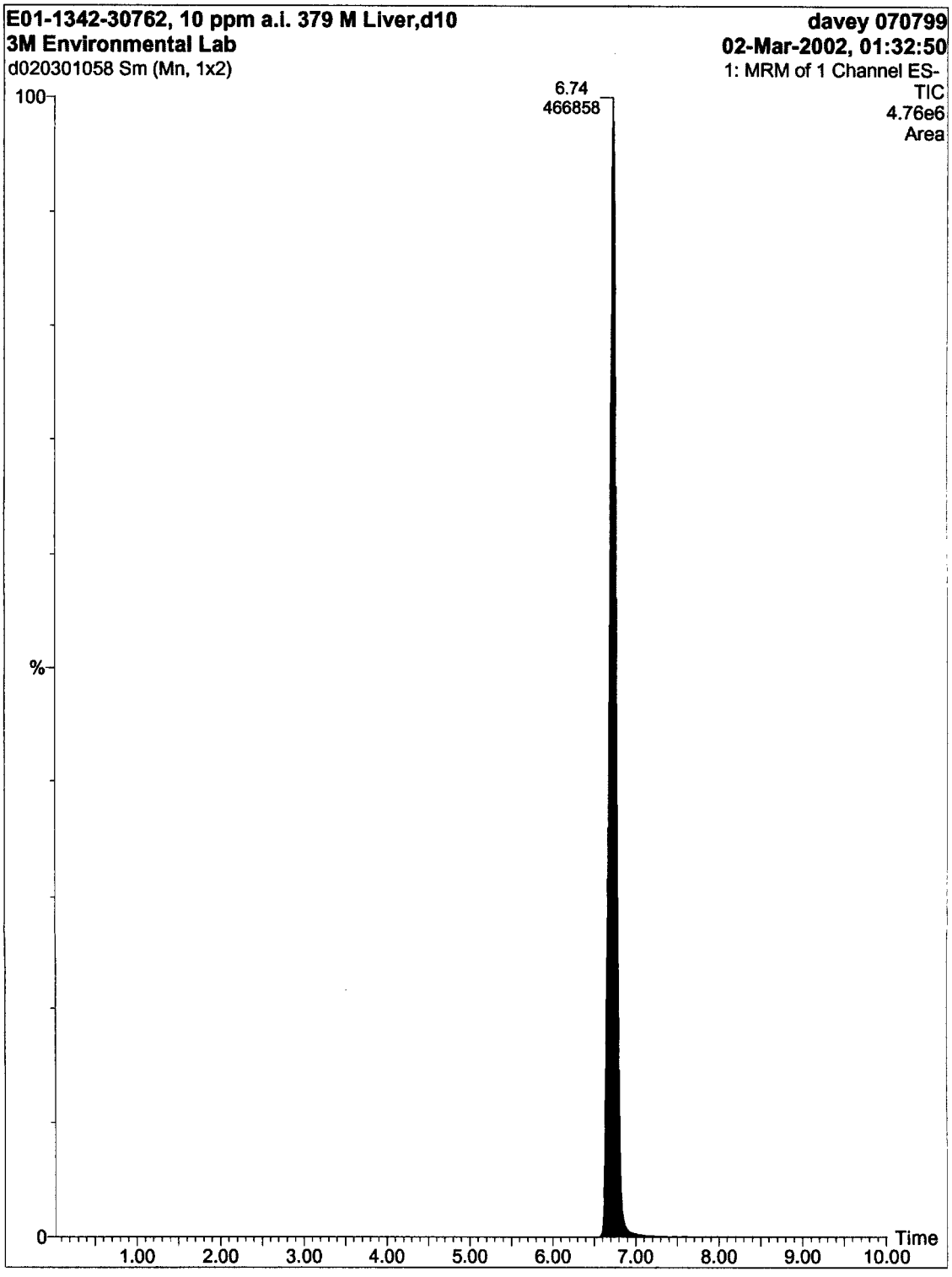


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

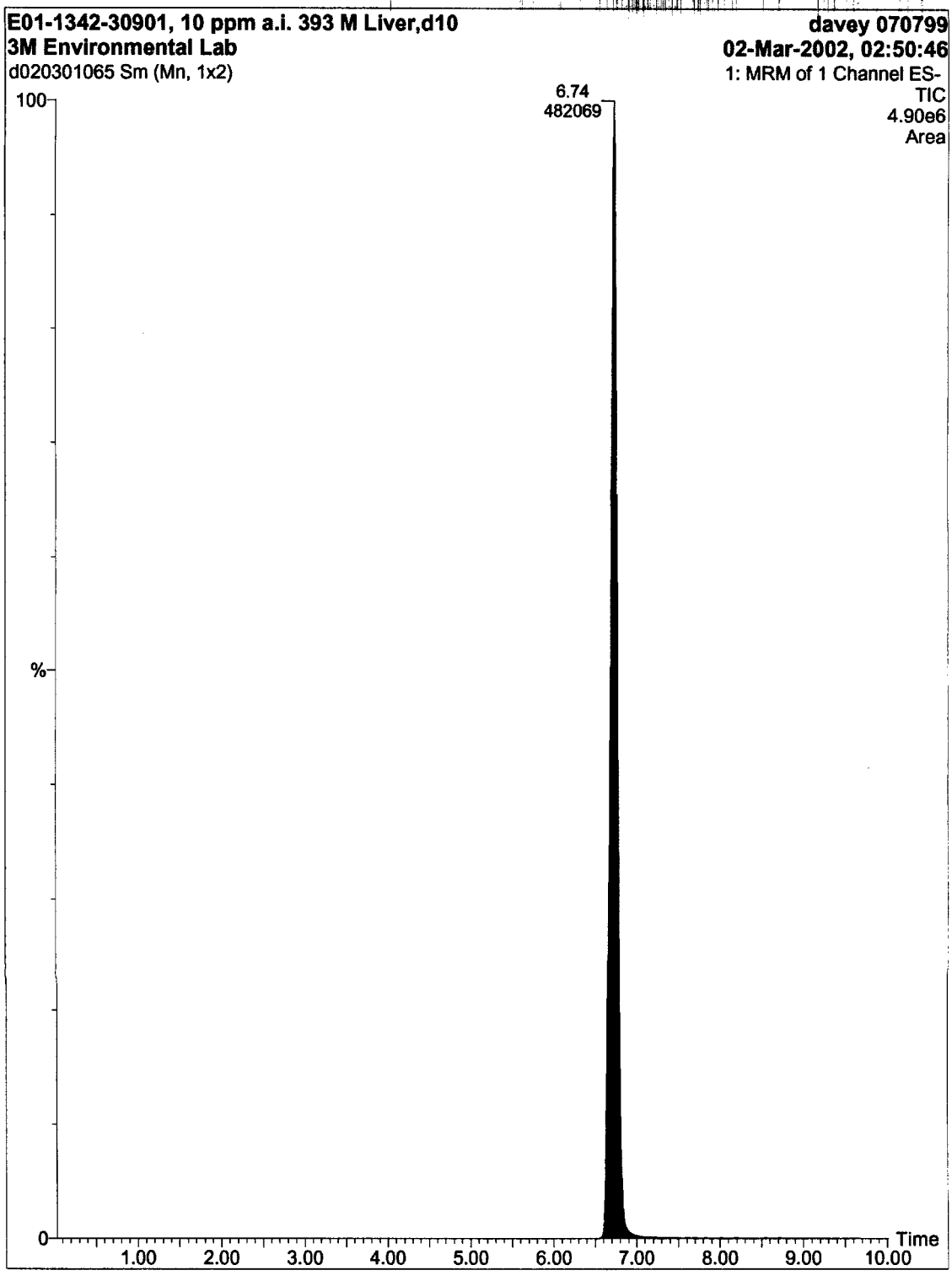


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

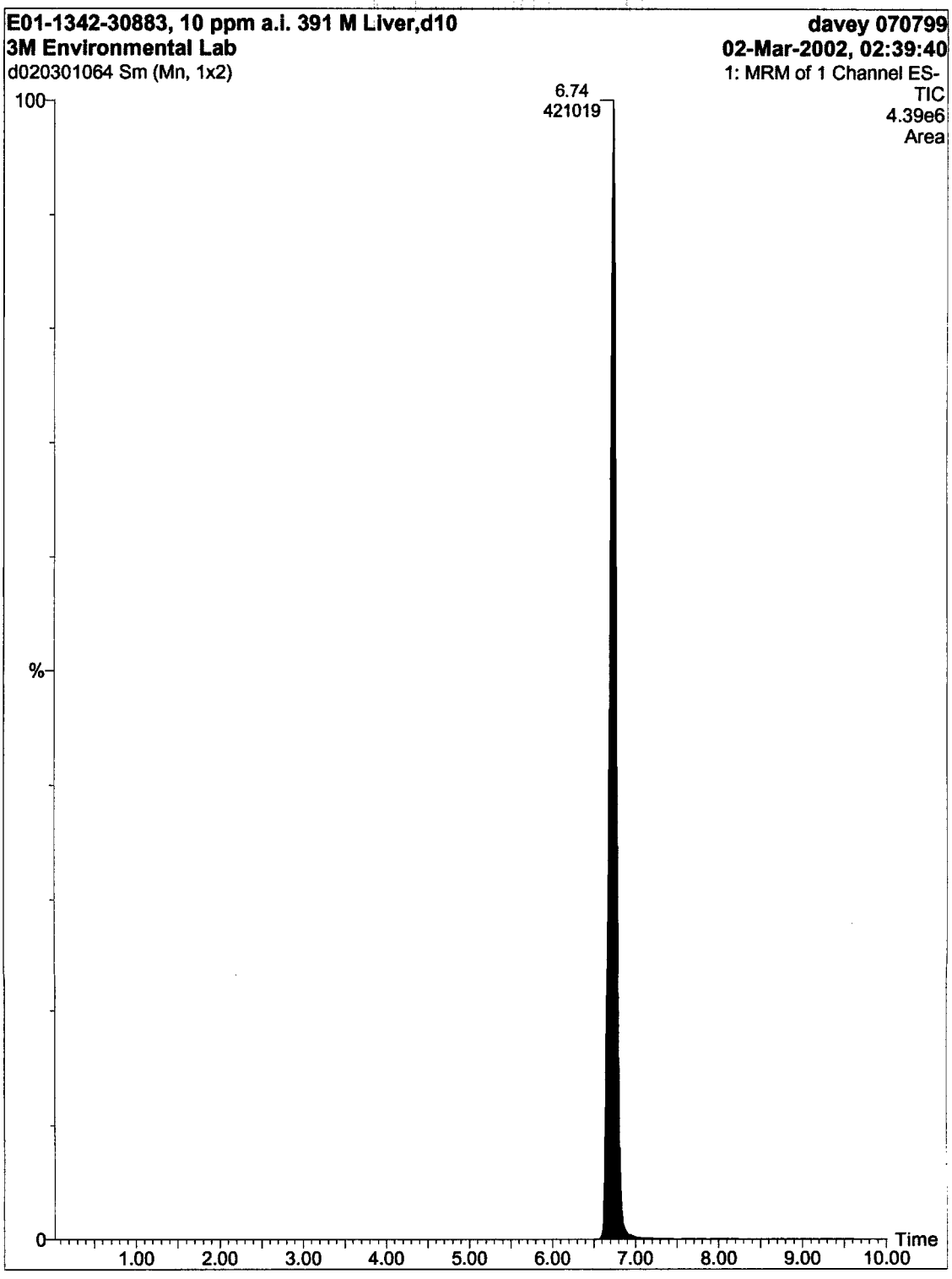
Dosed Group Male Liver Samples



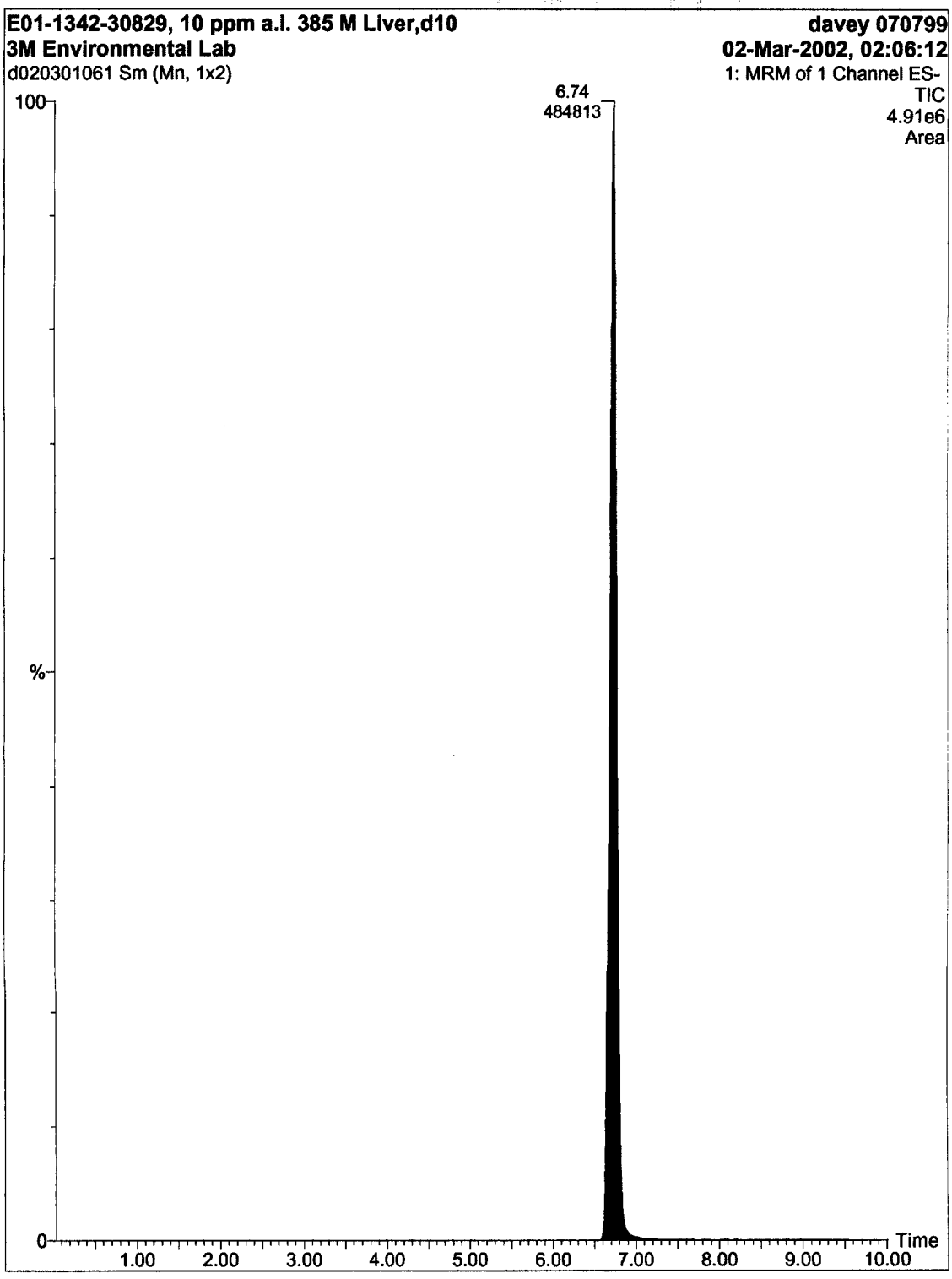
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



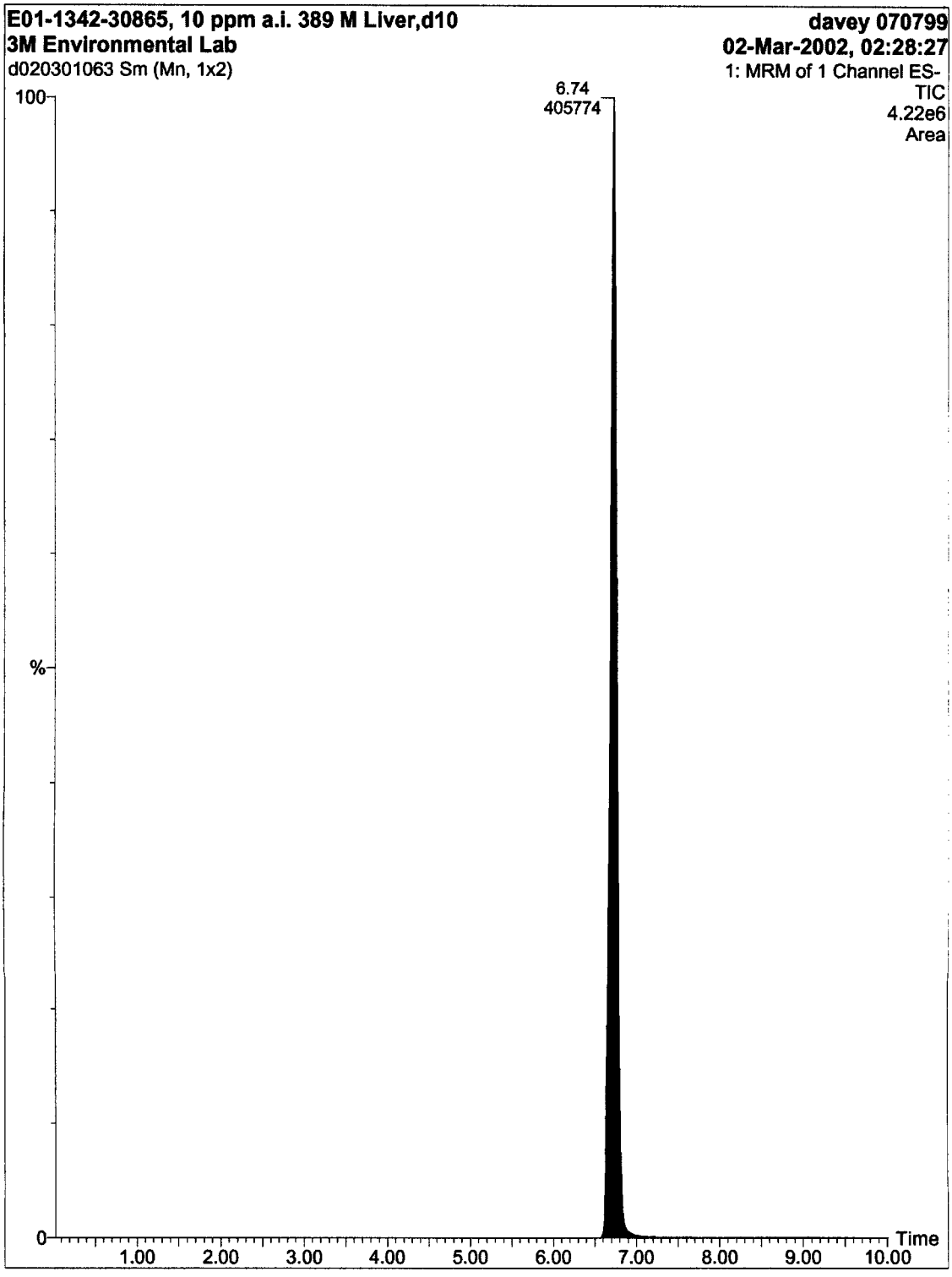
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



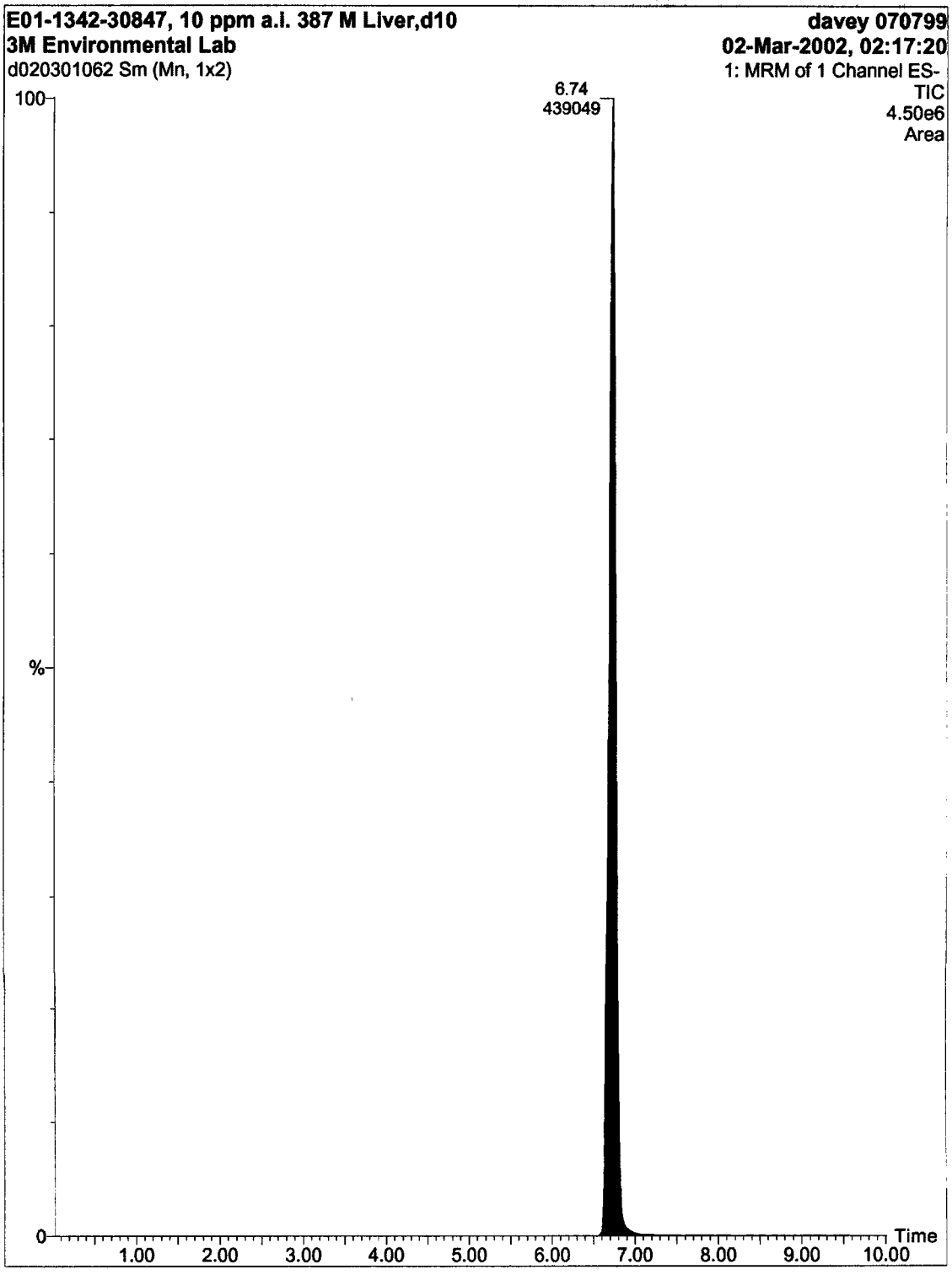
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



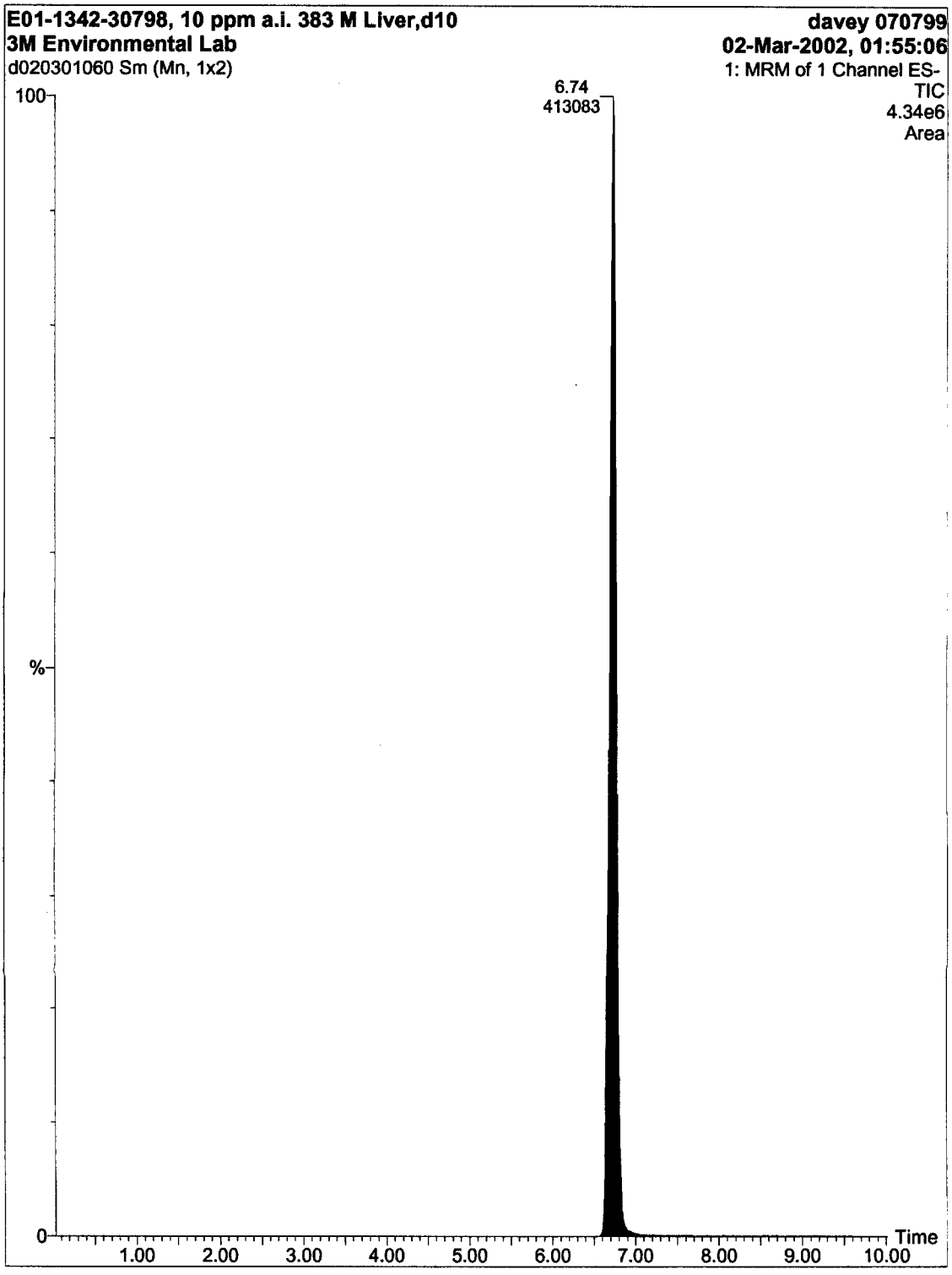
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



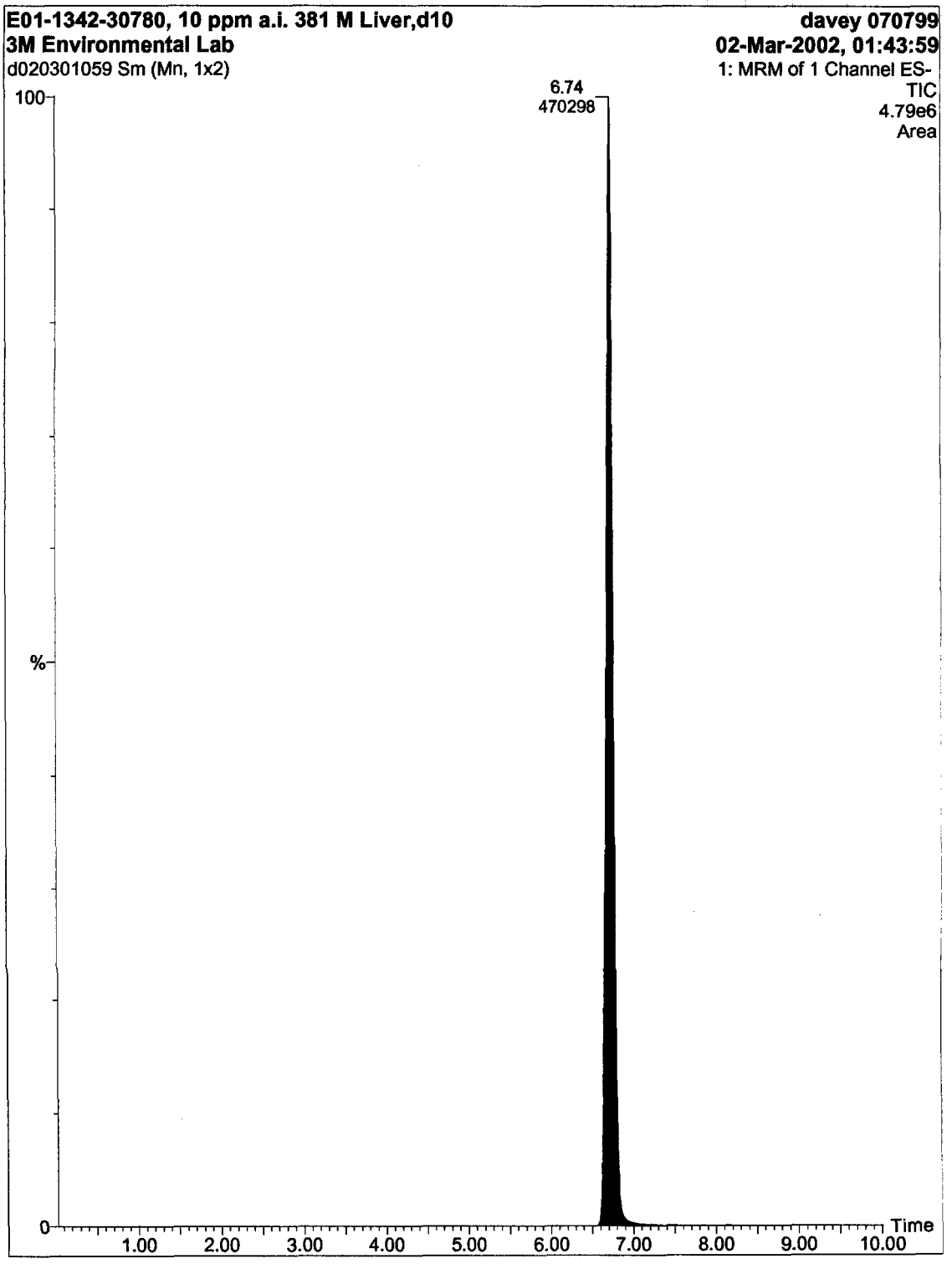
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



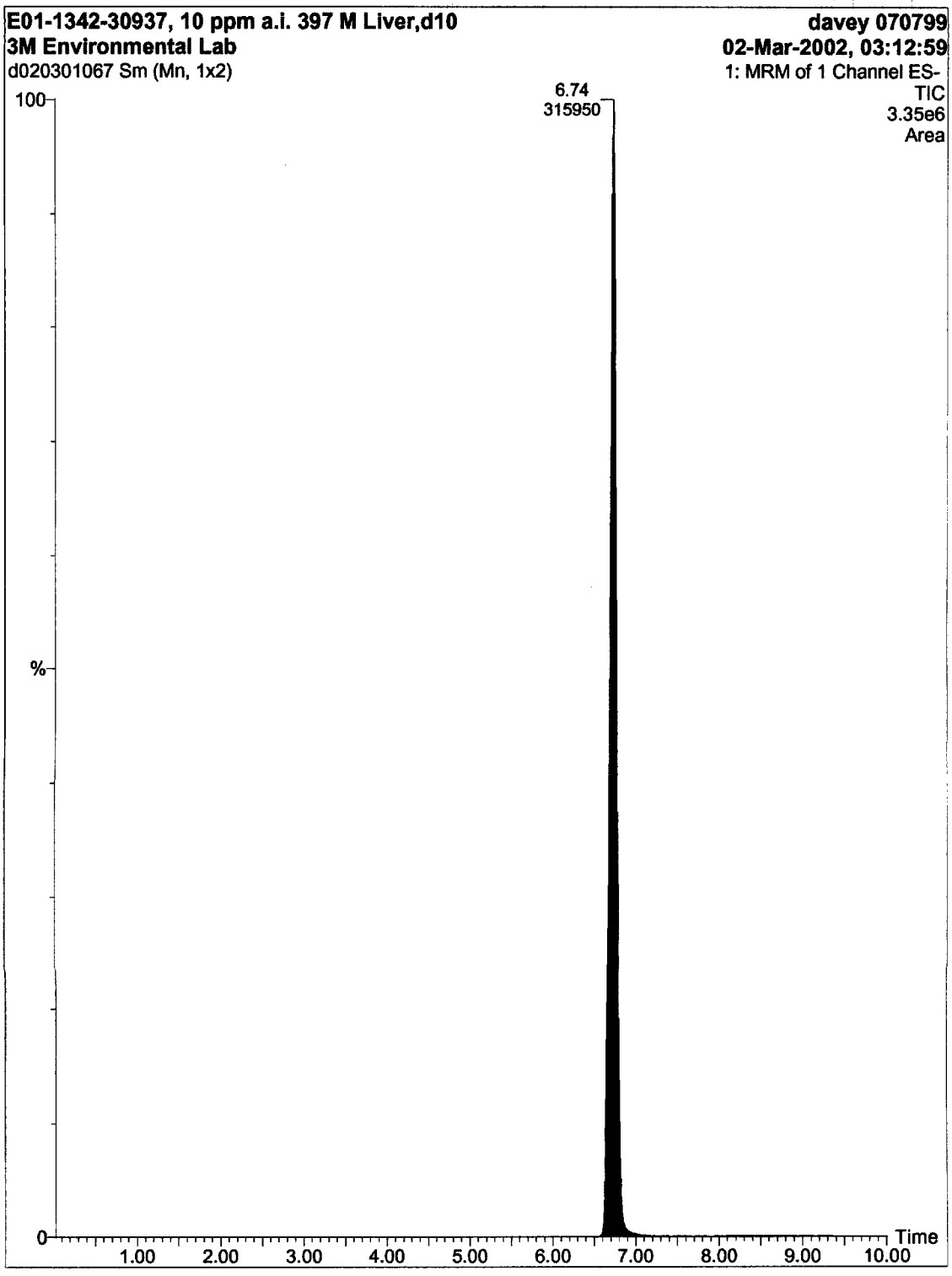
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



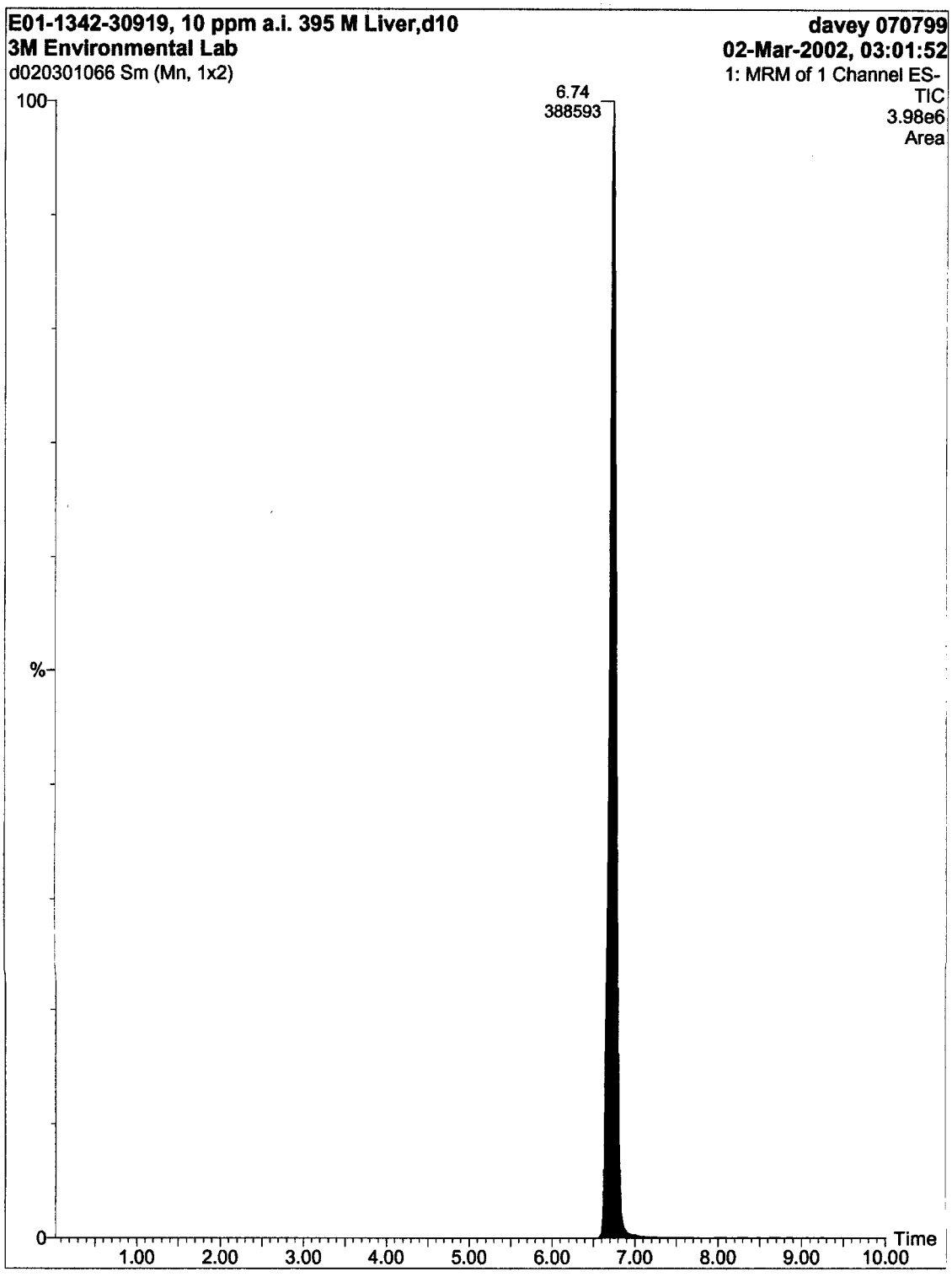
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

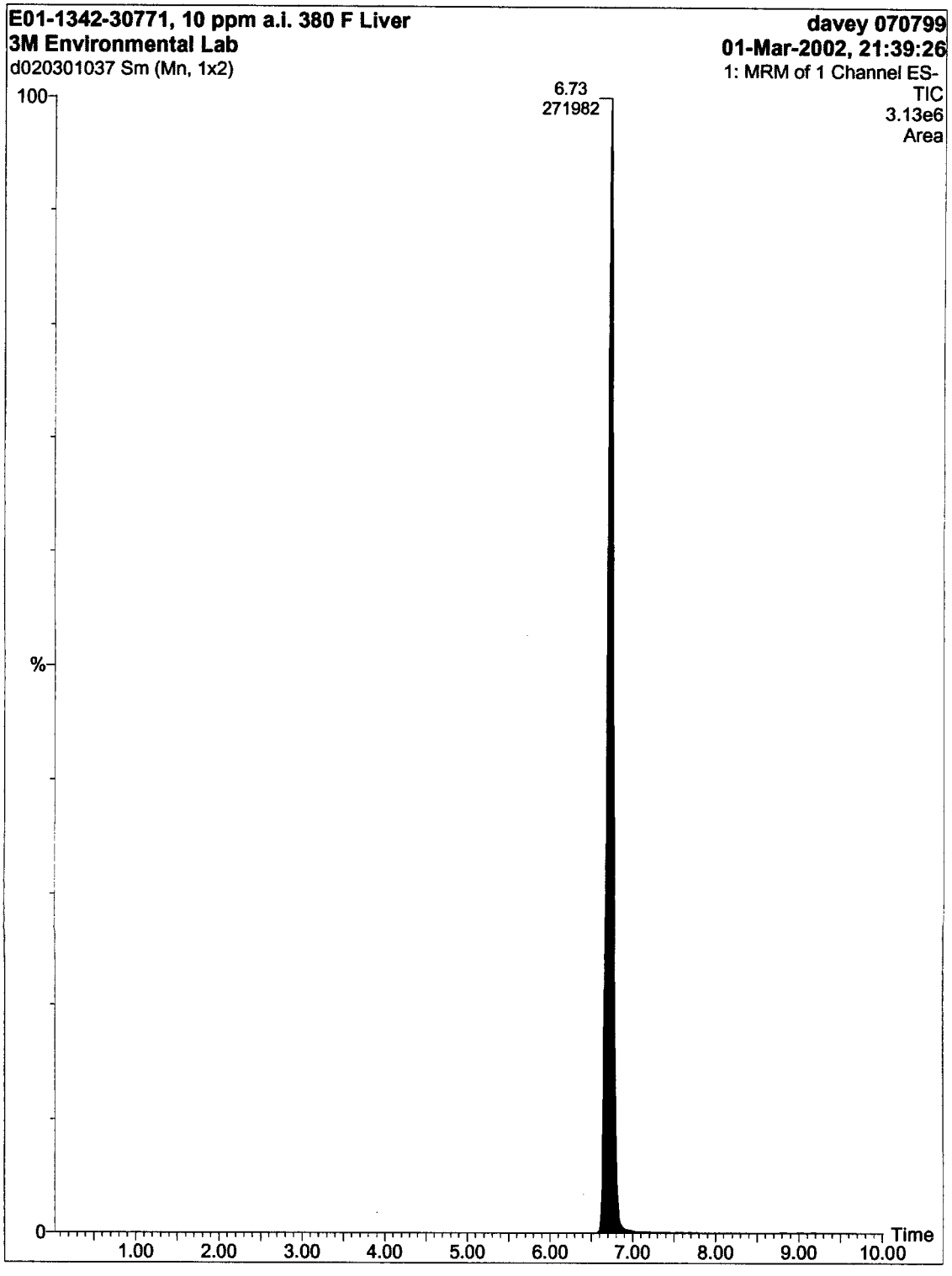


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

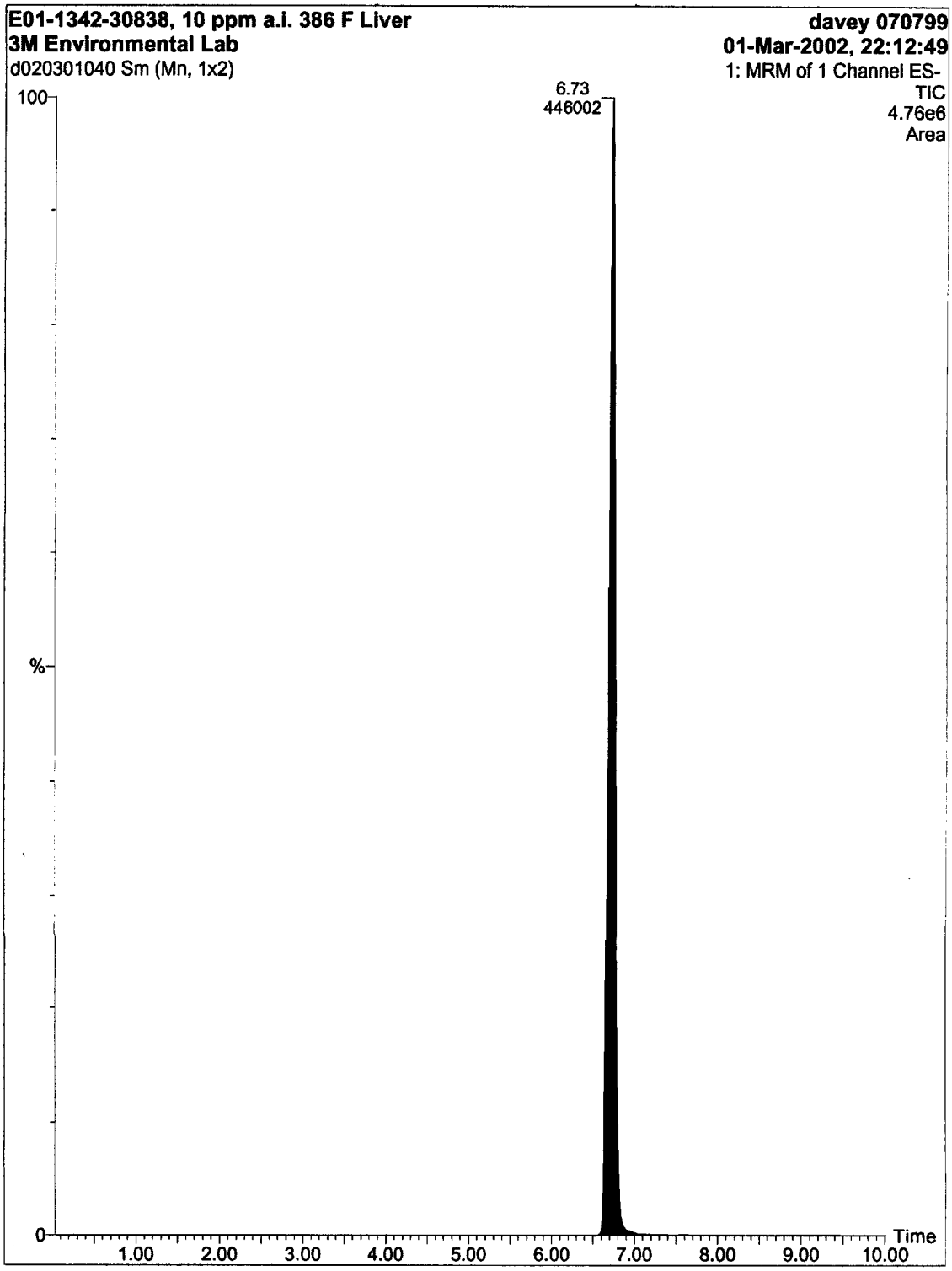


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

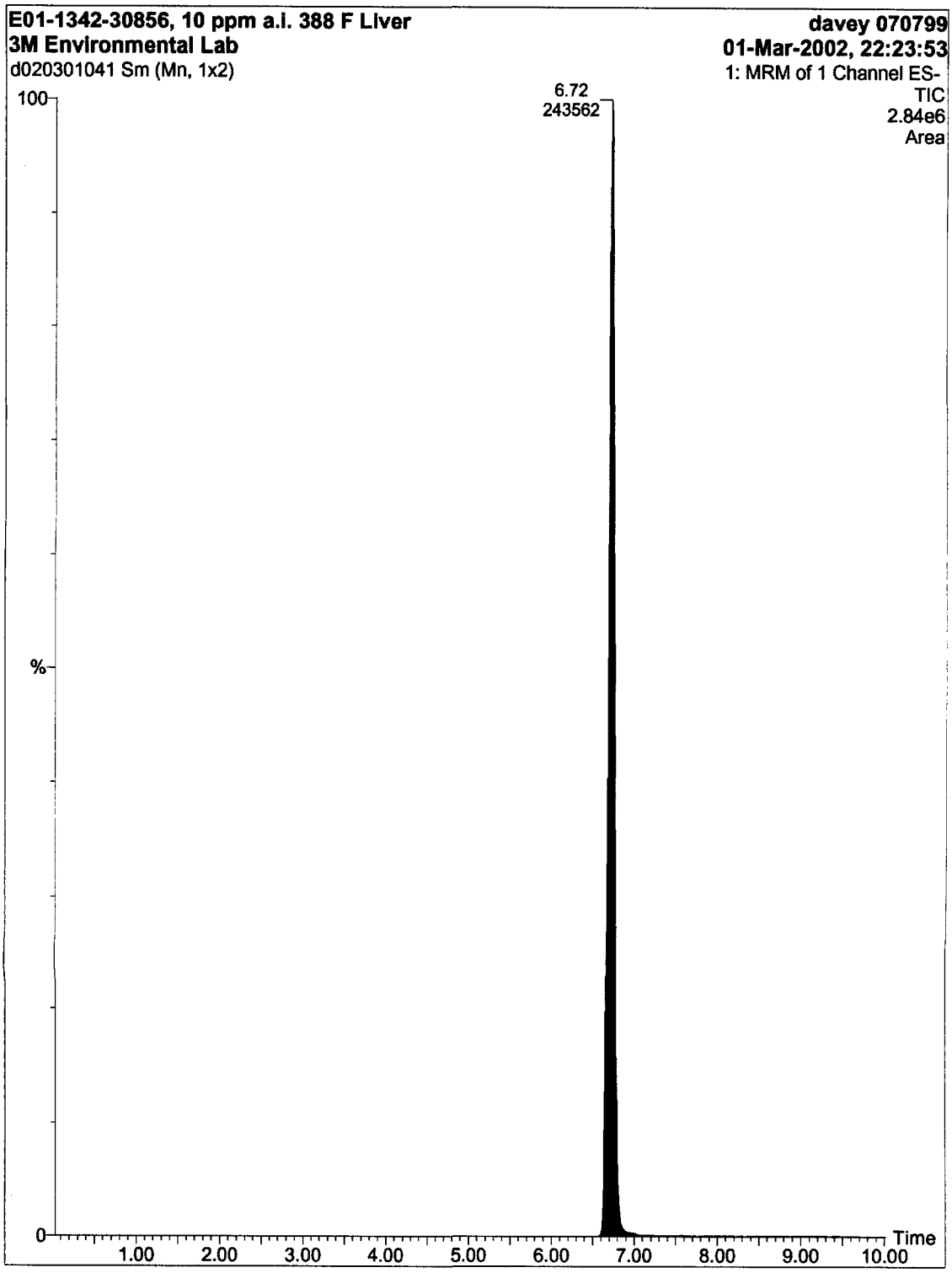
Dosed Group Female Liver Samples



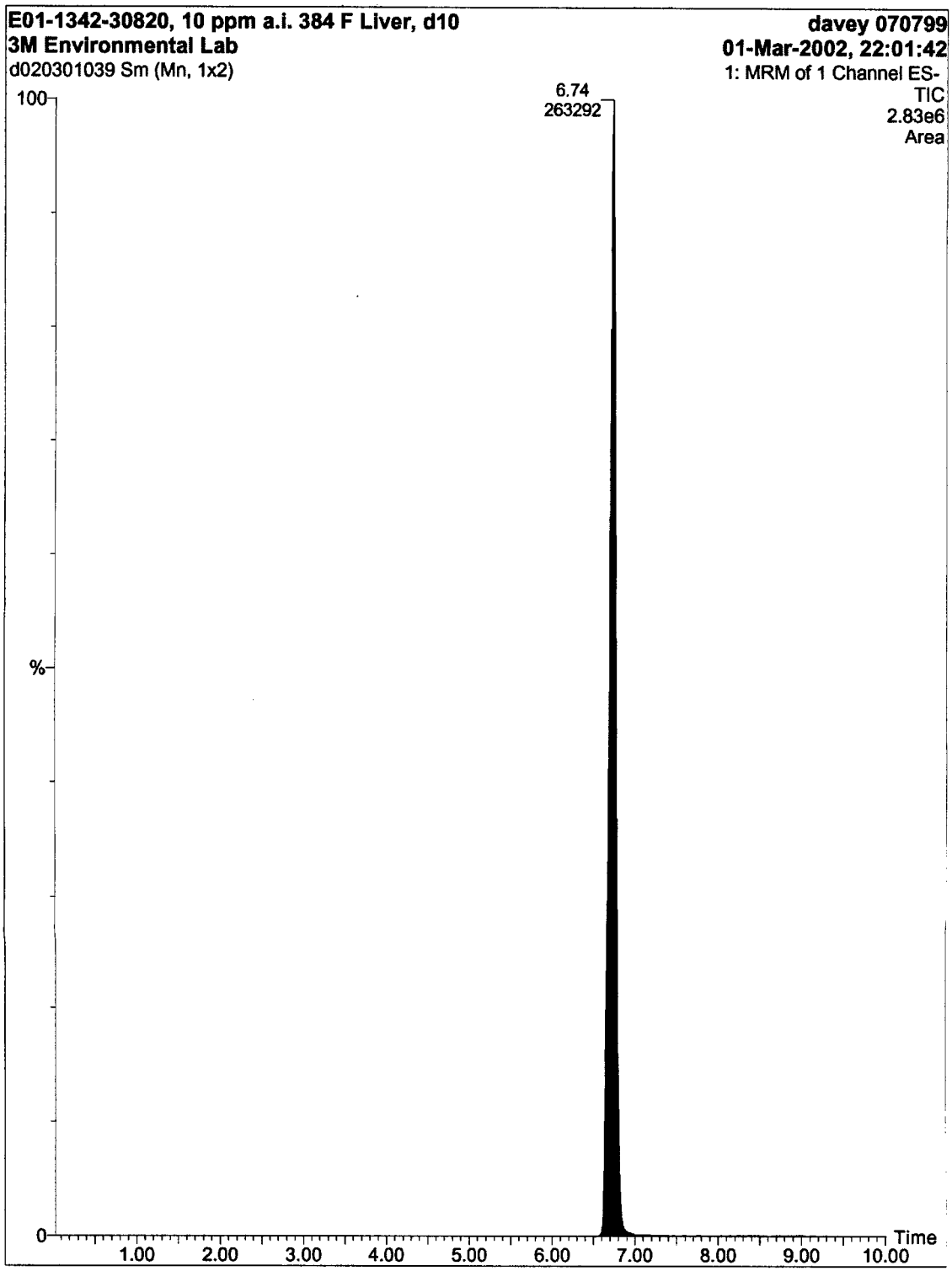
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



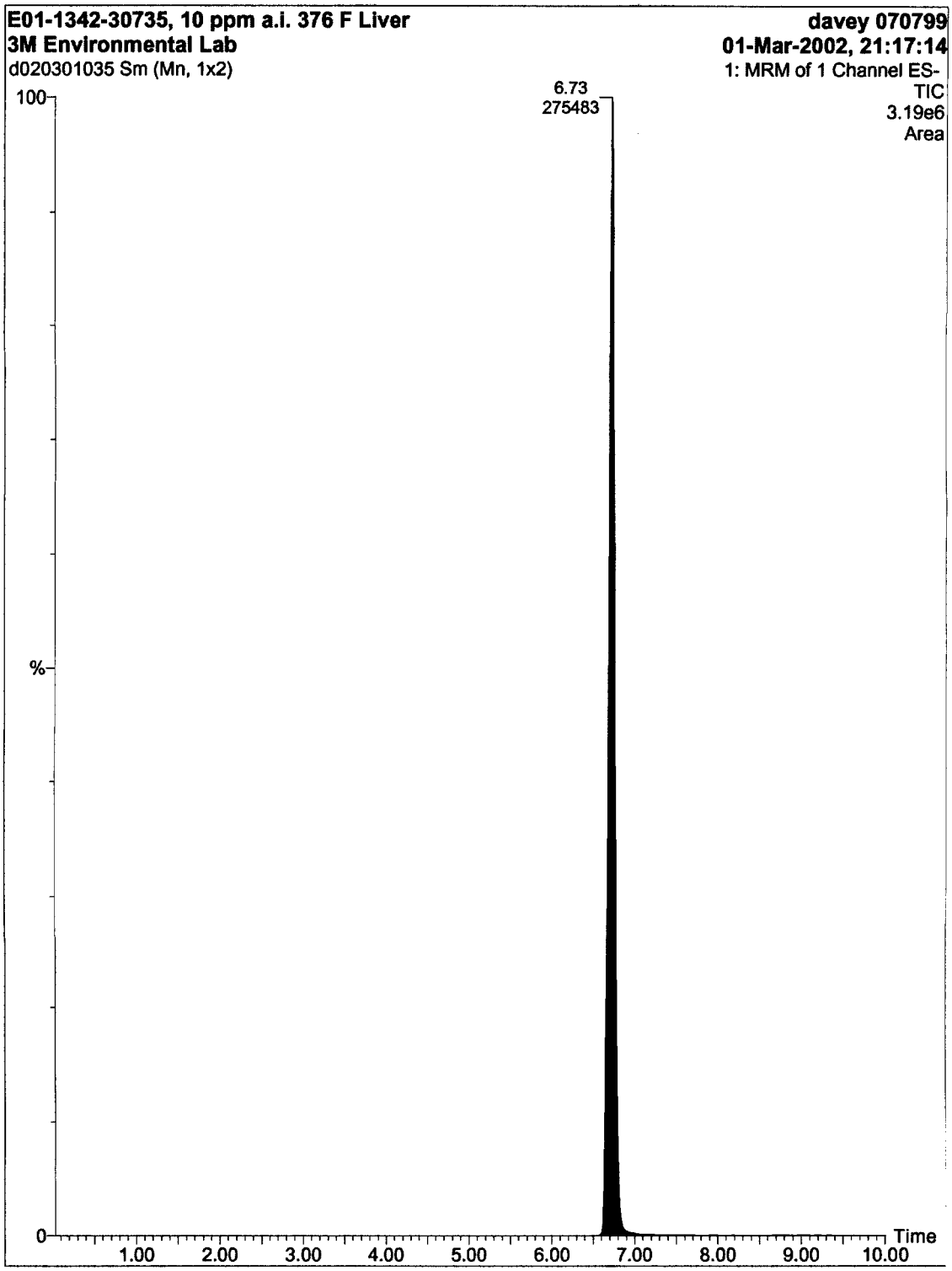
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



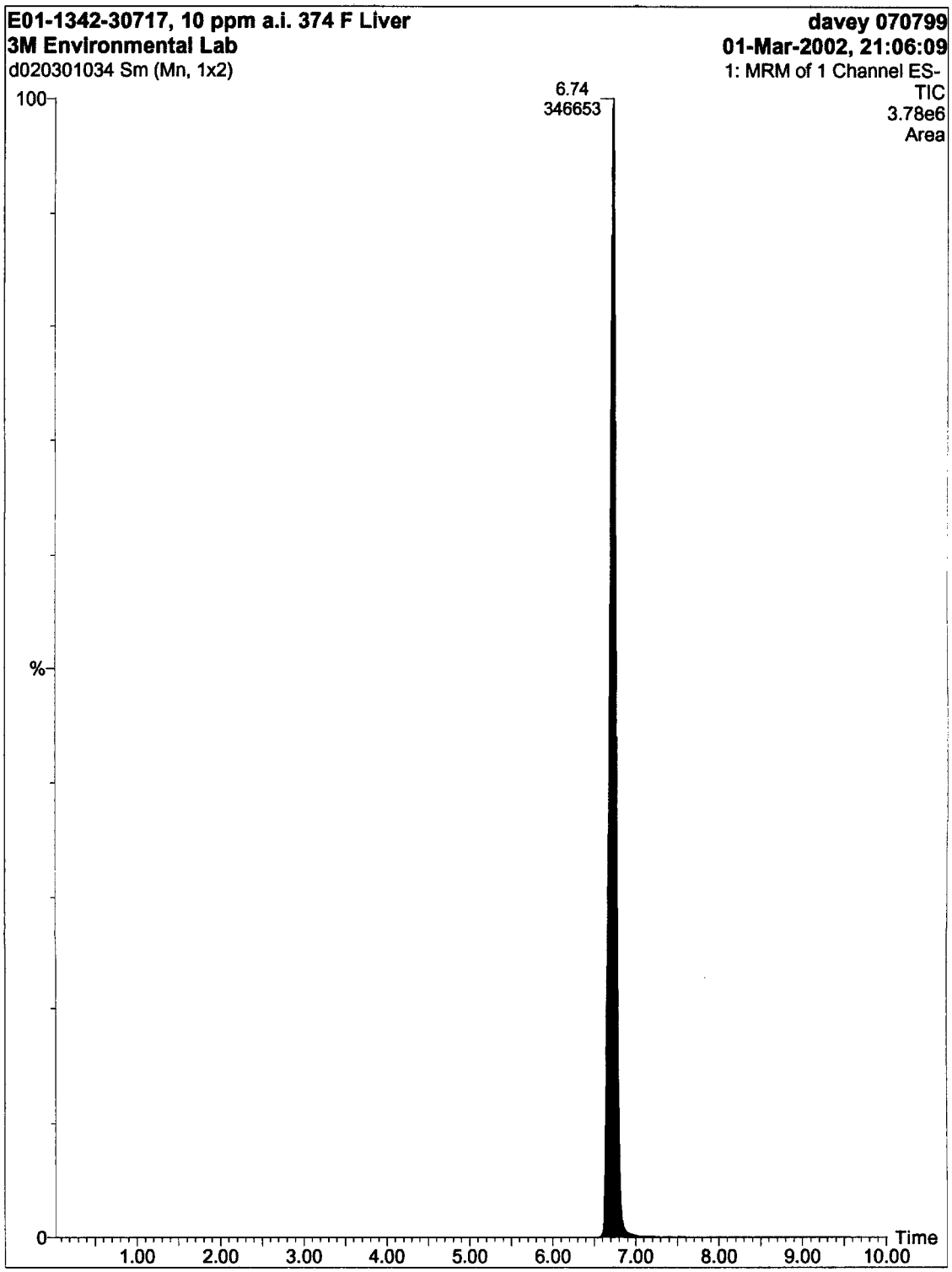
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



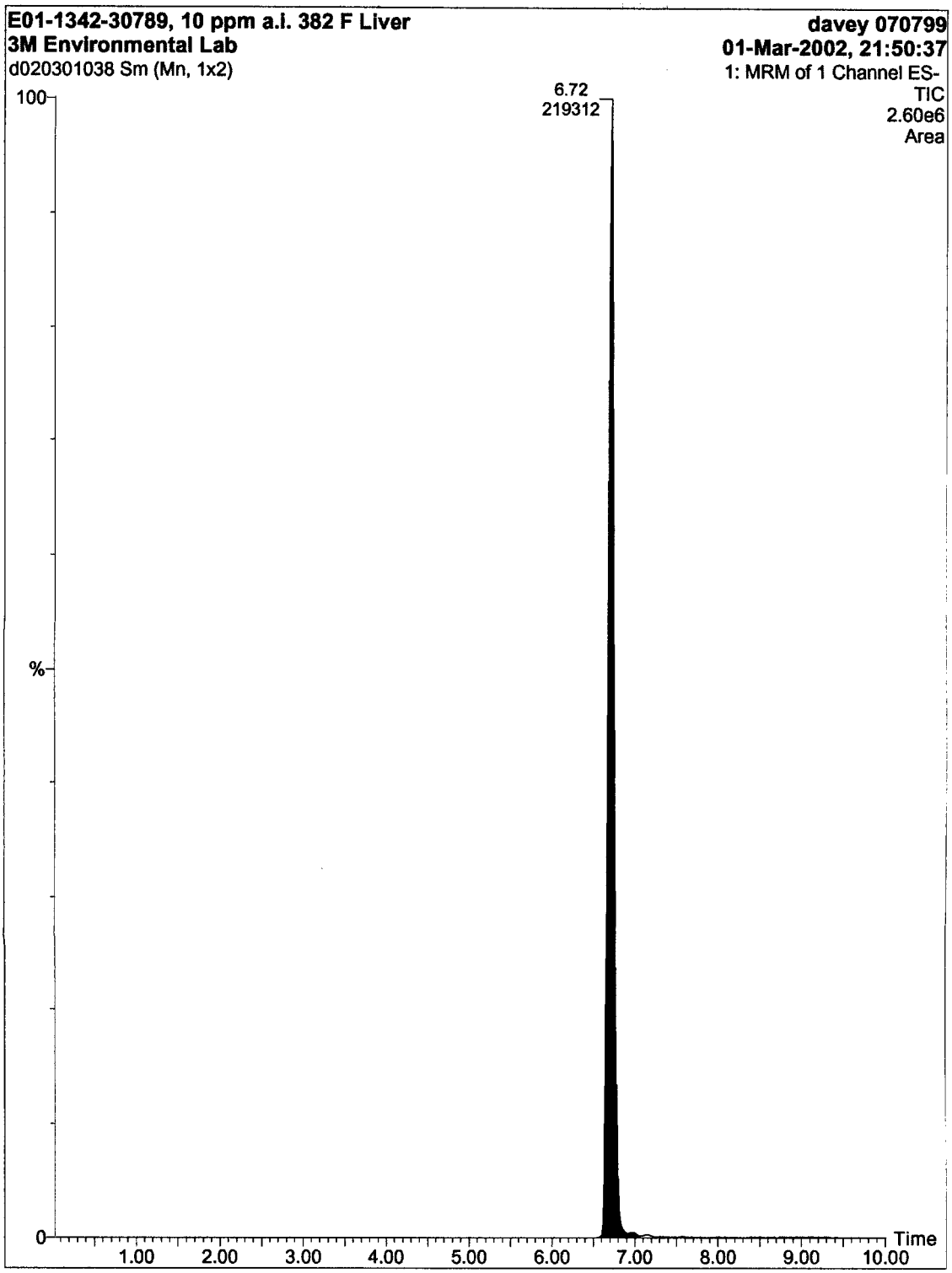
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



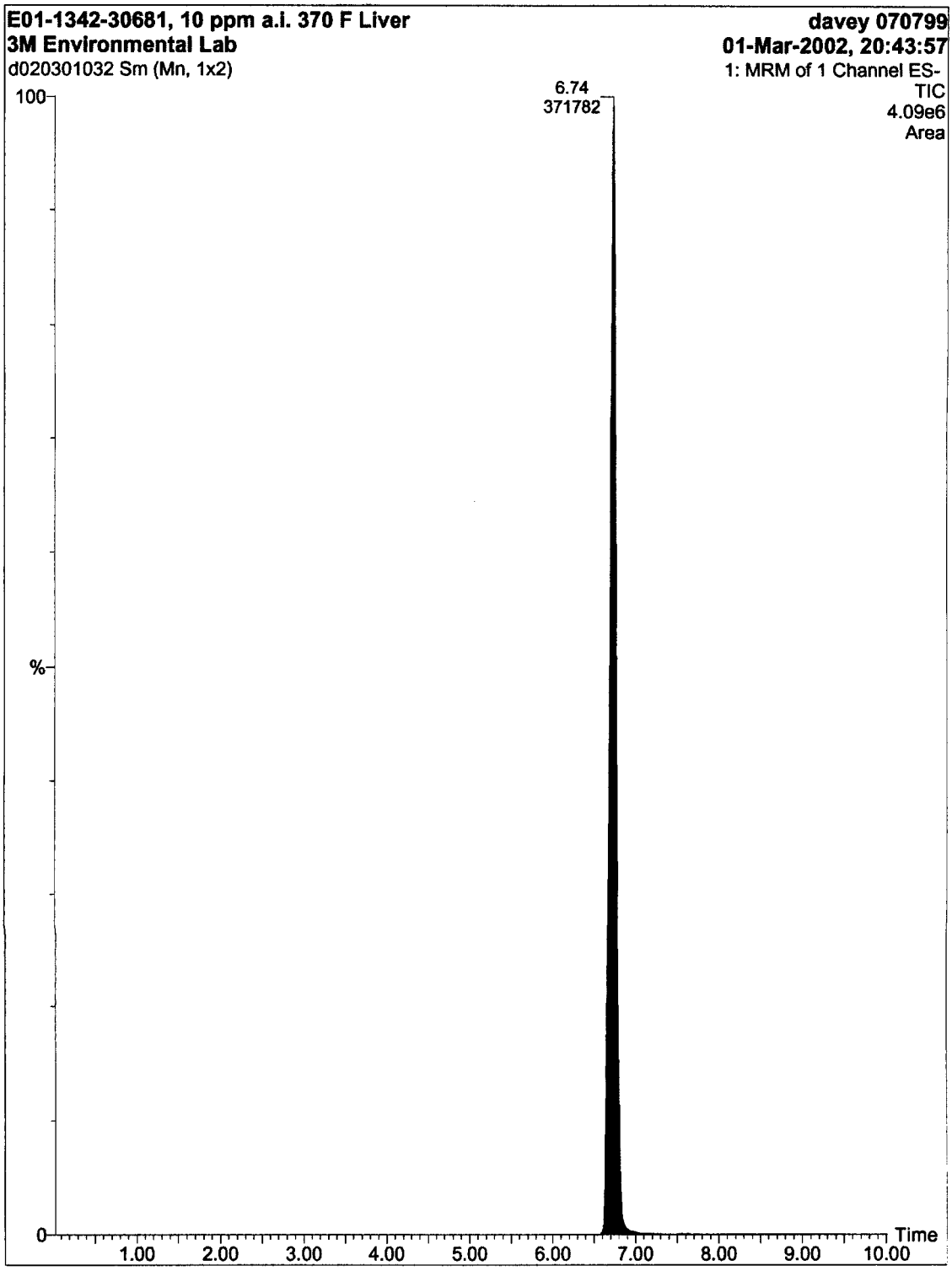
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



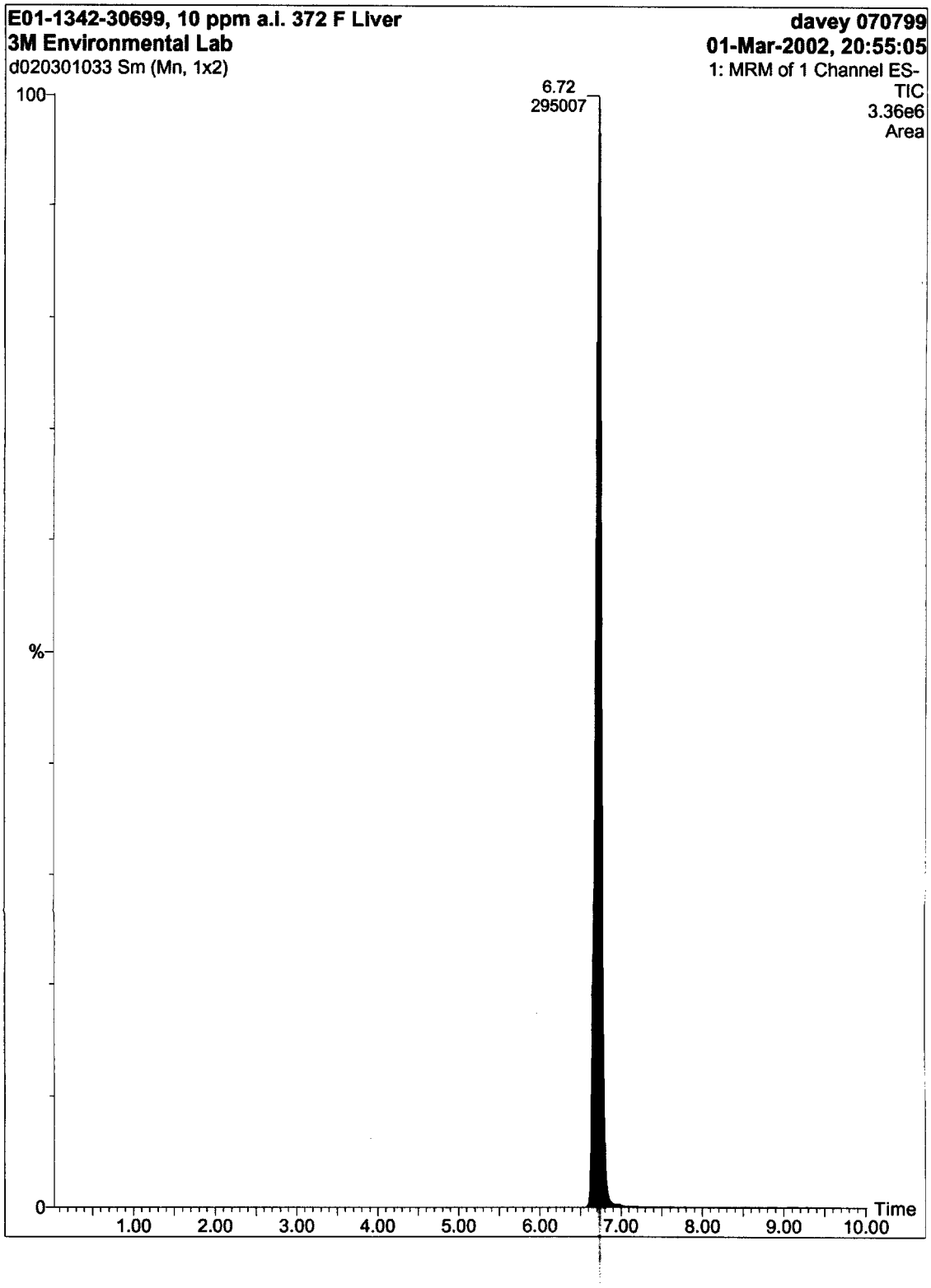
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



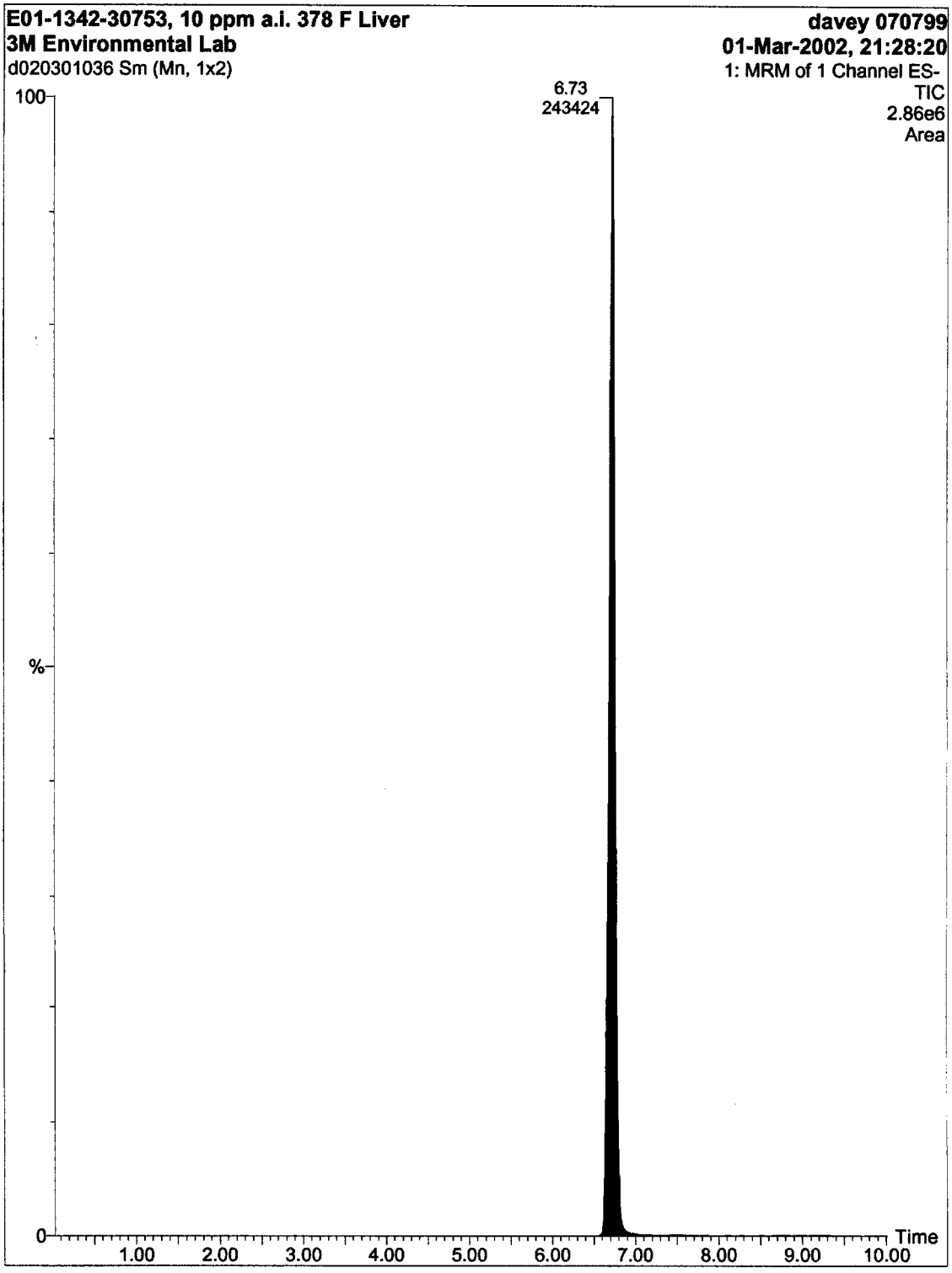
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

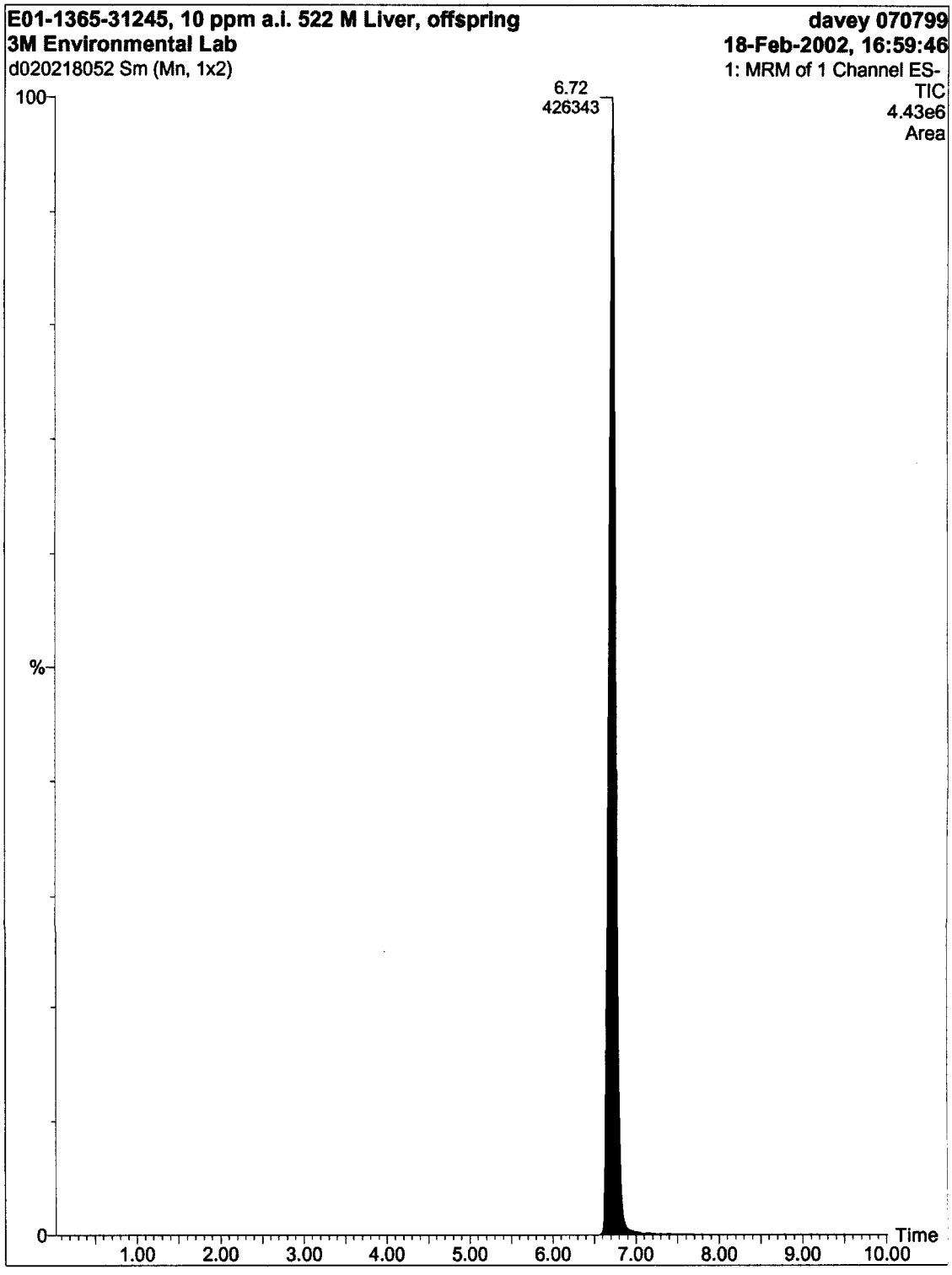


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

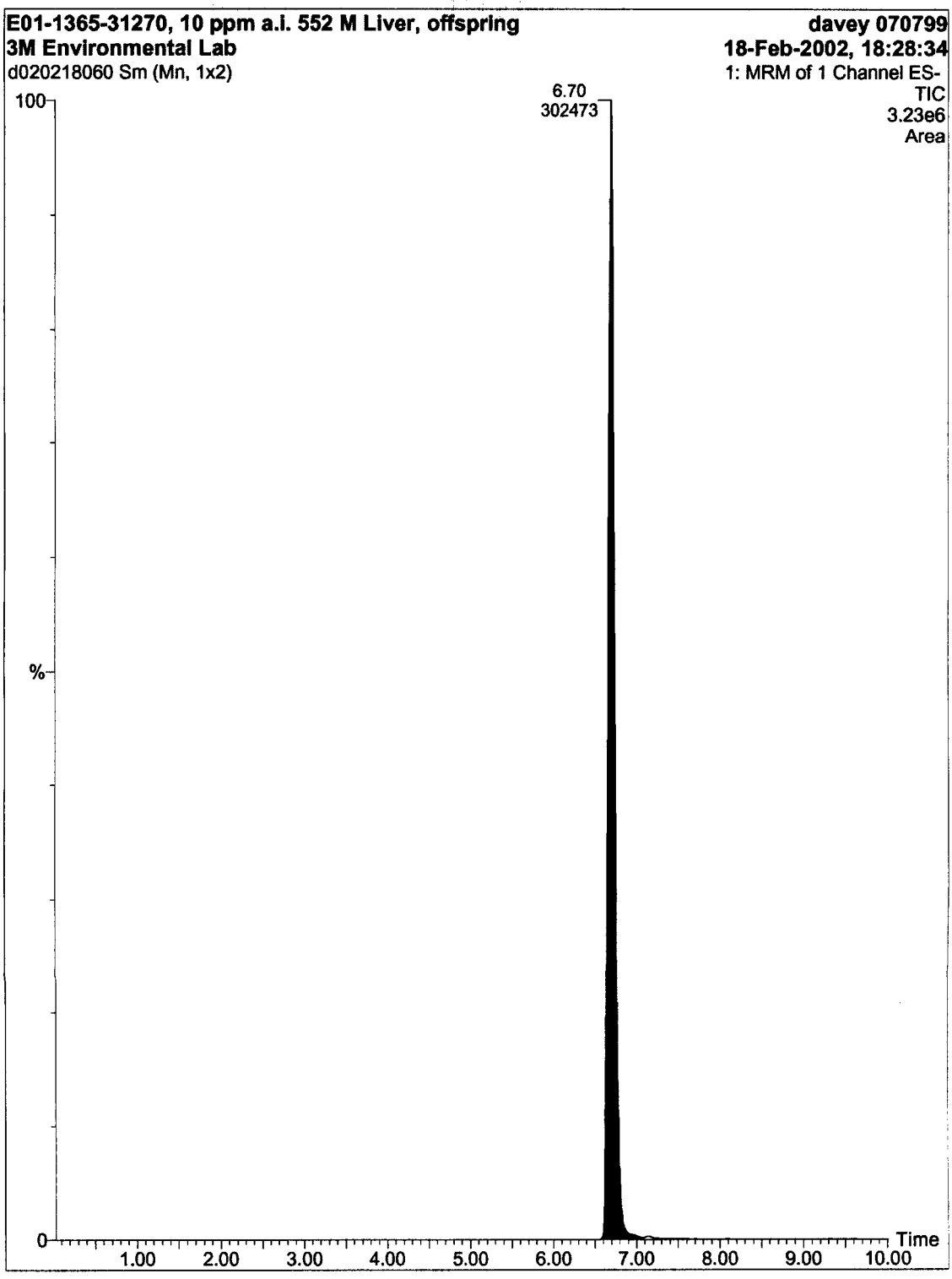


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

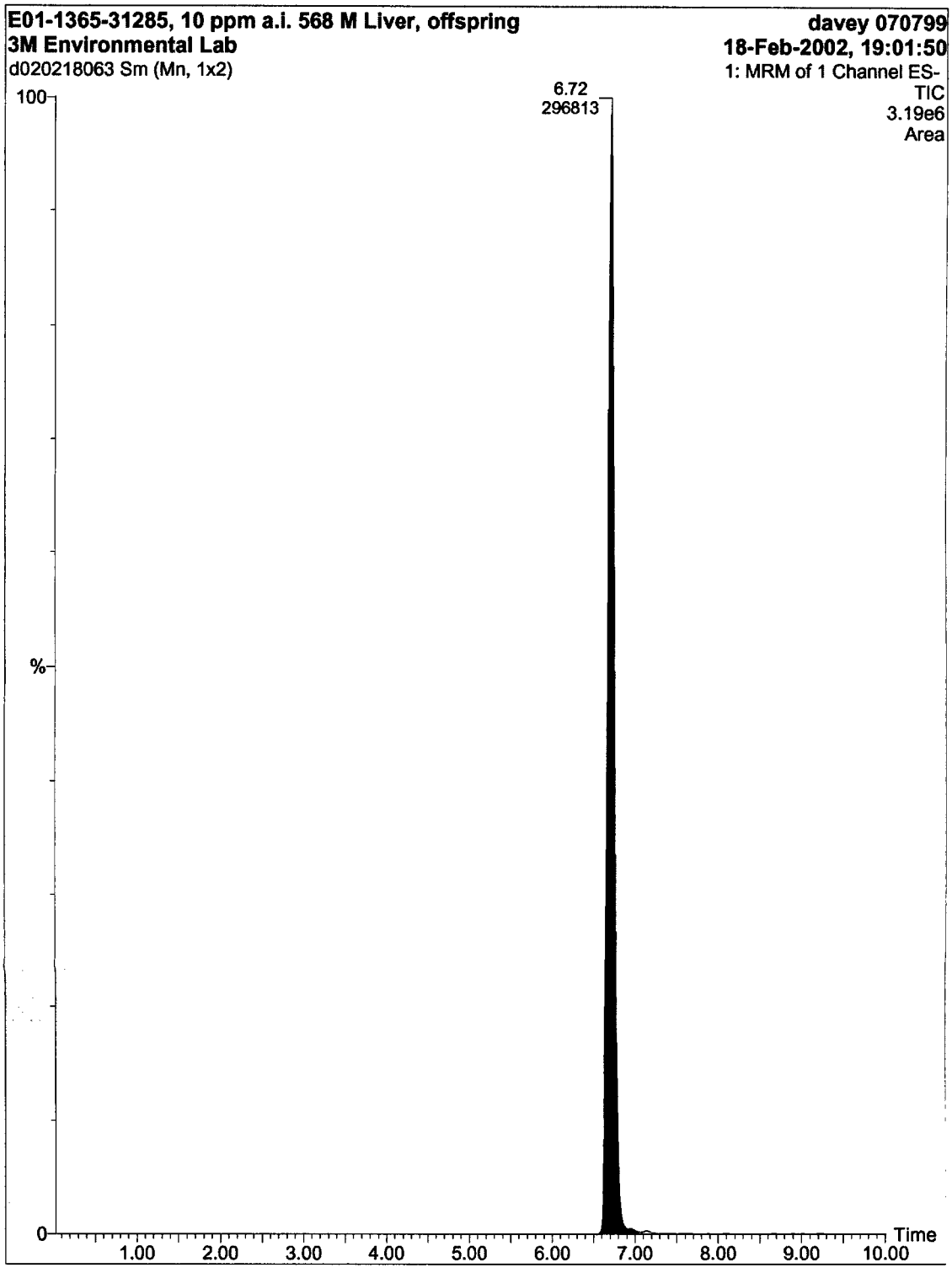
Dosed Group Male Offspring Liver Samples



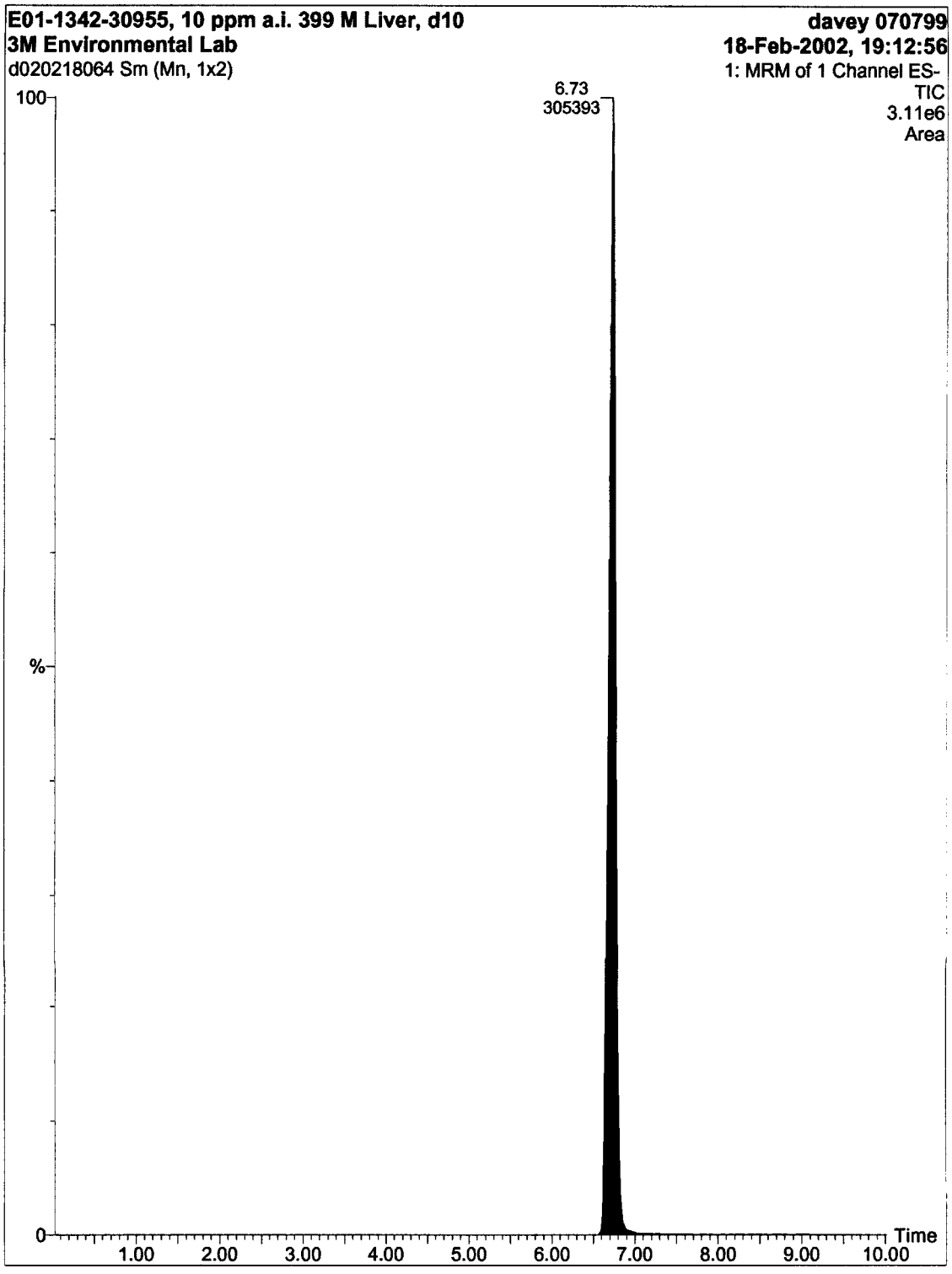
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



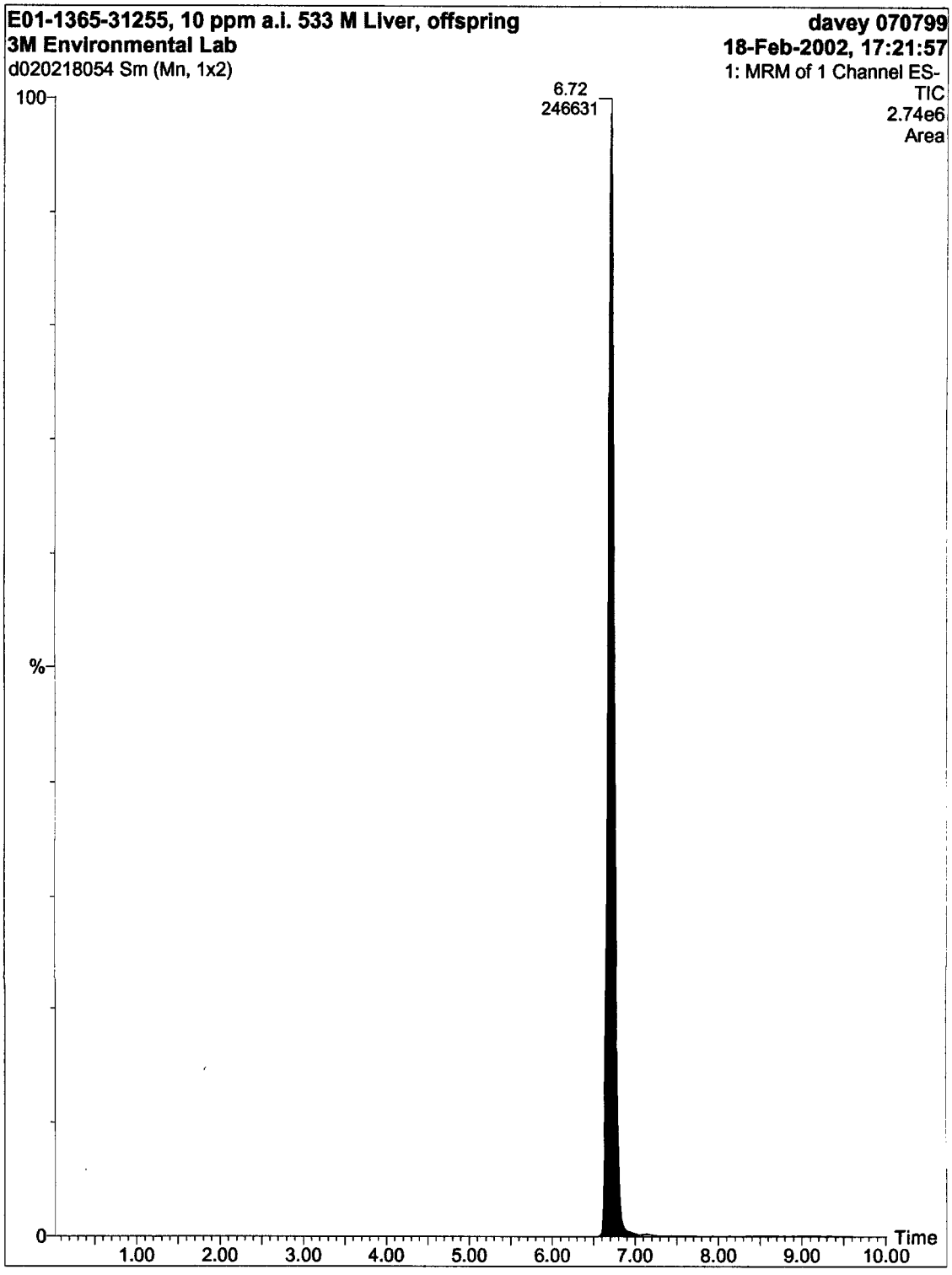
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



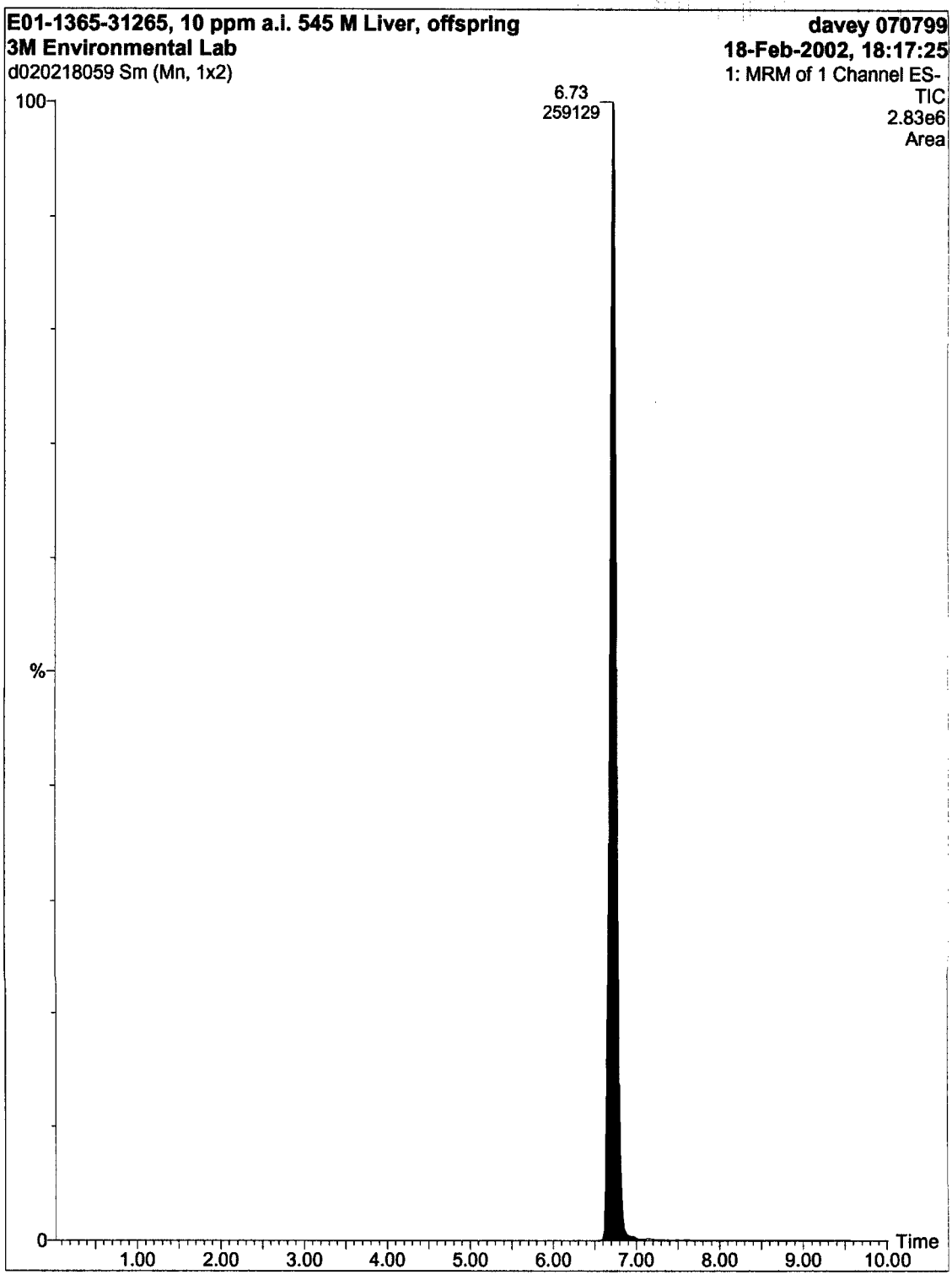
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

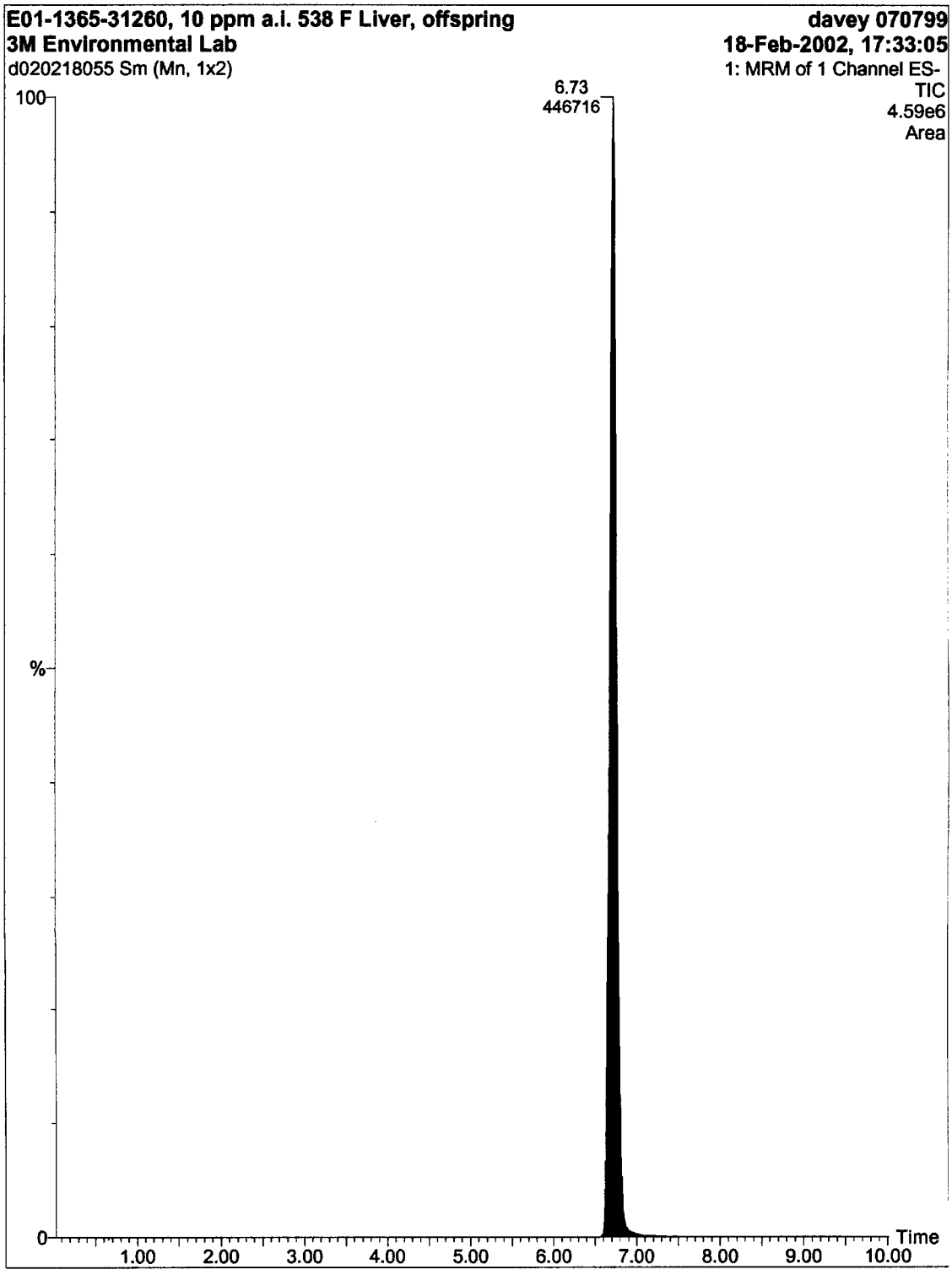


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

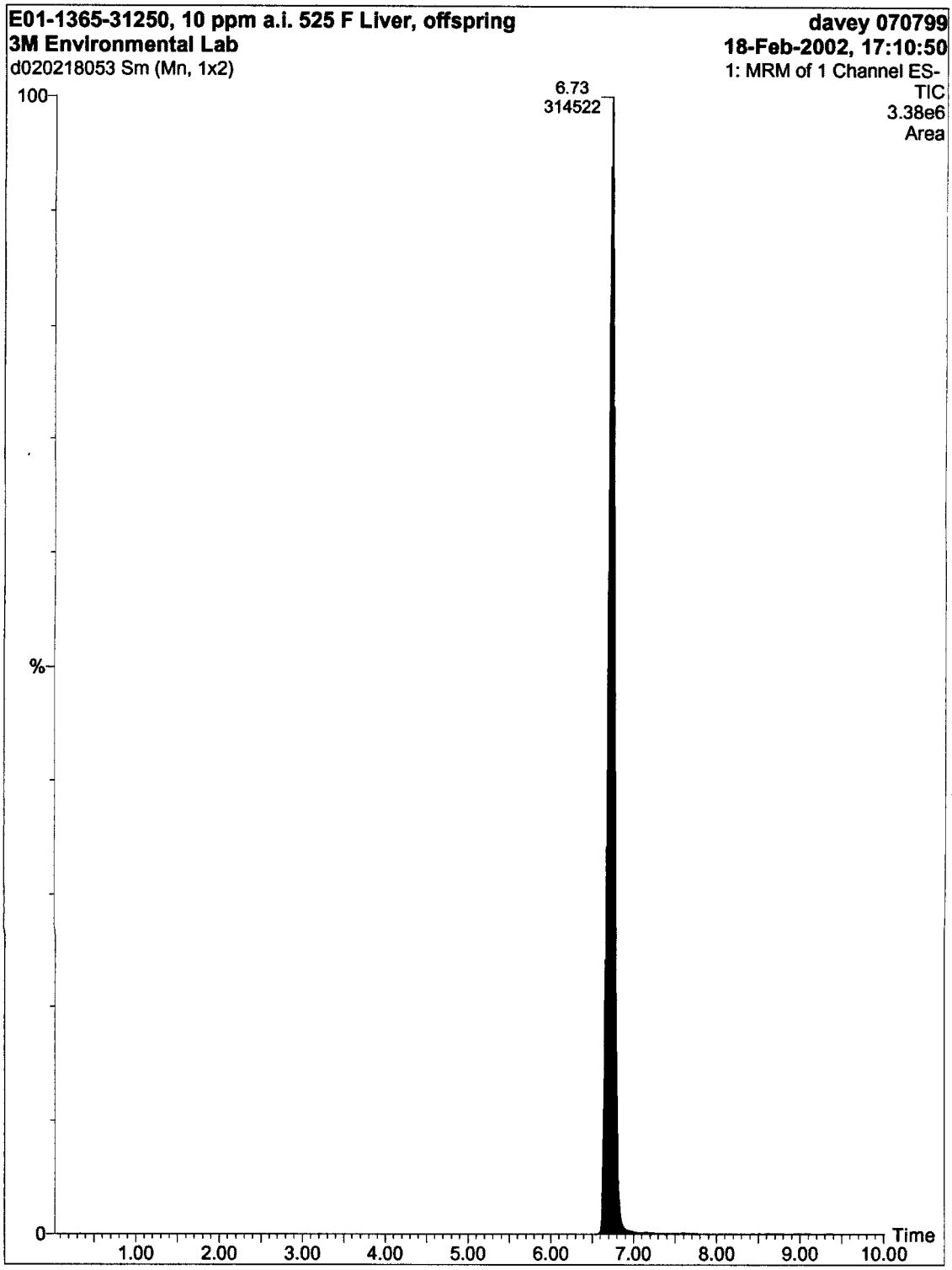


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

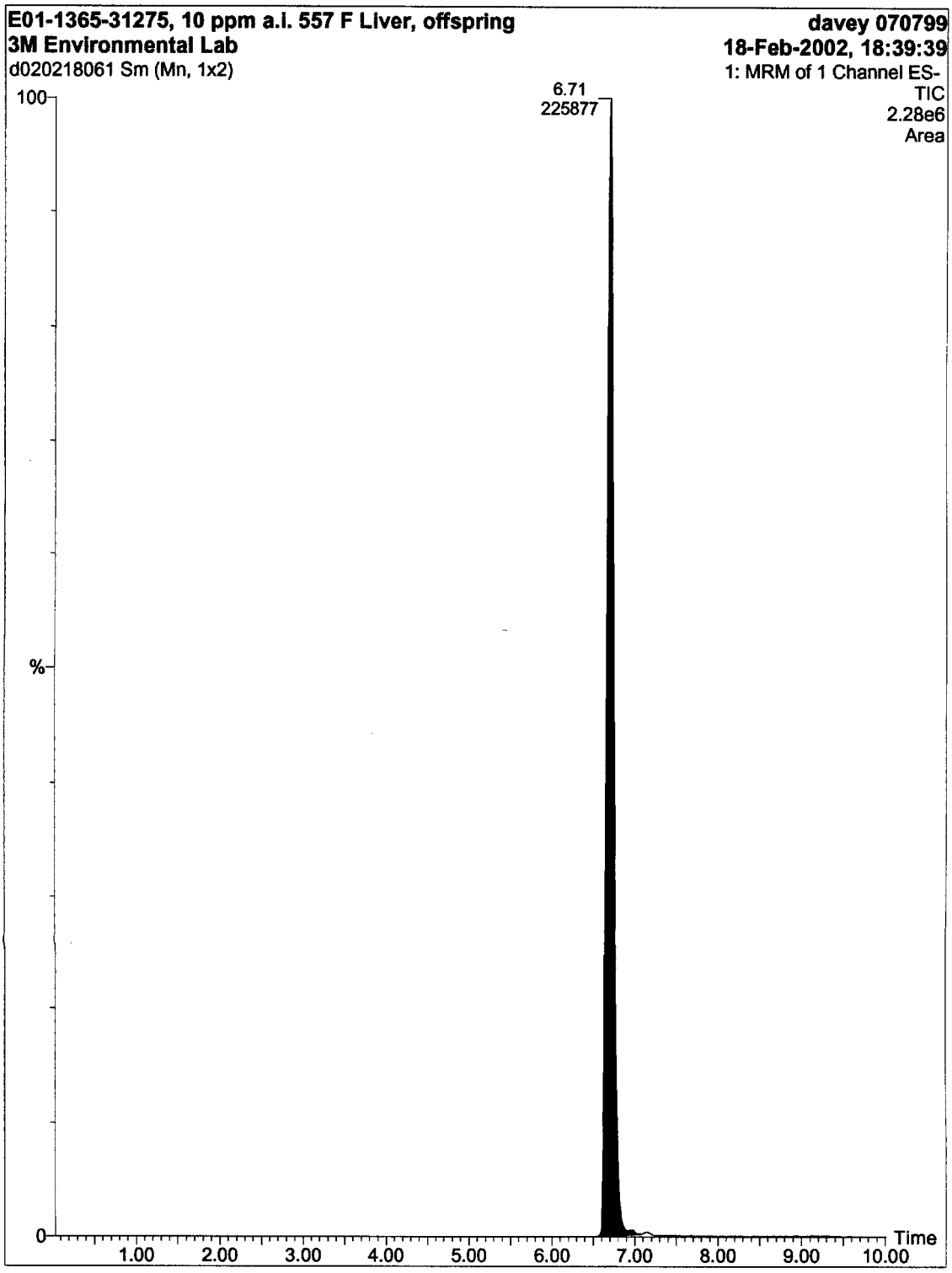
Dosed Group Female Offspring Liver Samples



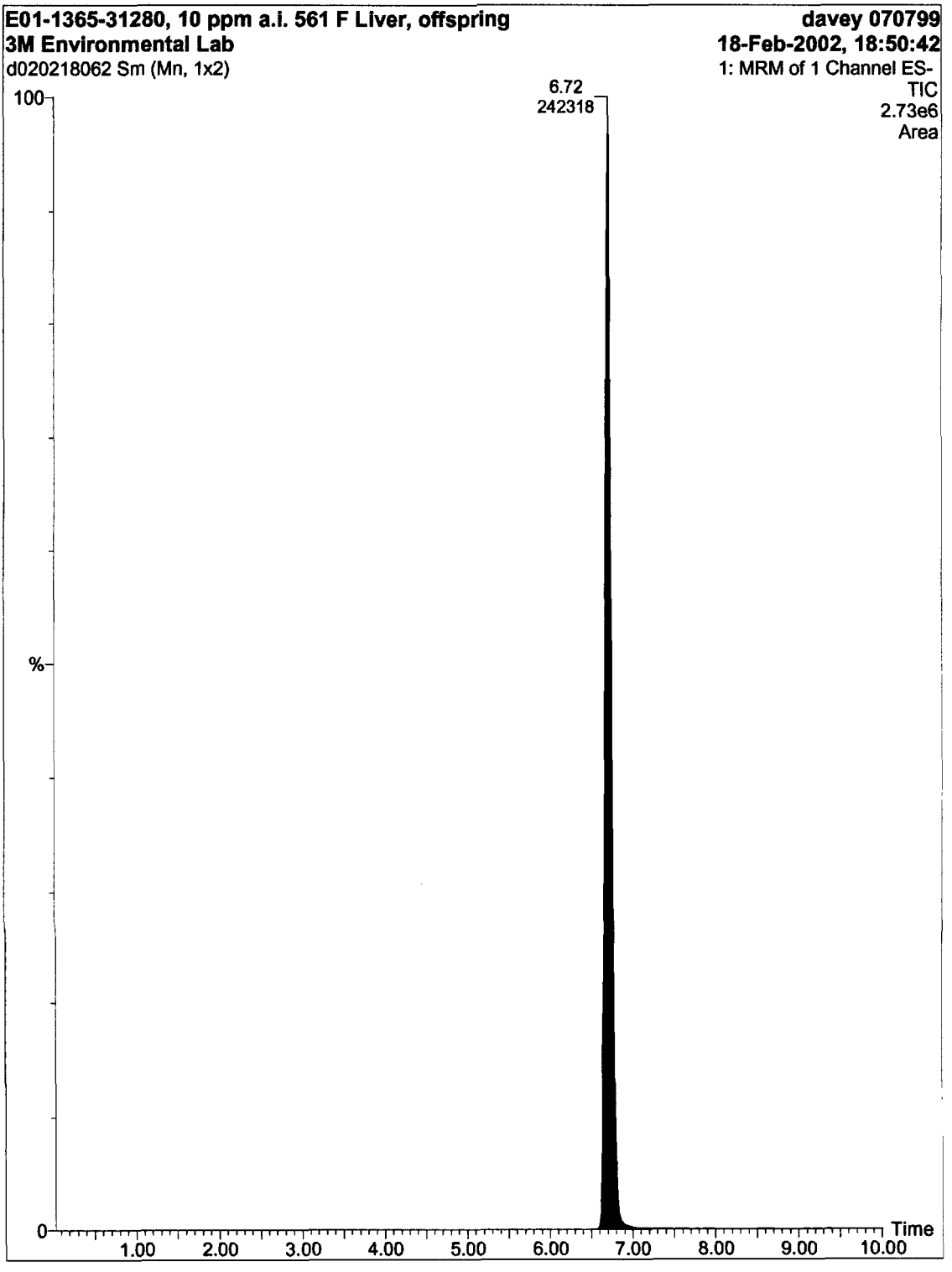
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



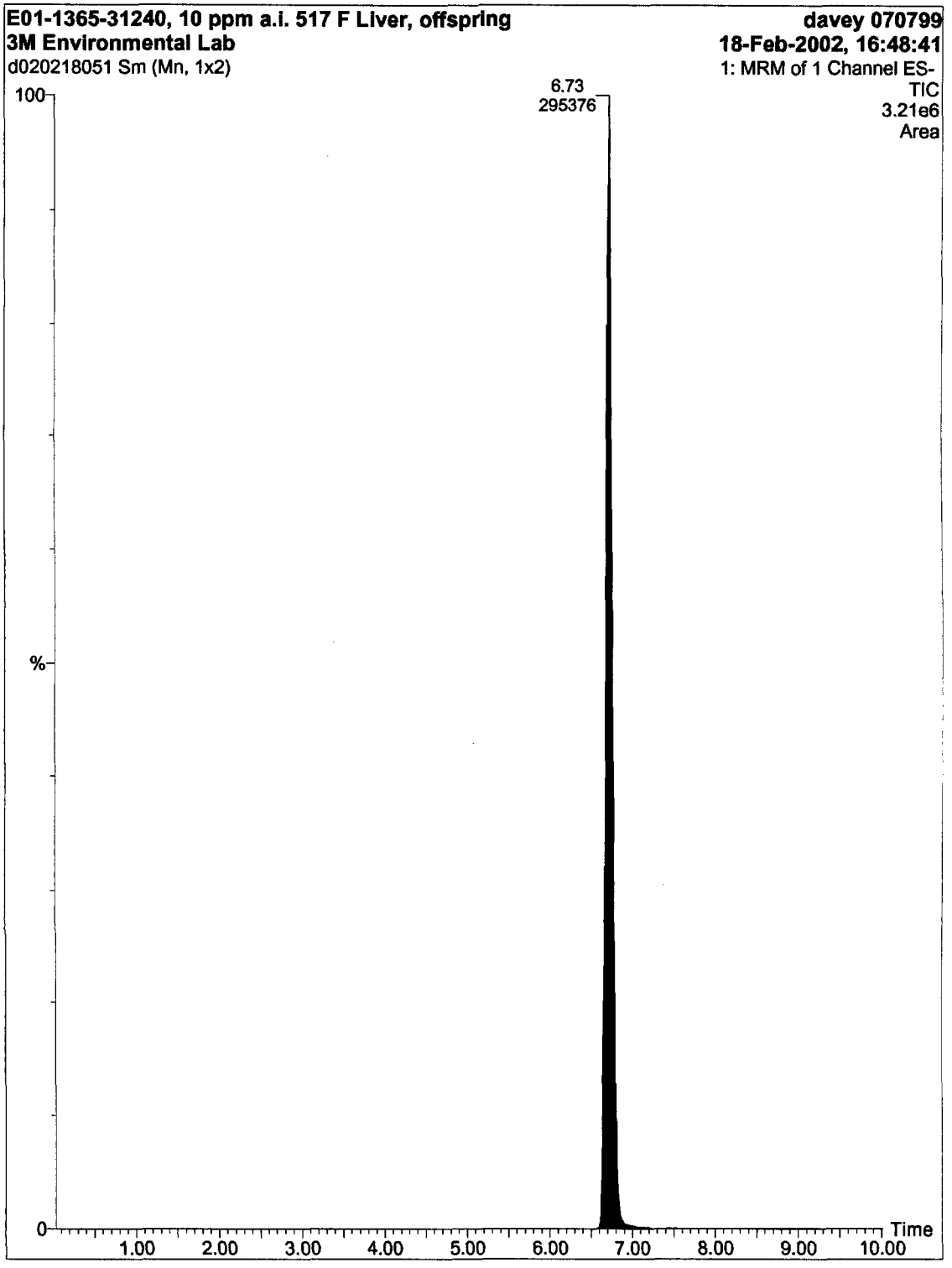
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

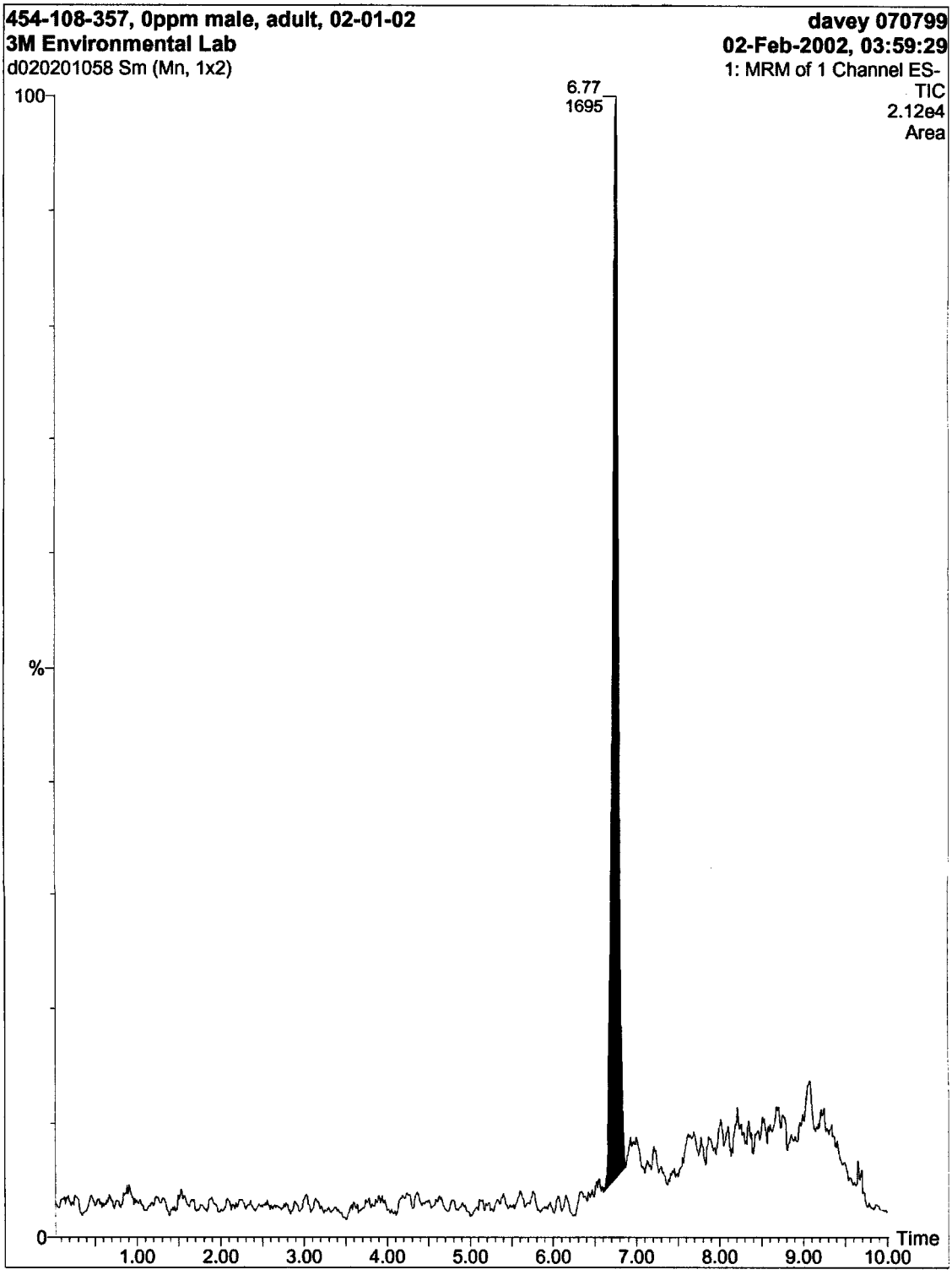


Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite



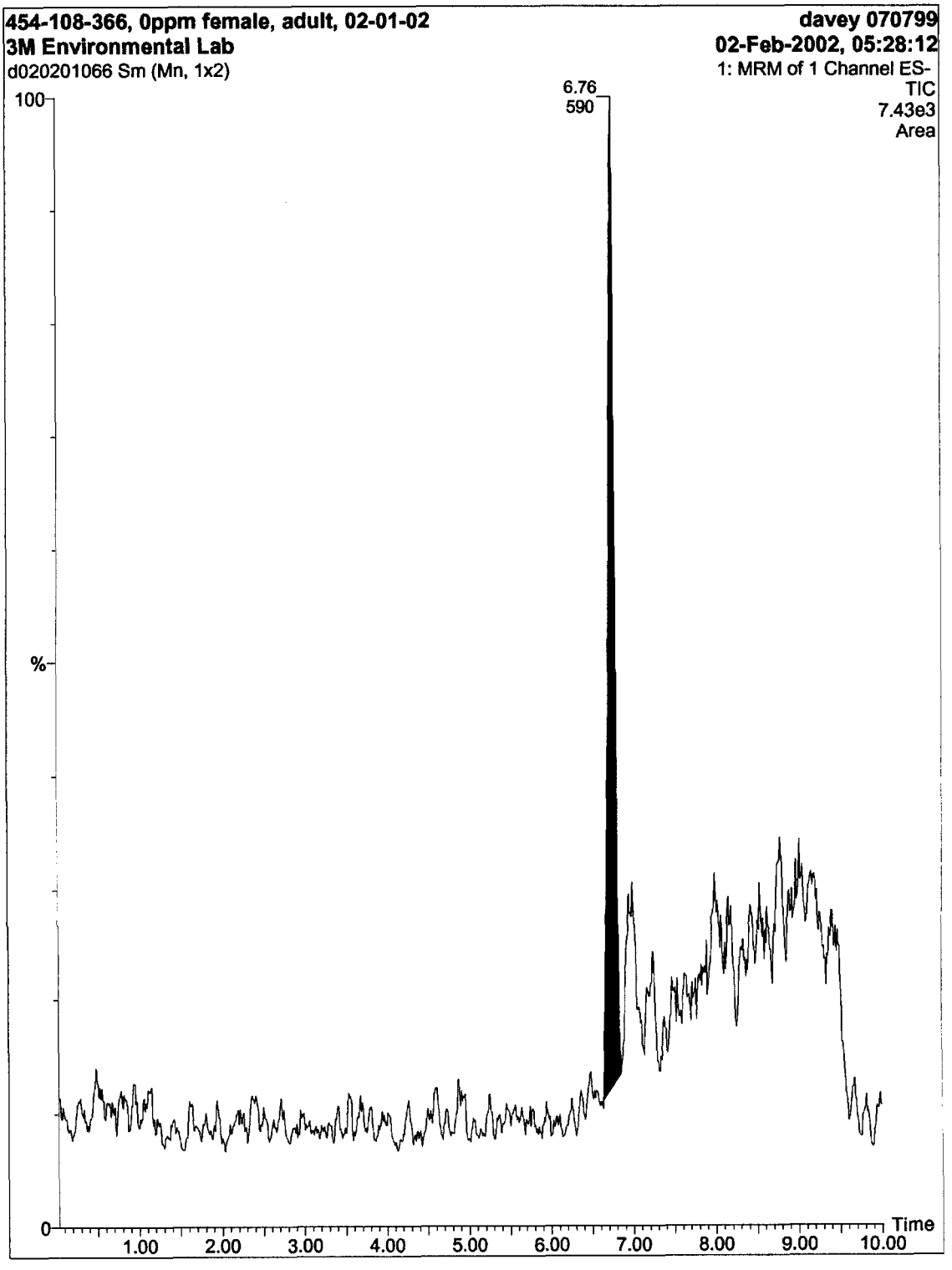
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Control Group Male Sera Sample



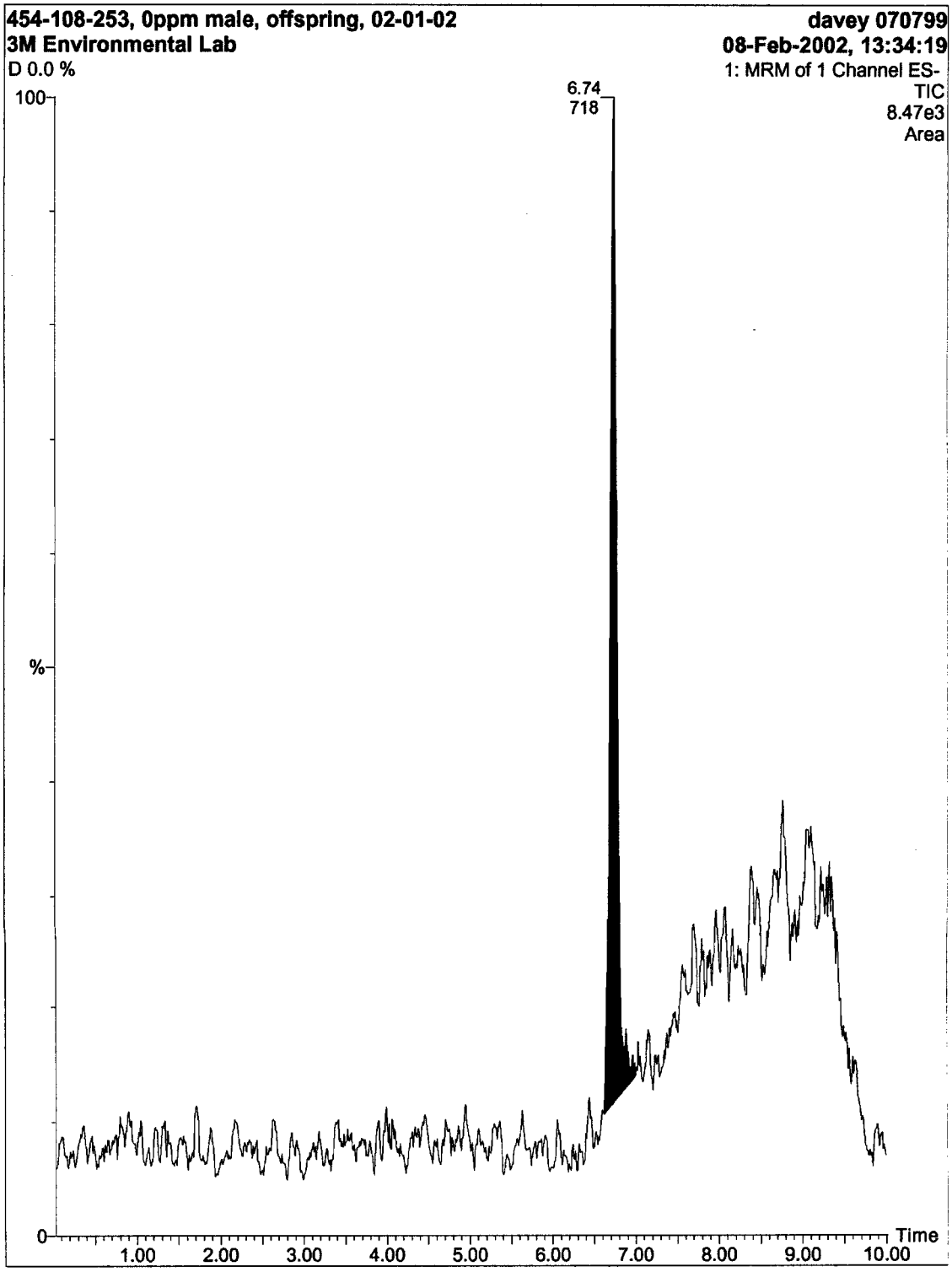
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Control Group Female Sera Sample



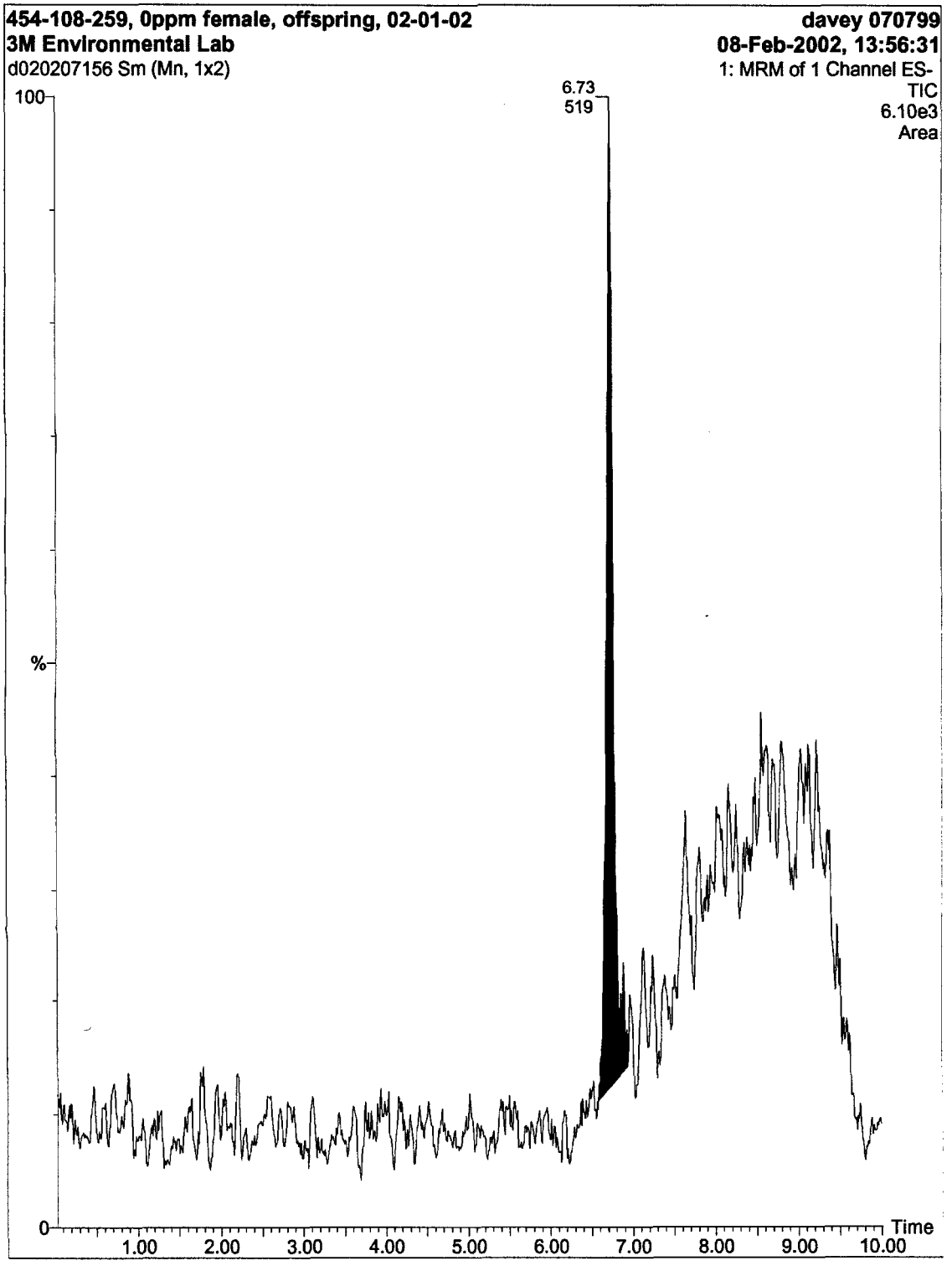
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Control Group Male Offspring Sera Sample



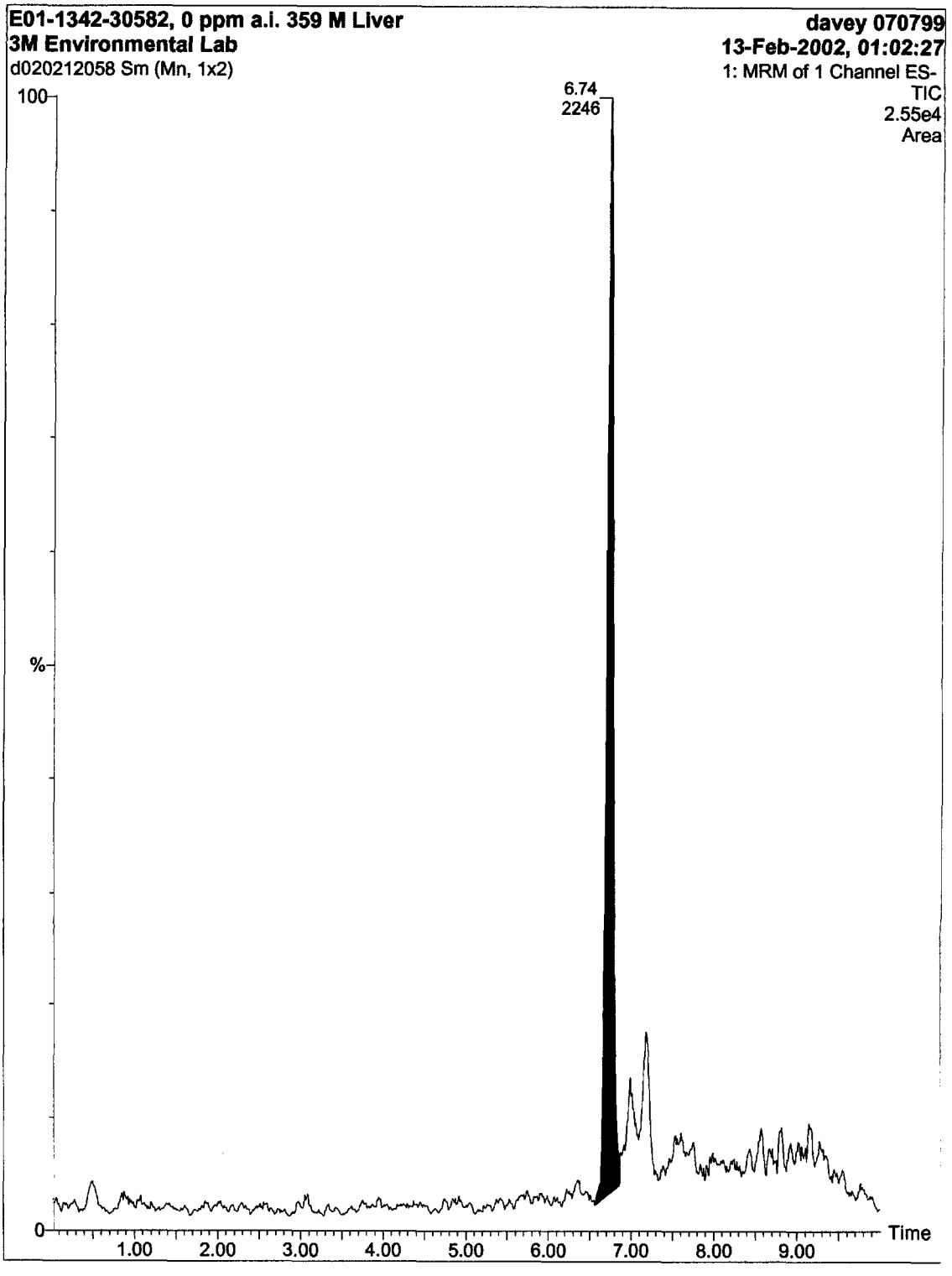
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Control Group Female Offspring Sera Sample



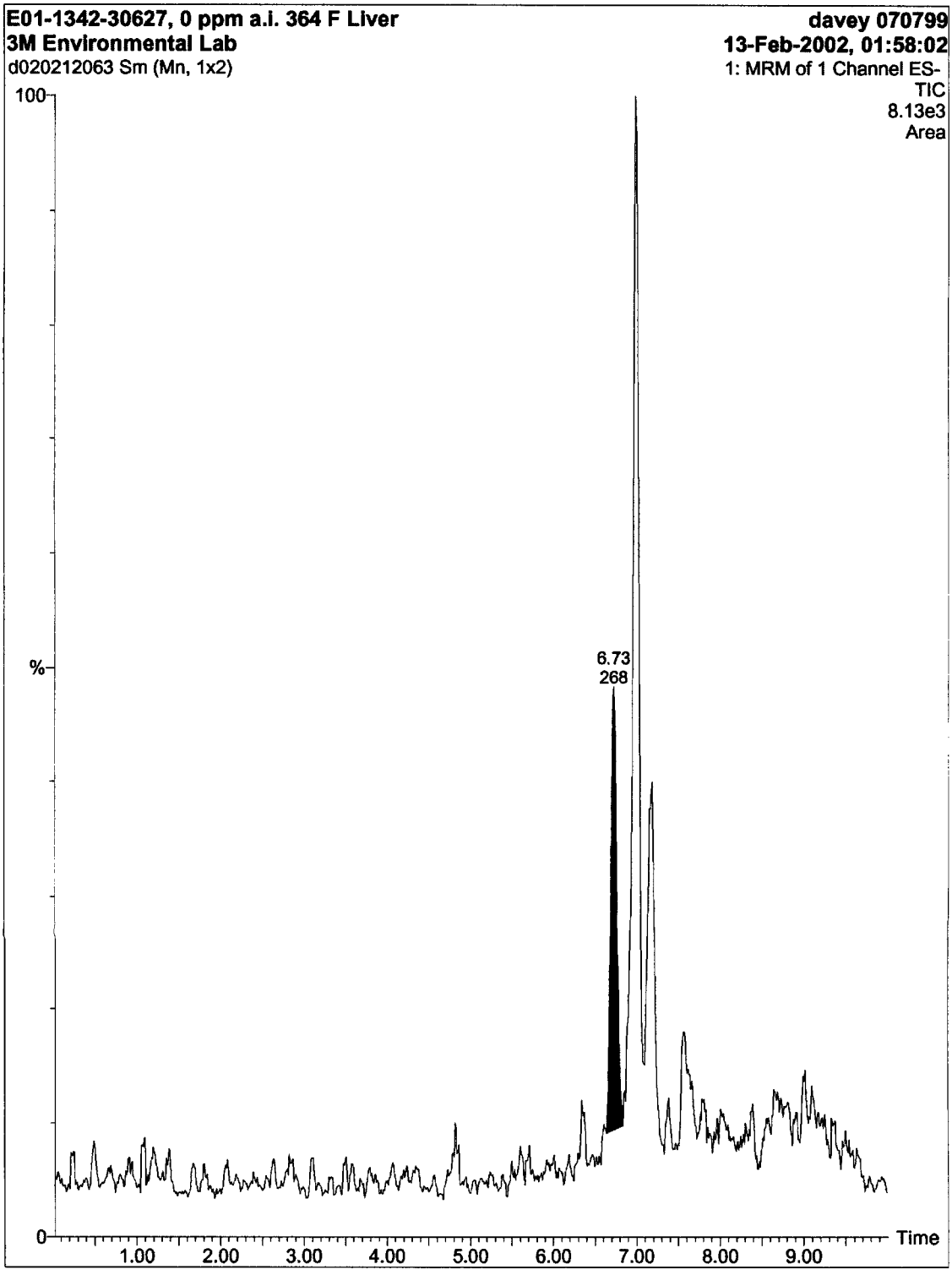
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Control Group Male Liver Sample



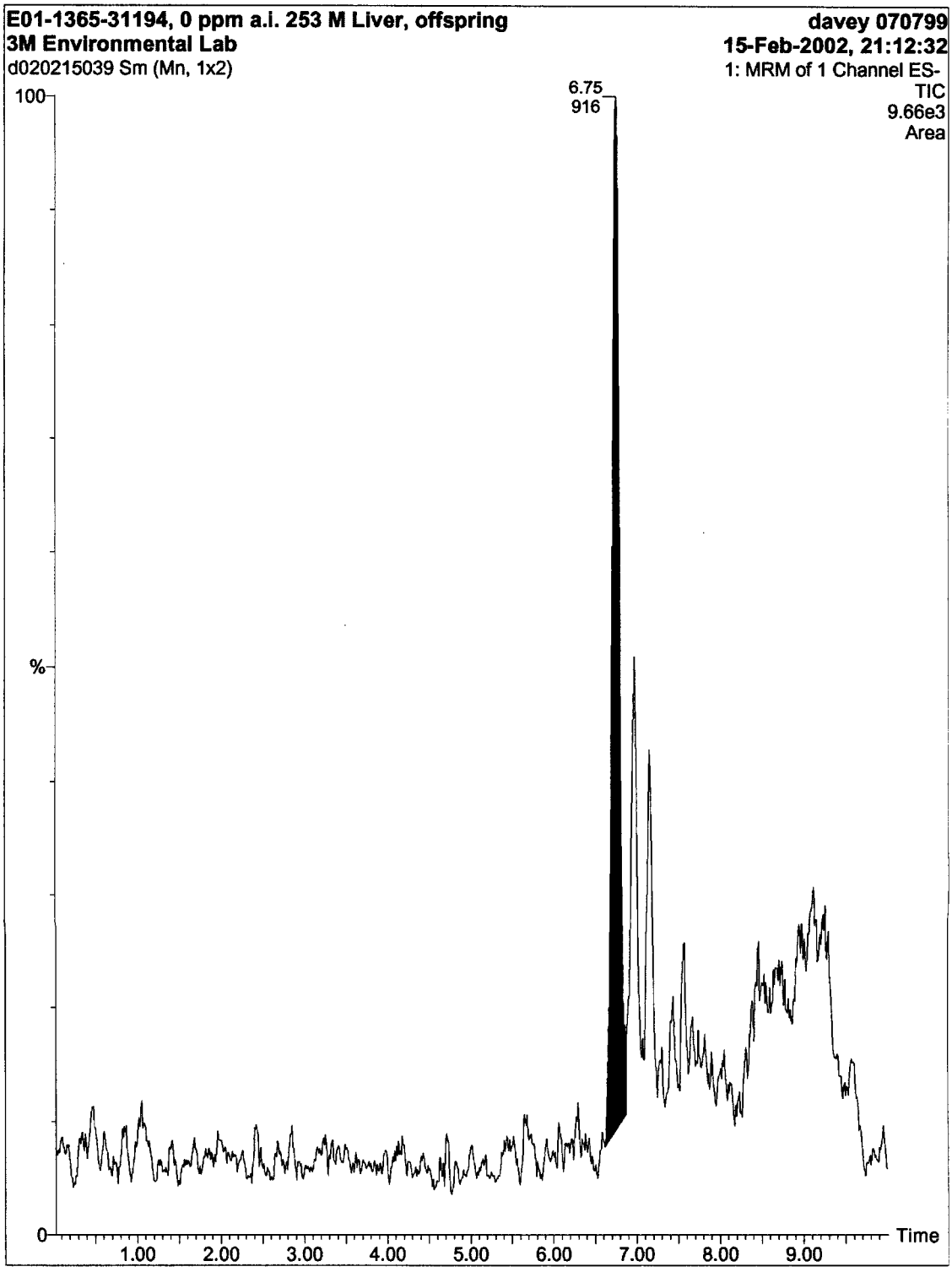
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Control Group Female Liver Sample



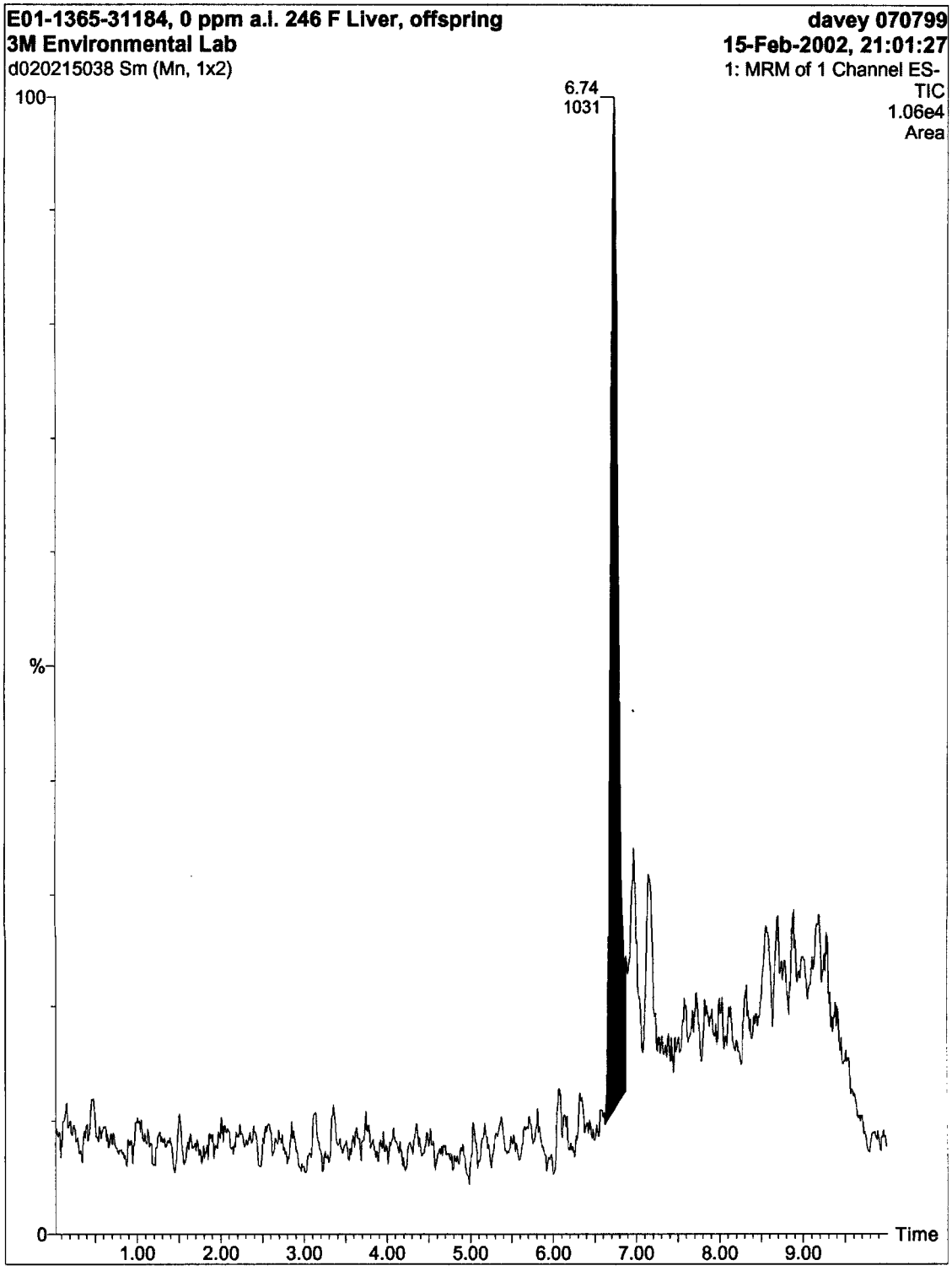
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Control Group Male Offspring Liver Sample



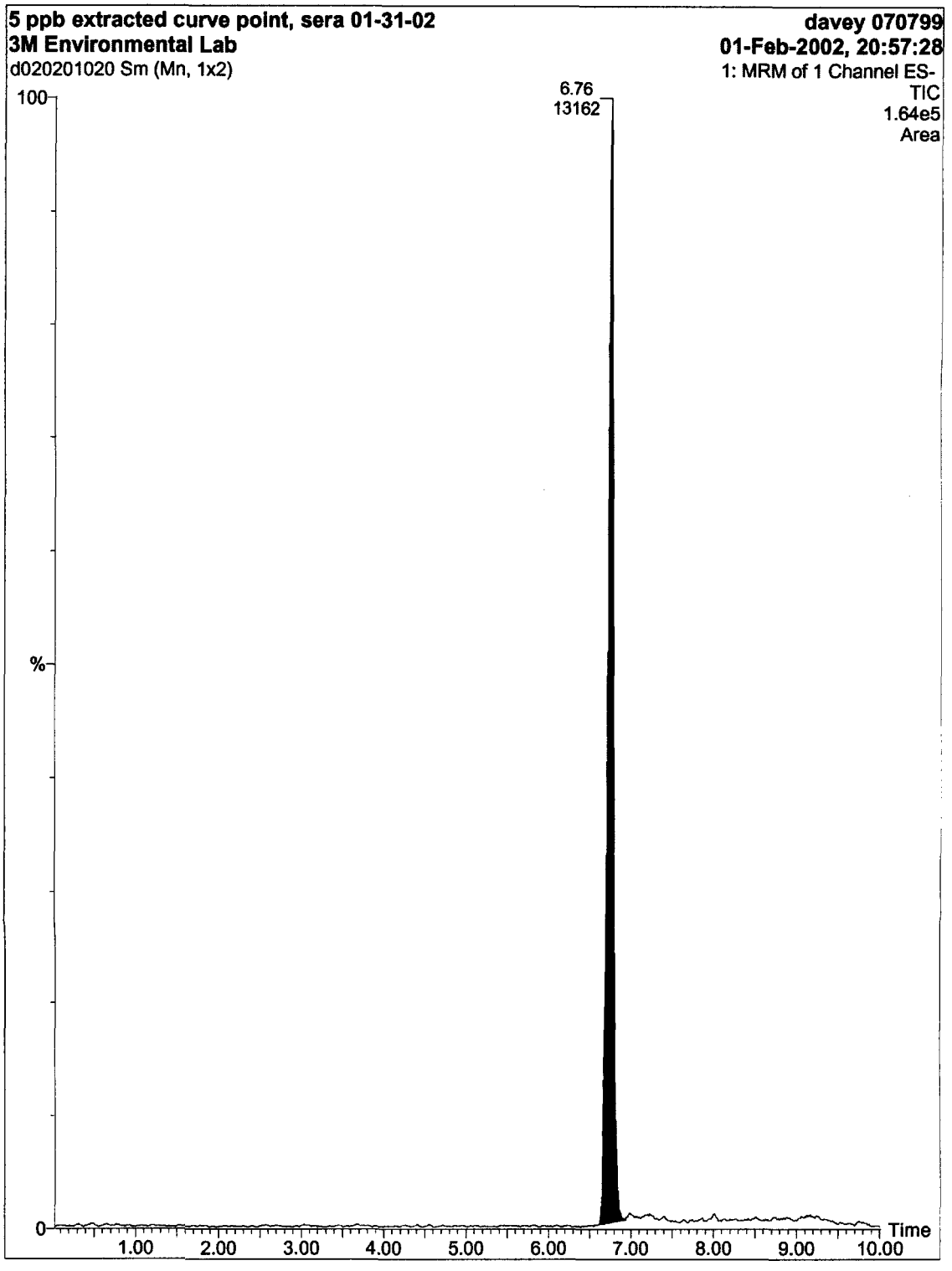
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Control Group Female Offspring Liver Sample



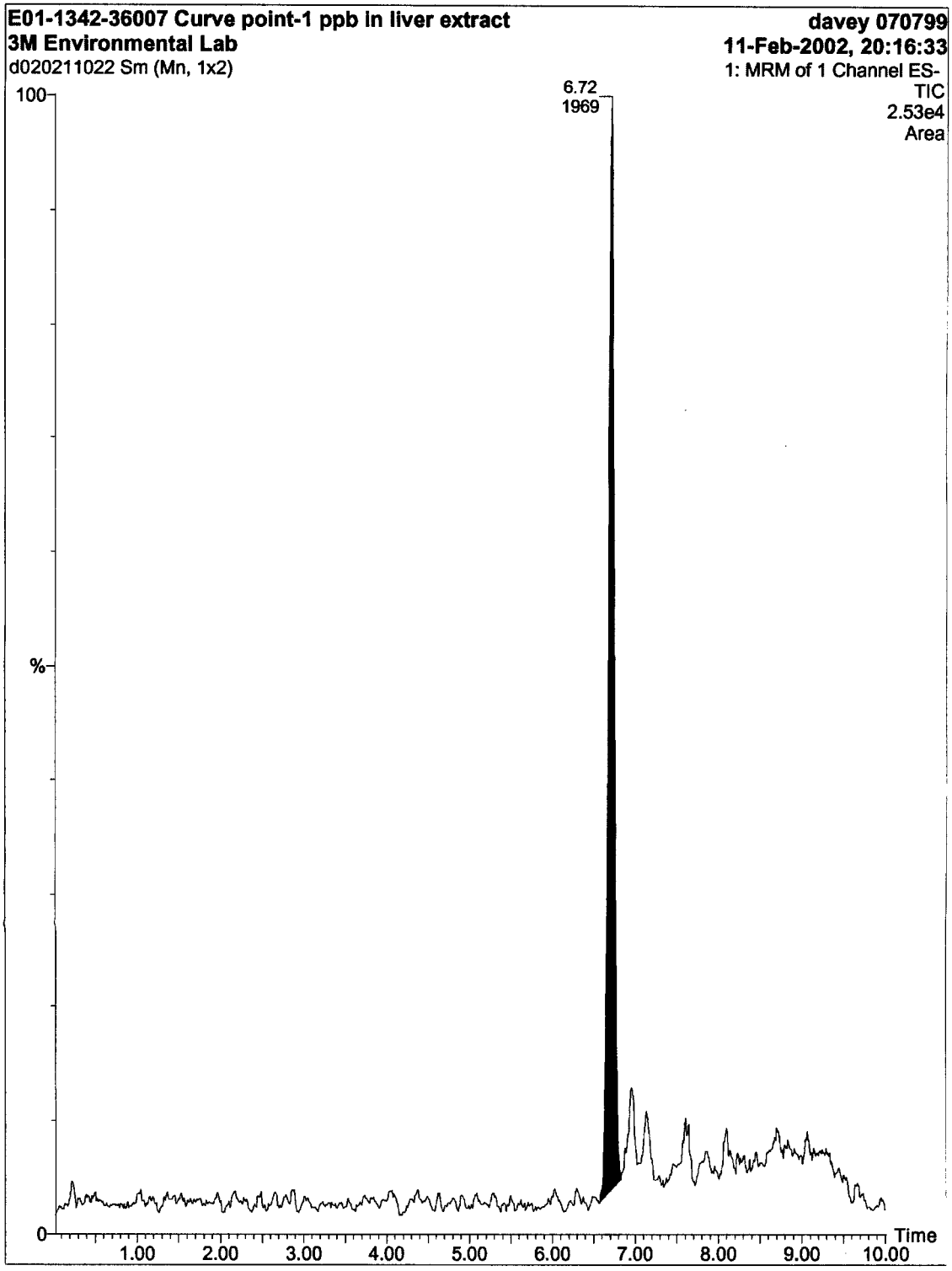
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Extracted Sera Calibration Curve Point



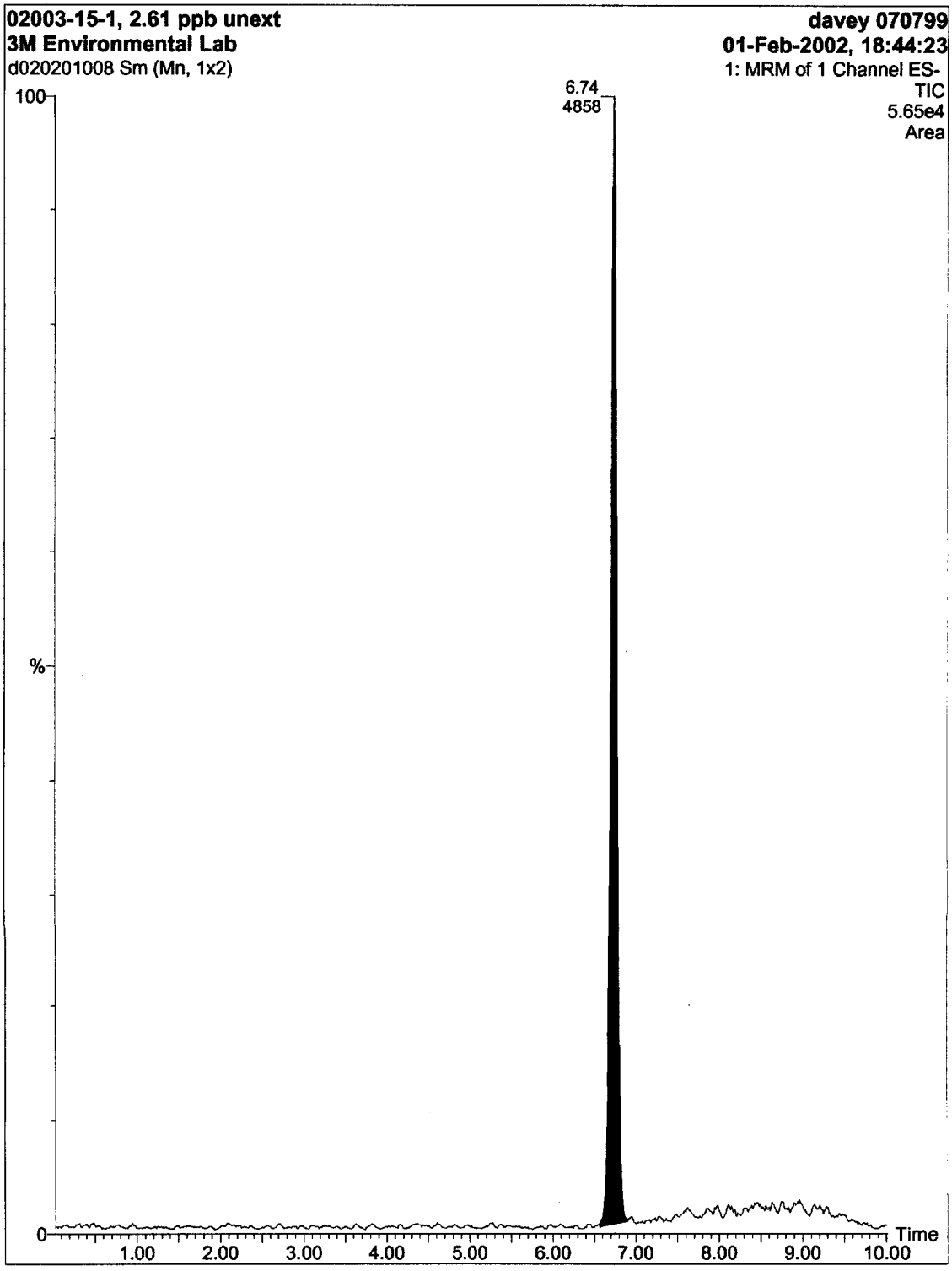
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Extracted Liver Calibration Curve Point



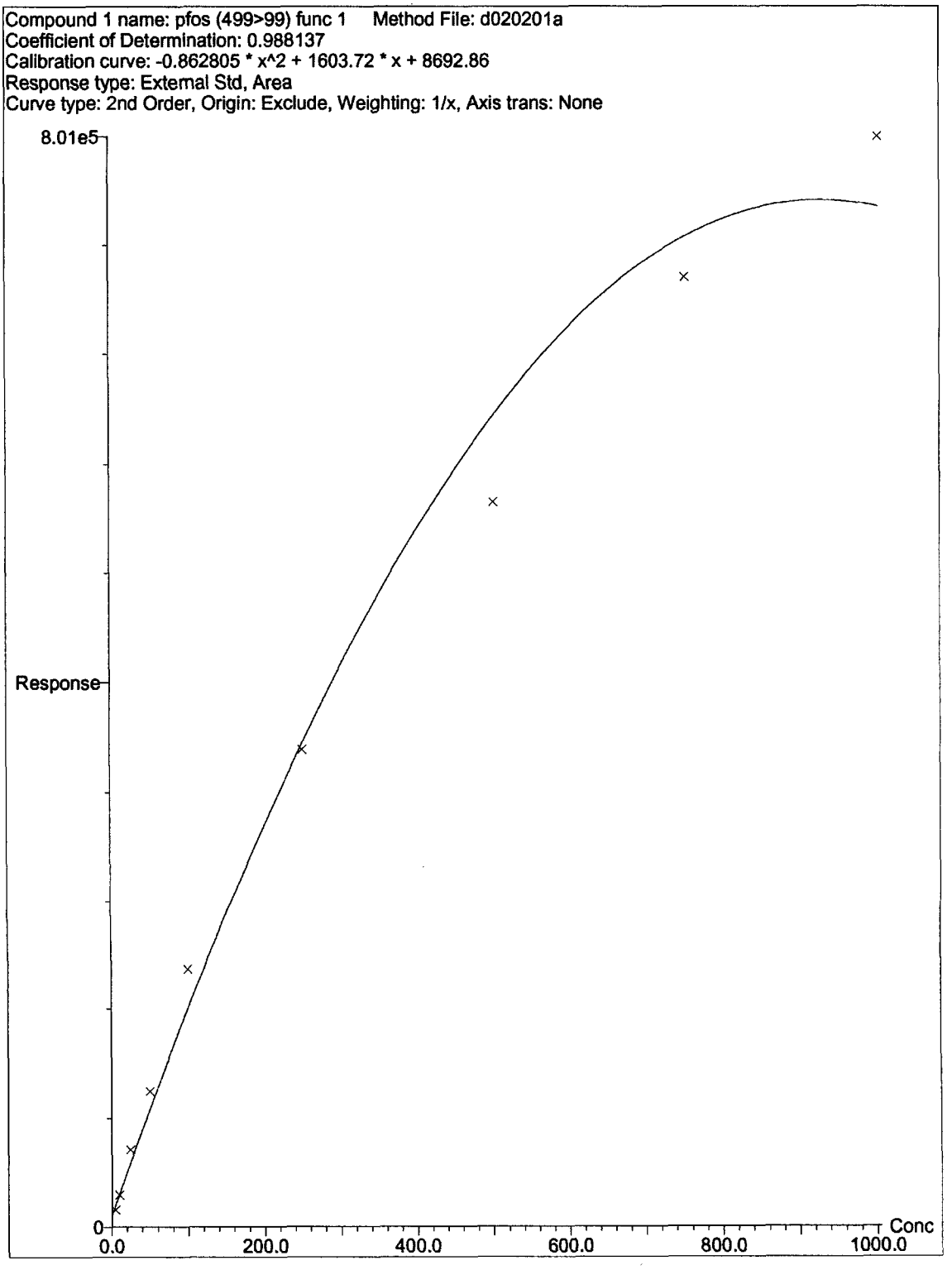
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Unextracted Calibration Curve Point



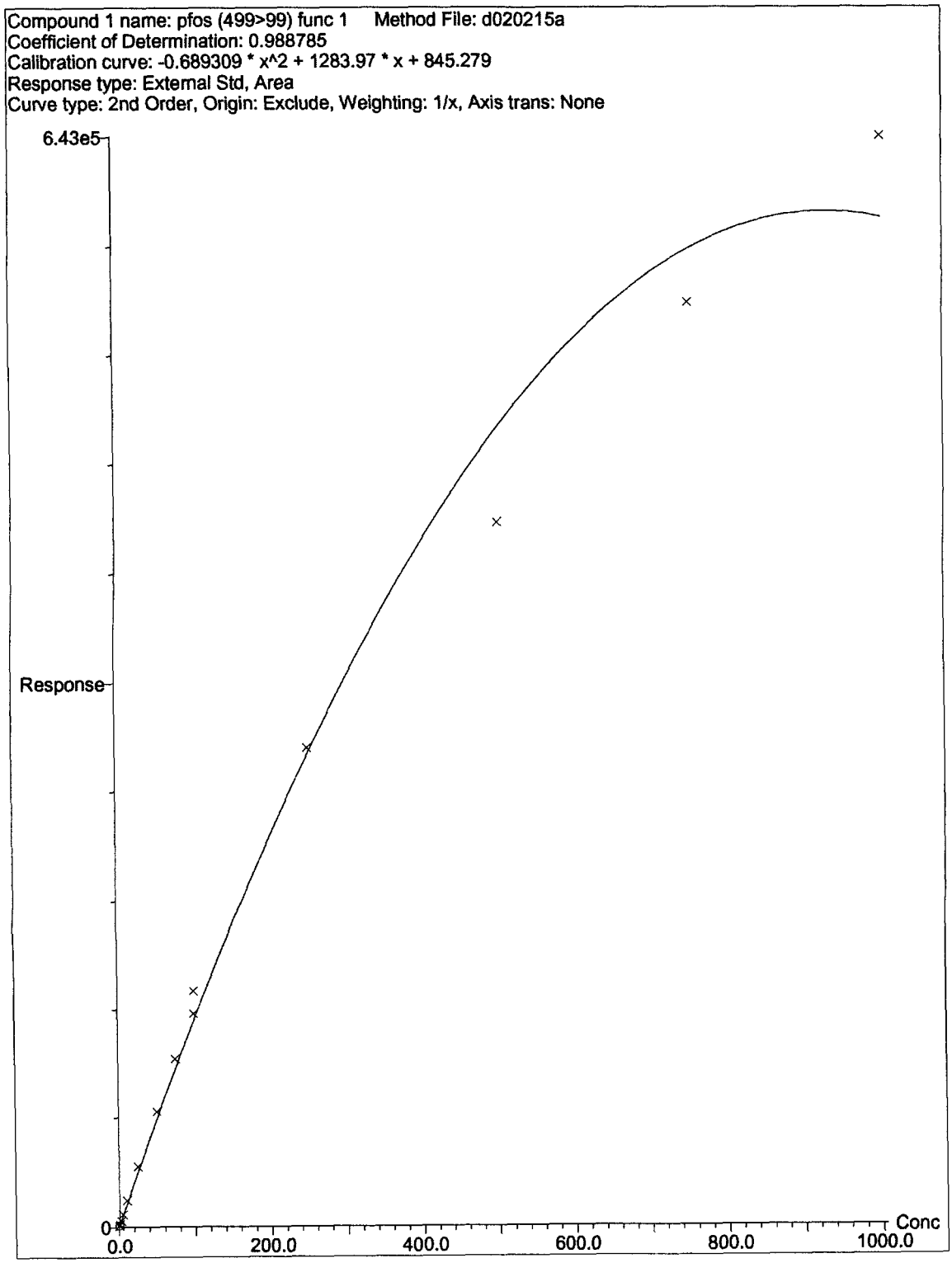
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Extracted Sera Calibration Curve



Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Extracted Liver Calibration Curve



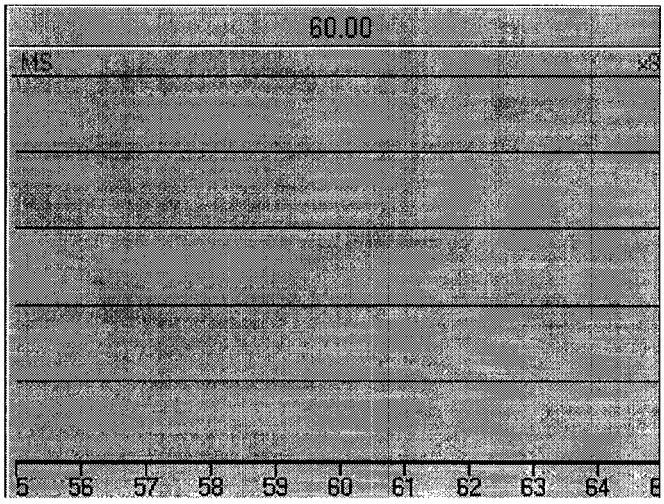
Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Mass Spectrometer Tune Settings

Tuning Method Report **Page 1**

Method: C:\MASSLYNX\DAVEY070799.PRO\ACQUDB\CENTRE1

Printed: Tue Mar 19 10:22:06 2002



MS

SOURCE (ESP-)	**Set**	**Rdbk**	**Analyser**	**Set**	**Rdbk**
Capillary	2.56	-2.48	LM Res 1	13.0	
Cone	20	-20	HM Res 1	13.0	
Hexapole 1	0.5		IEnergy 1	0.7	
Aperture 1	0.2		Entrance	2	1
Hexapole 2	0.8		Collision	11	11
Source Block Temp.	150	148	Exit	1	0
Desolvation Temp.	250	249	LM Res 2	11.0	
			HM Res 2	11.0	
			IEnergy 2	1.0	
			Multiplier	650	-646

Pressures	**Rdbk**	**Gas Flows**	**Rdbk**
Analyser Vacuum	3.8e-5	Cone Gas	150.5
Gas Cell	2.9e-3	Desolvation	701.5

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

HPLC Settings

Method Report **Page 1**

Method File: **C:\MASSLYNX\DAVEY070799.PRO\A(**
Last Modified: **Monday, March 11, 2002 08:01:01**

Printed: **Tuesday, March 19, 2002 08:37:50**

HP1100 LC Pump Initial Conditions

Solvents
A% 90.0
B% 10.0
C% 0.0
D% 0.0

Valve A set to channel
Valve B set to channel

Flow (ml/min) 0.300
Stop Time (mins) 10.0
Min Pressure (bar) 0
Max Pressure (bar) 400
Oven Temperature Left(°C) 40.0
Oven Temperature Right(°C) 40.0

HP1100 LC Pump Gradient Timetable

The gradient Timetable contains 5 entries which are :

Time	A%	B%	C%	D%	Flow
0.00	90.0	10.0	0.0	0.0	0.300
1.00	90.0	10.0	0.0	0.0	0.300
5.50	5.0	95.0	0.0	0.0	0.300
7.50	5.0	95.0	0.0	0.0	0.300
8.00	90.0	10.0	0.0	0.0	0.300

HP1100 LC Pump External Event Timetable

The Timetable contains 3 entries which are :

Time	Column Switch		Contact1	Contact2	Contac
Initial	Off	Off	Off	Off	Off
0.00	Off	On	On	Off	On
0.10	Off	Off	Off	Off	Off

HP1100 Autosampler Initial Conditions

Draw Speed 200.0
Eject Speed (µl/min) 200
Draw Position (mm) 0.00
Stop Time (mins) 10.00
Injection Volume(µl) 10.0
Vial Number 94
Thermostat On
Thermostat Temperature(°C) 20.0

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

Mass Spectrometer Scanning Parameters

Scanning Method Report **Page 1**

Method: **C:\MASSLYNX\DAVEY070799.PRO\ACQUDB\10MIN-PFOS-PFDA**
Last Modified: **Mon Jan 28 15:20:24 2002**

Printed: **Tue Mar 19 08:38:22 2002**

Solvent Delay (mins) : **0.00**

Function : 1 **MRM of 1 Mass Pair (ESP-)**

Inter Channel Delay (Secs) : 0.03
Span (Daltons) : 0.00
Start Time (Mins) : 0.00
End Time (Mins) : 10.00
Repeats : 1

Channel	Parent	Daughter	Dwell (Secs)	Coll Energy (eV)	Cone (V)
1	499.00	99.00	0.30	43	60

Function : 2 **MRM of 1 Mass Pair (ESP-)**

Inter Channel Delay (Secs) : 0.03
Span (Daltons) : 0.00
Start Time (Mins) : 0.00
End Time (Mins) : 10.00
Repeats : 1

Channel	Parent	Daughter	Dwell (Secs)	Coll Energy (eV)	Cone (V)
1	513.00	219.00	0.30	20	20

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

ATTACHMENT D: SAMPLE PREPARATION SHEETS

SPE Columns Extraction Worksheet

(1) Quail Sera 3 ENS 08/21/02
Page 1 of 2

Prep Date: 1/31/2002 1.31.02 OK
Analysts initials: OK/HOJ OK 1.31.02

Method Revision: ETS-8-231.0
Study Number: E01-1256 45 (RE) HOJ 1.31.02
Matrix: Sera

Lims Assgned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments 1.31.02
E01-1245-35766	Blank H2O-day 1	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	(2) (RE) OK 1-31-02
E01-1245-35767	QUAIL (RE) OK 1.31.02 Blank ~~mallard~~ sera-day 1	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	(2)
E01-1245-35768	QUAL (RE) OK 1.31.02 Mallard Sera MS-day 1	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 10ul of 02001-53	2ml of MeOH TN-A-5457	
E01-1245-35769	QUAIL (RE) OK 1.31.02 Mallard Sera MSD	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 10ul of 02001-53	2ml of MeOH TN-A-5457	
E01-1245-35770	5 ppb sera curve	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 2ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-35771	10 ppb sera curve	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 4ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-35772	25 ppb sera curve	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 10ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-35773	50 ppb sera curve	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 20ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-35774	100 ppb sera curve	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 4ul of 02001-53	2ml of MeOH TN-A-5457	
E01-1245-35775	250 ppb sera curve	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 10ul of 02001-53	2ml of MeOH TN-A-5457	
E01-1245-35776	500 ppb sera curve	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 20ul of 02001-53	2ml of MeOH TN-A-5457	
E01-1245-35777	750 ppb sera curve	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 30ul of 02001-53	2ml of MeOH TN-A-5457	
E01-1245-35778	1000 ppb sera curve	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 40ul of 02001-53	2ml of MeOH TN-A-5457	
E01-1245-35779	QC-25 ppb-1	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 10ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-35780	QC-25 ppb-2	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 10ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-35781	QC-25 ppb-3	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 10ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-35782	QC-40 ppm-1	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 36ul of 02001-15	2ml of MeOH TN-A-5457	
E01-1245-35783	QC-40 ppm-2	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 36ul of 02001-15	2ml of MeOH TN-A-5457	
E01-1245-35784	QC-40 ppm-3	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 36ul of 02001-15	2ml of MeOH TN-A-5457	
E01-1245-35785	QC-80 ppm-1	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 72ul of 02001-15	2ml of MeOH TN-A-5457	
E01-1245-35786	QC-80 ppm-2	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 72ul of 02001-15	2ml of MeOH TN-A-5457	
E01-1245-35787	QC-80 ppm-3	40+6	Waters, 1g, 6ml, lot 1290B2	✓ 72ul of 02001-15	2ml of MeOH TN-A-5457	

OK 8.28.02 RE

Blank Sera TN-A-5992 ; Amount of sera aliquated: 2 ml
Dilute Sera with Kandiyohi water; Amount of water added 38 ml
Aliquot 1ml of sera into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4145) to aliquated sample — DONE BY (RE) HOJ OK 8.28.02
Shake sample for 20 min @300 rpm (Shaker VWR-OSC-TAL) S/N041694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3M 769613)
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 02001-45 , conc. 100.7 ppm , added 2 µl to extract.
Transfer sample into appripriatelly marked autovial

(1) (RE) OK 8.28.02

(1) SERA SAMPLE RECEIVED FROM WILDLIFE WERE POOLED AT 3M PRIOR TO ANALYSIS SO ANY LEVELS IN THESE SAMPLE WOULD BE CONSISTANT FROM DAY TO DAY. HOJ 1.31.02

(2) USED 2ML OF POOLED SERA, DILUTED TO 40ML OF KANDIYOHI WATER. 1 ML WAS THEN USED FOR EXTRACTION. HOJ 1.31.02

✓ VISUAL VERIFICATION OF SPIKE PERFORMED BY OK VERIFIED BY HOJ OK 1.31.02

SPE Columns Extraction Worksheet (AC) Quail Sera } LAS 08/21/02

Prep Date: 1/31/2002 1.31.02
Analysts initials: OK/HOJ 012
1.31.02

Method Revision: ETS-8-231.0
Study Number: E01-1256 HOJ 1.31.02
Matrix: Sera 45

Lims Assgned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1245-28443	454-108-329, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200ul
E01-1245-28444	454-108-330, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	400ul
E01-1245-28445	454-108-331, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	400ul
E01-1245-28446	454-108-332, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	50ul
E01-1245-28447	454-108-333, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	400ul
E01-1245-28448	454-108-335, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200ul
E01-1245-28449	454-108-336, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300ul
E01-1245-28450	454-108-337, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100ul
E01-1245-28451	454-108-338, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300ul
E01-1245-28452	454-108-339, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200ul
E01-1245-28453	454-108-340, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200ul
E01-1245-28454	454-108-341, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200ul
E01-1245-28455	454-108-343, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100ul
E01-1245-28456	454-108-344, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200ul
E01-1245-28457	454-108-345, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200ul

OK 8.8.02

Blank Sera TN-A-5992 ; Amount of sera aliquated: 2 ml
Dilute Sera with Kandiyohi water; Amount of water added 38 ml
Aliquot 1ml of sera into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4145) to aliquated sample - DONE BY HOJ @ OK 8.8.02
Shake sample for 20 min @300 rpm (Shaker VWR ORBITAL/ SN 04/694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge SN# 7C9G13
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-1754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A- 5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 02001-45 , conc. 100.7ppm ; added 2ul to extract

Reviewed OK 4.9.02

FOR THE FOLLOWING SAMPLE VOLUMES, KANDIYOHI WATER WAS ADDED INTO QUANTITY LISTED TO MAKE A 1:20 DILUTION OF THE SERA.

SAMPLE VOLUME	H_2O ADDED
50ul	1 ML
100ul	1.9mL
200ul	3.8mL
300ul	5.7mL
400ul	7.6mL

(AC) 8 samples aliquated by OK. diluted by HOJ. OK 8.8.02

HOJ 1.31.02

Attachment D: Dilutions Summary Worksheet

Study: E01-1245
Dilution Date/Analyst:
Box Number: ~~E01-134~~ (RE) LAS 11/26/02

Solvent/TN Number: MeOH/5457 w/ IS PFDA
Extraction Date/Analyst: 1/31/02
Matrix/Timepoint: Quail Sera (AC) LAS 11/26/02

Sample Number or Description	Dilutions							
	1/1000	1/100	1/	1/	1/	1/	Comments	Verified By:
E01-1245-35785	x							
E01-1245-35786	x							
E01-1245-35787	x							
~~E01-1245-35779~~	(RE)	OK 11.26.02					OK 11.26.02	
E01-1245-35780								
E01-1245-35781								
E01-1245-35782		x						
E01-1245-35783		x						
E01-1245-35784		x						
							11.26.02 OK	

Notes:
1/10 dilution = 100µl of sample + 900µl of solvent
1/100 dilution = 10µl of sample + 990µl of solvent
1/1000 dilution = 1µl of sample + 1000µl of solvent

dilutions were done on date of extraction.
OK 11.26.02
Dilution information was confirmed by the autovial sample info. (AC) 12.2.02 OK

Form Completion Verified By: LAS 11/26/02

SPE Standard Curves--Fluids

Prep Date(s): 01/31/02, 02/01/02, 02/13/02
Analyst(s): OK/HOJ
Sample Matrix: Quail Sera
Method/Revision: ETS-8-231.1
Target Analyte(s): PFOS

Study Number: E01-1245
Equipment Number: NA
Final Solvent & TN Number: MeOH/TN-A-05457
Blank Tissue/Identifier: Quail Sera-TN-A-5992

PFOS Std: 5.02 ppm: 02001-52
PFOS Std: 50.2 ppm: 02001-53
PFOS Std: 2241 ppm: 02001-15
Surr.Std Approx. 100.7 ppm: 02001-45 PFDA

Blank sera initial dilution (mL/mL) for curves: 0.0500
Final solvent volume (mL) for curves: 2.000

Actual Concentrations of Standards in the FC Mix

PFOS Std Conc. ug/mL	PFOS Am't Spiked mL	PFDA Std Conc. ug/mL	PFDA Am't Spiked mL	All Initial Dilution mL/mL	All Final Vol. mL
5.02	0.0020	100.7	0.002	0.0500	2.000
5.02	0.0040	100.7	0.002	0.0500	2.000
5.02	0.0100	100.7	0.002	0.0500	2.000
5.02	0.0200	100.7	0.002	0.0500	2.000
50.2	0.0040	100.7	0.002	0.0500	2.000
50.2	0.0100	100.7	0.002	0.0500	2.000
50.2	0.0200	100.7	0.002	0.0500	2.000
50.2	0.0300	100.7	0.002	0.0500	2.000
50.2	0.0400	100.7	0.002	0.0500	2.000
5.02	0.0100	100.7	0.002	0.0500	2.000
2241	0.0360	100.7	0.002	0.0500	2.000
2241	0.0720	100.7	0.002	0.0500	2.000
50.2	0.0100	100.7	0.002	0.0500	2.000
50.2	0.0100	100.7	0.002	0.0500	2.000
5.02	0.0020	100.7	0.002	0.0500	2.000
5.02	0.0800	100.7	0.002	0.0500	2.000

Calculation sheet added for clarity
UAS 08/27/02

Calculated Concentrations of Standards in the sample matrix

Standard Number	PFOS Std Conc. ng/mL	PFDA Std Conc. ng/mL
E01-1245-35770, 5.0 ppb	201	4028
E01-1245-35771, 10 ppb	402	4028
E01-1245-35772, 25 ppb	1004	4028
E01-1245-35773, 50 ppb	2008	4028
E01-1245-35774, 100 ppb	4016	4028
E01-1245-35775, 250 ppb	10040	4028
E01-1245-35776, 500 ppb	20080	4028
E01-1245-35777, 750 ppb	30120	4028
E01-1245-35778, 1000 ppb	40160	4028
QC-25 ppb	1004	4028
QC-40 ppm	1613520	4028
QC-80 ppm	3227040	4028
Quail Sera MS/MSD-Day 1	10040	4028
Quail Sera MS/MSD-Day 2	10040	4028
Quail Sera MS-5 ppb	201	4028
Quail Sera MS-200 ppb	8032	4028

Calculated Concentrations of Standards in the final solvent

Standard Number	PFOS Std Conc. ng/mL	PFDA Std Conc. ng/mL
E01-1245-35770, 5.0 ppb	5.02	101
E01-1245-35771, 10 ppb	10.0	101
E01-1245-35772, 25 ppb	25.1	101
E01-1245-35773, 50 ppb	50.2	101
E01-1245-35774, 100 ppb	100	101
E01-1245-35775, 250 ppb	251	101
E01-1245-35776, 500 ppb	502	101
E01-1245-35777, 750 ppb	753	101
E01-1245-35778, 1000 ppb	1004	101
QC-25 ppb	25.1	101
QC-40 ppm	40338	101
QC-80 ppm	80676	101
Quail Sera MS/MSD-Day 1	251	101
Quail Sera MS/MSD-Day 2	251	101
Quail Sera MS-5 ppb	5.02	101
Quail Sera MS-200 ppb	201	101

Verified by: RWM - 8/27/02

SPE Columns Extraction Worksheet

(HC) Quail Sera OK 02/31/02

Prep Date: 2/1/2002
Analysts initials: OK/HOJ 2.1.02 OK 2.1.02

Method Revision: ETS-8-231.0
Study Number: E01-1245
Matrix: Sera

Lims Assgned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1245-35825	Blank H2O-day 2	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1245-35826	(RE) QUAIL OK 1.31.02 Blank mallard sera-day 2	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	USED 1mL OF DILUTED SERA THAT
E01-1245-35827	QUAIL (RE) OK 1.31.02 Mallard Sera MS-day 2	40+6	Waters, 1g, 6ml, lot 1290B2	10ul of 02001-53	2ml of MeOH TN-A-5457	WAS PREPD ON 1.31.02
E01-1245-35828	QUAIL OK 1.31.02 Mallard Sera MSD-Day2	40+6	Waters, 1g, 6ml, lot 1290B2	10ul of 02001-53	2ml of MeOH TN-A-5457	
E01-1245-28458	454-108-346, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28459	454-108-347, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	200uL
E01-1245-28460	454-108-348, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	300uL
E01-1245-28461	454-108-349, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	20uL
E01-1245-28462	454-108-350, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	400uL
(1) E01-1245-28463	454-108-351, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	400uL (RE) OK 2.1.02
E01-1245-28464	454-108-352, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	4. (RE) OK 2.1.02 150uL
E01-1245-28465	454-108-353, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	3. (RE) OK 2.1.02 300uL
E01-1245-28466	454-108-354, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	300uL
E01-1245-28467	454-108-355, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	200uL
E01-1245-28468	454-108-356, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	300uL
E01-1245-28469	454-108-357, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	70uL
E01-1245-28470	454-108-358, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	300uL
E01-1245-28471	454-108-359, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	200uL
E01-1245-28472	454-108-360, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	300uL
E01-1245-28473	454-108-361, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	200uL
E01-1245-28474	454-108-362, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	200uL
E01-1245-28475	454-108-363, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2		2ml of MeOH TN-A-5457	100uL

(1) NO SERA SAMPLE FOR THIS NUMBER. ONLY BLOOD. 2.1.02 OK

OK 2.1.02

ONE POINT CALIBRATION (RE) 2.1.02

FOR THE ABOVE SAMPLE VOLUMES THE FOLLOWING AMOUNT OF KANDIYOHI WATER WAS ADDED TO ACHIEVE A 1:20 DILUTION

- 50uL - 1mL
- 70uL - 1.4mL (RE) OK 2.1.02
- 100uL - 1.9mL
- 200uL - 3.8mL
- 300uL - 5.7mL
- 400uL - 7.6mL
- 500uL - 9.5mL

60uL = 1.2mL
80uL = 1.6mL
90uL = 1.8mL

Spike samples accordingly OK 2.1.02
Add 5ml of ACN (TN-A-4145) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker VWR-OSCILLATING S/N 041694)
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3M #769613)
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 62061-95, conc. 100.7 ppm, amount added 2mL TO EXTRACT OK 2.1.02
Transfer sample into appripriatelly marked autovial

MZE OK 2.2.02 (ACCR) SAMPLES WERE ALIQUOTED BY OK/

SPE Columns Extraction Worksheet

(AC) Quail Sera LAS 08/31/02
Page 2 of 4

Prep Date: 2/1/2002 2.1.02 OK
Analysts initials: OK/HOJ
HOJ 2.1.02

Method Revision: ETS-8-231.0
Study Number: E01-1245
Matrix: Sera

Lims Assgned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1245-28476	454-108-365, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	400uL
E01-1245-28477	454-108-366, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28478	454-108-367, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28479	454-108-368, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL
E01-1245-28480	454-108-369, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28481	454-108-370, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL
E01-1245-28482	454-108-371, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28483	454-108-372, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	400uL
E01-1245-28484	454-108-373, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	1000uL
E01-1245-28485	454-108-374, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28486	454-108-375, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL
E01-1245-28487	454-108-376, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL
E01-1245-28488	454-108-377, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	700uL
E01-1245-28489	454-108-378, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28490	454-108-379, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28491	454-108-380, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28492	454-108-381, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28493	454-108-382, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL
E01-1245-28494	454-108-383, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL
E01-1245-28495	454-108-385, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28496	454-108-386, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	400uL
E01-1245-28497	454-108-387, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL

OK 8.28.02

(AC) (RE) OK 8.28.02

(RE) 2.1.02

Blank Sera TN-A-5752; Amount of sera aliquated: N/A ml
Dilute Sera with Kandiyohi water; Amount of water added NA ml
Aliquot 1ml of sera into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4145) to aliquated sample — DONE BY HOJ (RE) OK 8.28.02
Shake sample for 20 min @300 rpm (Shaker VWR ORBITAL S/N 041694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge IEC 769617
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 02001-45, conc. 0.71/ml, amount added 2mL to extract.

- Samples were aliquoted by OK & diluted by HOJ!
(AC) (RE) OK 8.28.02

(1) (RE) OK 8.28.02

OK 8.28.02

SPE Columns Extraction Worksheet

(AC) Quantisera Page 3 of 4 } LAS 08/21/02

Prep Date: 2/1/2002 2.1.02 OK
Analysts initials: OK/HOJ 2.1.02

Method Revision: ETS-8-231.0
Study Number: E01-1245
Matrix: Sera

Lims Assgned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1245-28498	454-108-388, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL
E01-1245-28499	454-108-389, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28500	454-108-390, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28501	454-108-391, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	60uL
E01-1245-28502	454-108-392, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	80uL
E01-1245-28503	454-108-393, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	20uL
E01-1245-28504	454-108-394, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL
E01-1245-28505	454-108-395, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28506	454-108-396, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	90uL
E01-1245-28507	454-108-397, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	300uL
E01-1245-28508	454-108-398, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28509	454-108-399, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28510	454-108-401, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28511	454-108-402, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	400uL
E01-1245-28512	454-108-403, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28513	454-108-404, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28514	454-108-405, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28515	454-108-406, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28516	454-108-407, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28517	454-108-408, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28518	454-108-246, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28519	454-108-253, 0 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	20uL (1uL Hex 8.30.02)

Blank Sera TN-A-5992 ; Amount of sera aliquated: N/A ml
Dilute Sera with Kandiyohi water; Amount of water added N/A ml
Aliquot 1ml of sera into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4495) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker Van Ormstal 5/w241694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge Za # 769613
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (Meoh TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 82001-95 , conc. 100.7 ug/ml, amount added 2uL TOP-SHOT

OK 8.30.02 OK 8.30.02

samples were aliquoted by OK & diluted by HOJ. (AC) (QC) OK 8.30.02 (D)

(D) (RE) OK 8.30.02

SPE Columns Extraction Worksheet

(AC) Quant Sera } LAS 08/21/02
Page 4 of 4 }

Prep Date: 2/1/2002 2-1-02 OK
Analysts initials: OK/HOJ
LAS 2-1-02

Method Revision: ETS-8-231.0
Study Number: E01-1245
Matrix: Sera

Lims Assgned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1245-28520	454-108-256, 0 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	20uL (*)
E01-1245-28521	454-108-259, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
E01-1245-28522	454-108-266, 0 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	70uL
E01-1245-28523	454-108-270, 0 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28524	454-108-273, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	80uL
E01-1245-28525	454-108-277, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	200uL
E01-1245-28526	454-108-283, 0 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	70uL
E01-1245-28527	454-108-291, 0 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	50uL
E01-1245-28528	454-108-517, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	20uL (*)
E01-1245-28529	454-108-522, 10 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	70uL
E01-1245-28530	454-108-525, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	30uL (*)
E01-1245-28531	454-108-533, 10 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	70uL
E01-1245-28532	454-108-538, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	80uL
E01-1245-28533	454-108-545, 10 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	40uL (*)
E01-1245-28534	454-108-552, 10 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	70uL
E01-1245-28535	454-108-557, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	60uL
E01-1245-28536	454-108-561, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	50uL
E01-1245-28537	454-108-568, 10 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	100uL
			LAS 08/22/02			

Blank Sera TN-A-5992 ; Amount of sera aliquated: N/A ml
Dilute Sera with Kandiyohi water; Amount of water added N/A ml
Aliquot 1ml of sera into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4695) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker VAR ORBITAL SN 041694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3M# 769613
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 02001-95 , conc. 100.7 ppm amount added 2uL TO EXTRACT

(*)(AC) 1ml of H2O added to these samples. 4.9.02 OK

- All samples aliquoted by OK & diluted by HOJ.

(AC) OK 8.8.02

(D) RE OK 82802

OK 8.8.02 (AC) (*) (D)

OK 8.9.02

(AC) OK 8.8.02

BOTTLE–TOP DISPENSER DAILY CALIBRATION CHECK

Manufacturer and Model:	VWR 5ml
Date:	2·1·02
Initials:	OK
Dispenser ID:	0799
Balance ID:	900
Volume:	5ml
Solvent:	ACN
ID #:	IN-A- 4145

	Mass of solvent (g)	Density[1] (g/ml)	Volume of Solvent
1	3.9368	0.7857	5.0106
2	3.9413	0.7857	5.0163
3	3.93821	0.7857	5.0123
4	3.9474	0.7857	5.0241
5	3.9417	0.7857	5.0268
		Average Volume:	5.0160
		%Accuracy:	100.3%
		Std. Dev.:	0.005208
		%C.V.:	0.1%
		Pass/Fail:	(P) / F

Performance Specifications		
Volume, (mL)	Accuracy, (%)	C.V., (%)
1 to 10	98.0 - 102 %	± 1.0 %

Corrective Actions:

None taken. 2·1·02
OK

[1]Density values taken from the Merck Index, 12th Edition, Copyright 1996
d^{y}_{x} =specific gravity at $y^{degrees\ C}$ referred to water at $x^{degrees\ C}$
n-Hexane d^{20}_{4} =0.660
Acetone d^{25}_{0} =0.788
Methanol d^{25}_{4} =0.7866
Methylene Chloride d^{20}_{4} =1.3255

(AC) Page 1 of 5 03-08/22/02

Prep Date: 2-7-02/2-8-02
Analysts initials: OK/HCJ

Method Revision: ES-S-231.1
Study Number: E01-1245 AC 2.8.02
Matrix: Liver

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Amount of liver weighed (g)	Comments
E01-1342-30312	0 ppm a.i. 329 M Liver					1.0032g	NA
E01-1342-30321	0 ppm a.i. 330 F Liver					1.0749 g	
E01-1342-30330	0 ppm a.i. 331 M Liver					1.0530g	
E01-1342-30339	0 ppm a.i. 332 F Liver					1.0469g	
E01-1342-30348	0 ppm a.i. 333 M Liver					1.0378g	
E01-1342-30357	0 ppm a.i. 334 F Liver					1.0232g	
E01-1342-30366	0 ppm a.i. 335 M Liver					1.0062g	
E01-1342-30375	0 ppm a.i. 336 F Liver					1.0188g	
E01-1342-30384	0 ppm a.i. 337 M Liver					1.0269g	
E01-1342-30393	0 ppm a.i. 338 F Liver		PFOS			1.0543g	
E01-1342-30402	0 ppm a.i. 339 M Liver					1.0986g	
E01-1342-30411	0 ppm a.i. 340 F Liver					1.0182g	
E01-1342-30420	0 ppm a.i. 341 M Liver					1.0235g	
E01-1342-30429	0 ppm a.i. 342 F Liver					1.0904g	
E01-1342-30438	0 ppm a.i. 343 M Liver					1.0090g	
E01-1342-30447	0 ppm a.i. 344 F Liver					1.0087g	
E01-1342-30456	0 ppm a.i. 345 M Liver					1.1046g	
E01-1342-30465	0 ppm a.i. 346 F Liver					1.0993g	
E01-1342-30474	0 ppm a.i. 347 M Liver					1.0586g	
E01-1342-30483	0 ppm a.i. 348 F Liver					1.1036g	
E01-1342-30492	0 ppm a.i. 349 M Liver					1.0358g	
E01-1342-30501	0 ppm a.i. 350 F Liver					1.0237g	

Blank Liver TN-A-_____; Amount of liver: _____g
Homogenize liver with Kandiyohi water; Amount of water added _____ml
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-_____) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker _____)
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge _____)
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-_____)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (_____TN-A-_____) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number _____, conc._____, amount added_____
Transfer sample into appripriatelly marked autovial

OK 2.8.02

Samples were weighed out by OK 2.8.02

SPE Columns Extraction Worksheet

(AC) Quality Level Page 2 of 5 LAS 08/22/02

Prep Date: 2.7.02/2.8.02
Analysts initials: OK/HOJ

Method Revision: C75-8-231.1
Study Number: E01.1245
Matrix: Liver

HOJ 2.8.02

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Amount of liver weighed (g)	Comments
E01-1342-30510	0 ppm a.i. 351 M Liver					1.0583g	NA
E01-1342-30519	0 ppm a.i. 352 F Liver					1.1509g	
E01-1342-30528	0 ppm a.i. 353 M Liver					1.0183g	
E01-1342-30537	0 ppm a.i. 354 F Liver					1.0578g	
E01-1342-30546	0 ppm a.i. 355 M Liver					1.0899g	
E01-1342-30555	0 ppm a.i. 356 F Liver					1.0312g	
E01-1342-30564	0 ppm a.i. 357 M Liver					1.0352g	
E01-1342-30573	0 ppm a.i. 358 F Liver					1.1031g	
E01-1342-30582	0 ppm a.i. 359 M Liver					1.0941g	
E01-1342-30591	0 ppm a.i. 360 F Liver					1.0586g	
E01-1342-30600	0 ppm a.i. 361 M Liver					1.0084g	
E01-1342-30609	0 ppm a.i. 362 F Liver					1.0424g	
E01-1342-30618	0 ppm a.i. 363 M Liver					1.0818g	
E01-1342-30627	0 ppm a.i. 364 F Liver					1.0364g	
E01-1342-30636	0 ppm a.i. 365 M Liver					1.0916g	
E01-1342-30645	0 ppm a.i. 366 F Liver					1.0577g	
E01-1342-30654	0 ppm a.i. 367 M Liver					1.0327g	
E01-1342-30663	0 ppm a.i. 368 F Liver					1.0920g	
E01-1342-30672	10 ppm a.i. 369 M Liver					1.0010g	
E01-1342-30681	10 ppm a.i. 370 F Liver					1.0868g	
E01-1342-30690	10 ppm a.i. 371 M Liver					1.0856g	
E01-1342-30699	10 ppm a.i. 372 F Liver					1.0748g	

Blank Liver TN-A-_____; Amount of liver: _____g
Homogenize liver with Kandiyohi water; Amount of water added _____ml
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-_____) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker _____)
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge _____)
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-_____)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (_____TN-A-_____) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number _____, conc._____, amount added_____
Transfer sample into appripriatelly marked autovial

3.8.02 OK

Samples were weighed out by OK & homogenized by HOJ. (AC) (LS) 8.8.02 OK

Prep Date: 2.7.02 / 2.8.02
Analysts initials: OK / HOJ

Method Revision: EPA-821.1
Study Number: E01-1245
Matrix: Liver 2.8.02

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Amount of liver weighed (g)	Comments
E01-1342-30708	10 ppm a.i. 373 M Liver					1.0276g	NA
E01-1342-30717	10 ppm a.i. 374 F Liver					1.0790g	
E01-1342-30726	10 ppm a.i. 375 M Liver					1.1005g	
E01-1342-30735	10 ppm a.i. 376 F Liver					1.1036g	
E01-1342-30744	10 ppm a.i. 377 M Liver					1.0236g	
E01-1342-30753	10 ppm a.i. 378 F Liver					1.0021g	
E01-1342-30762	10 ppm a.i. 379 M Liver					1.0312g	
E01-1342-30771	10 ppm a.i. 380 F Liver					1.0825g	
E01-1342-30780	10 ppm a.i. 381 M Liver					1.0501g	
E01-1342-30789	10 ppm a.i. 382 F Liver					1.0122g	
E01-1342-30798	10 ppm a.i. 383 M Liver					1.0110g	
E01-1342-30820	10 ppm a.i. 384 F Liver					0.6864g	
E01-1342-30829	10 ppm a.i. 385 M Liver					1.0078g	
E01-1342-30838	10 ppm a.i. 386 F Liver					1.1215g	
E01-1342-30847	10 ppm a.i. 387 M Liver					~~1.0078g~~ (Re)	1.0315g
E01-1342-30856	10 ppm a.i. 388 F Liver					1.0878g	
E01-1342-30865	10 ppm a.i. 389 M Liver					1.0310g	
E01-1342-30874	10 ppm a.i. 390 F Liver					1.0018g	
E01-1342-30883	10 ppm a.i. 391 M Liver					1.0947g	
E01-1342-30892	10 ppm a.i. 392 F Liver					1.0060g	
E01-1342-30901	10 ppm a.i. 393 M Liver					1.0834g	
E01-1342-30910	10 ppm a.i. 394 F Liver					1.0238g	

OK 2.8.02

Blank Liver TN-A-5143 (FEMALE*); Amount of liver: 5.0353 g ; TN-A-5142 ; → 1.0015g - MALE*
Homogenize liver with Kandiyohi water; Amount of water added 95 ml FOR FEMALE (AE) 4 ml FOR MALE OK 4.9.02
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-_____) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker _____)
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge _____)
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-_____)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (_____TN-A-_____) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number _____, conc._____, amount added_____
Transfer sample into appripriatelly marked autovial

All samples were weighed out & homogenized on 2.8.02 & 2.7.02 by OK & HOJ, including blanks.

(*) AC TRUE FOR ALL BLANKS IN THIS STUDY OK 4.9.02

samples were weighed out by OK & homogenized by HOJ. (AE) OK 2.8.02

SPE Columns Extraction Worksheet (AC) Quail Liver } LAS 08/22/02
Page 4 of 5

Prep Date: 9K 2.7.02/2.8.02
Analysts initials: OK/HOJ

Method Revision: EPA-E-231.1
Study Number: E01-1245
Matrix: Liver

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Amount of liver weighed (g)	Comments
E01-1342-30919	10 ppm a.i. 395 M Liver					1.0489g	NA
E01-1342-30928	10 ppm a.i. 396 F Liver					1.0370g	
E01-1342-30937	10 ppm a.i. 397 M Liver					1.0309g	
E01-1342-30946 (RO) 2.18.02 OK (AC)	10 ppm a.i. 398 F ~~Brain~~ Liver					1.0240g	
E01-1342-30955	10 ppm a.i. 399 M Liver					1.0276g	
E01-1342-30964	10 ppm a.i. 400 F Liver					1.0181g	
E01-1342-30973	10 ppm a.i. 401 M Liver					1.0361g	
E01-1342-30982	10 ppm a.i. 402 F Liver					1.0876g	
E01-1342-30991	10 ppm a.i. 403 M Liver					1.0019g	
E01-1342-31000	10 ppm a.i. 404 F Liver					1.0173g	
E01-1342-31009	10 ppm a.i. 405 M Liver					1.0336g	
E01-1342-31018	10 ppm a.i. 406 F Liver					1.0047g	
E01-1342-31027	10 ppm a.i. 407 M Liver					1.0956g	
E01-1342-31036	10 ppm a.i. 408 F Liver					1.0044g	
						NA	

Blank Liver TN-A-_____; Amount of liver: _____g
Homogenize liver with Kandiyohi water; Amount of water added _____ml
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-_____) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker _____)
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge _____)
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-_____)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (_____TN-A-_____) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number _____, conc._____, amount added_____
Transfer sample into appripriatelly marked autovial

8.8.02 OK

Samples were weighed out by OK & homogenized by HOJ. (AC) 8.8.02

SPE Columns Extraction Worksheet (AC) Quail Liver } LAS 08/22/02 Page 5 of 5

Prep Date: 2-7-02/2-8-02
Analysts initials: DK/HOJ

Method Revision: E75.8.231.1
Study Number: E01.1245
Matrix: Liver AC/2-8-02

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Amount of liver weighed (g)	Comments
E01-1365-31184	0 ppm a.i. 246 F Liver					0.7302g	NA
E01-1365-31194	0 ppm a.i. 253 M Liver					0.3806g	
E01-1365-31199	0 ppm a.i. 256 M Liver					0.5947g	
E01-1365-31204	0 ppm a.i. 259 F Liver					0.4434g	
E01-1365-31209	0 ppm a.i. 266 M Liver					0.2673g	
E01-1365-31214	0 ppm a.i. 270 M Liver					0.5900g	
E01-1365-31219	0 ppm a.i. 273 F Liver					0.5044g	
E01-1365-31224	0 ppm a.i. 277 F Liver					0.4326g	
E01-1365-31229	0 ppm a.i. 283 M Liver					0.6666g	
E01-1365-31234	0 ppm a.i. 291 M Liver					0.6072g	
E01-1365-31240	10 ppm a.i. 517 F Liver					0.6578g	
E01-1365-31245	10 ppm a.i. 522 M Liver					0.7325g	
E01-1365-31250	10 ppm a.i. 525 F Liver					0.5720g	
E01-1365-31255	10 ppm a.i. 533 M Liver					0.6905g	
E01-1365-31260	10 ppm a.i. 538 F Liver					0.5211g	
E01-1365-31265	10 ppm a.i. 545 M Liver					0.4624g	
E01-1365-31270	10 ppm a.i. 552 M Liver					0.5353g	
E01-1365-31275	10 ppm a.i. 557 F Liver					0.4654g	
E01-1365-31280	10 ppm a.i. 561 F Liver					0.9417g	
E01-1365-31285	10 ppm a.i. 568 M Liver					0.7581g	

Blank Liver TN-A-_____; Amount of liver: _____g
Homogenize liver with Kandiyohi water; Amount of water added _____ml
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-_____) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker _____)
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge _____)
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-_____)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (_____TN-A-_____) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number _____, conc._____, amount added_____
Transfer sample into appripriatelly marked autovial

3.8.02 OK

Sample weights were rounded to the nearest 0.1g and had Kandiyohi water added as summarized below: AC) 2-8 02

0.3g - 2.7mL
0.4g - 3.6 mL
0.5g - 4.5 mL
0.6g - 5.4 mL
0.7g - 6.3mL
0.8g - 7.2mL
0.9g - 8.1 mL
1.0g - 9.0mL

(AC) This is true for all the livers weighed in this study. 4.9.02 OK.

(AC) (DK) 8.8.02 OK
Samples were wighed out by OK; homogenized by HOJ. and is true for all samples in this study.

SPE Columns Extraction Worksheet

(AC) Quant Level Page 1 of 23 LAS 08/22/02

Prep Date: 2/11/2002 2.11.02
Analysts initials: OK.HOJ OK

Method Revision: ETS-8-231.0
Study Number: E01-~~1342/1365~~ ,1245 (Re) MW 2.11.02
Matrix: Liver

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1342-36000	Blank H2O-day 1	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	N.A
E01-1342-36001	Blank female liver-day 1	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-36002	Blank male liver-day 1	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-36003	Female liver MS-day 1	40+6	C18, 1g, 6cc, lot W2025B1	50ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36004	Female liver MSD-day 1	40+6	C18, 1g, 6cc, lot W2025B1	50ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36005	Curve point-0.2 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	0.4ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36006	Curve point-0.5 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	1ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36007	Curve point-1 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	2ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36008	Curve point-2.5 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	5ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36009	Curve point-5 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	10ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36010	Curve point-10 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	20ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36011	Curve point-25 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	50ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36012	Curve point-50 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	100ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36013	Curve point-75 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	150ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36014	Curve point-100 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	200ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36062	Curve point-100 ppb-2 in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	4ul of 02001-53	2ml MeOH TN-A-5754	
E01-1342-36015	Curve point-250 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	10ul of 02001-53	2ml MeOH TN-A-5754	
E01-1342-36016	Curve point-500 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	20ul of 02001-53	2ml MeOH TN-A-5754	
E01-1342-36060	Curve point-750 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	30ul of 02001-53	2ml MeOH TN-A-5754	
E01-1342-36061	Curve point-1000 ppb in liver extract	40+6	C18, 1g, 6cc, lot W2025B1	40ul of 02001-53	2ml MeOH TN-A-5754	

FEMALE* MALE** (AC) OK 4.9.02

Blank Liver TN-A-5143; Amount of liver: 5.0353 g Blank Liver TN-A-5142; Amount of liver: 1.0015 g
Homogenize liver with Kandiyohi water; Amount of water added 45 ml Amount of water added 9 ml
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4145) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker VWR SHAKER S/N: 041694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3.4# 769613
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 02001-45, conc. 100.7ppm, amount added 2ul
Transfer sample into appripriatelly marked autovial

(AC) (AC). Entire extraction done by OK. 8.8.02 OK

SPE Columns Extraction Worksheet

(AC) Quail Liver } LAS 08/22/02
Page 2 of 2

Prep Date: 2.11.02
Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0
Study Number: E01-~~1342/136~~ (R) 1245 AG 2.11.02
Matrix: LIVER

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1342-36017	QC@10 ppb-1	40+6	C18, 1g, 6cc, lot W2025B1	4ul of 02001-52	2ml MeOH TN-A-5754	NA
E01-1342-36018	QC@10 ppb-2	40+6	C18, 1g, 6cc, lot W2025B1	4ul of 02001-52	2ml MeOH TN-A-5754	
E01-1342-36019	QC@10 ppb-3	40+6	C18, 1g, 6cc, lot W2025B1	4ul of 02001-52	2ml MeOH TN-A-5754	
E01-1342-36020	QC@75 ppb-1	40+6	C18, 1g, 6cc, lot W2025B1	30ul of 02001-52	2ml MeOH TN-A-5754	
E01-1342-36021	QC@75 ppb-2	40+6	C18, 1g, 6cc, lot W2025B1	30ul of 02001-52	2ml MeOH TN-A-5754	
E01-1342-36022	QC@75 ppb-3	40+6	C18, 1g, 6cc, lot W2025B1	30ul of 02001-52	2ml MeOH TN-A-5754	
E01-1342-36023	QC@150 ppb-1	40+6	C18, 1g, 6cc, lot W2025B1	60ul of 02001-52	2ml MeOH TN-A-5754	
E01-1342-36024	QC@150 ppb-2	40+6	C18, 1g, 6cc, lot W2025B1	60ul of 02001-52	2ml MeOH TN-A-5754	
E01-1342-36025	QC@150 ppb-3	40+6	C18, 1g, 6cc, lot W2025B1	60ul of 02001-52	2ml MeOH TN-A-5754	
E01-1342-30312	0 ppm a.i. 329 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30321	0 ppm a.i. 330 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30330	0 ppm a.i. 331 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30339	0 ppm a.i. 332 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30348	0 ppm a.i. 333 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30357	0 ppm a.i. 334 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30366	0 ppm a.i. 335 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30375	0 ppm a.i. 336 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30384	0 ppm a.i. 337 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30393	0 ppm a.i. 338 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
—	—	—	—	—	—	
—	—	—	—	—	—	

Blank Liver TN-A-5143; Amount of liver: 2.0353 g Blank Liver TN-A-5142; Amount of liver: 1.0045 g
Homogenize liver with Kandiyohi water; Amount of water added 45 ml Amount of water added 9 ml
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4145) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker VWR S/N041694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3HH 769CB
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 02001-45, conc. 100.7ppm, amount added 2ml
Transfer sample into appripriatelly marked autovial

- Liver extraction reviewed by OK. (AC) (R) (D)

SPE Standard Curves--Solids

Prep Date(s): 02/11/02, 02/12/02, 02/13/02, 02/15/02
Analyst(s): OK/HOJ
Sample Matrix: Quail Liver
Method/Revision: ETS-8-231.1
Target Analyte(s): PFOS

Study Number: E01-1245
Equipment Number: NA
Final Solvent & TN Number: MeOH/TN-A-5754
Blank Tissue/Identifier: Quail Liver(female)/TN-A-5143
Quail Liver(male)/TN-A-5142

FC Mix Std Approx. 1.00 ppm: 02001-66
FC Mix Std Approx. 5.02 ppm: 02001-52
FC Mix Std Approx. 50.2 ppm: 02001-53
Surr. Std Approx. 100.7 ppm: 02001-45 PFDA

Initial female homogenate = 5.0353g/50.0353g+mL = 0.1006 g/mL 0.1006
Initial male homogenate = 1.0015g/10.0015g+mL = 0.1001 g/mL 0.1001

Blank liver initial homogenate (g/mL) for curves, MS, QC: 0.1006
Final solvent volume (mL) for curves, MS, QC: 2.000

Actual Concentrations of Standards in the FC Mix

Standard Number	PFOS Std Conc. ug/mL	PFOS Am't Spiked mL	PFDA Std Conc. ug/mL	All Am't Spiked mL	All Initial Homogenate g/mL	All Final Vol. mL
E01-1342-36005	1.004	0.0004	100.7	0.002	0.1006	2.000
E01-1342-36006	1.004	0.0010	100.7	0.002	0.1006	2.000
E01-1342-36007	1.004	0.0020	100.7	0.002	0.1006	2.000
E01-1342-36008	1.004	0.0050	100.7	0.002	0.1006	2.000
E01-1342-36009	1.004	0.0100	100.7	0.002	0.1006	2.000
E01-1342-36010	1.004	0.0200	100.7	0.002	0.1006	2.000
E01-1342-36011	1.004	0.0500	100.7	0.002	0.1006	2.000
E01-1342-36012	1.004	0.1000	100.7	0.002	0.1006	2.000
E01-1342-36013	1.004	0.1500	100.7	0.002	0.1006	2.000
E01-1342-36014	1.004	0.2000	100.7	0.002	0.1006	2.000
E01-1342-36062	50.20	0.0040	100.7	0.002	0.1006	2.000
E01-1342-36015	50.20	0.0100	100.7	0.002	0.1006	2.000
E01-1342-36016	50.20	0.0200	100.7	0.002	0.1006	2.000
E01-1342-36060	50.20	0.0300	100.7	0.002	0.1006	2.000
E01-1342-36061	50.20	0.0400	100.7	0.002	0.1006	2.000
QC-10 ppb	5.020	0.0040	100.7	0.002	0.1006	2.000
QC-75 ppb	5.020	0.0300	100.7	0.002	0.1006	2.000
QC-150 ppb	5.020	0.0600	100.7	0.002	0.1006	2.000
Quail Liver-MS/MSD-Day 1	1.004	0.0500	100.7	0.002	0.1006	2.000
Quail Liver-MS/MSD-Day 2	1.004	0.0500	100.7	0.002	0.1006	2.000
Quail Liver-MS/MSD-Day 3	1.004	0.0500	100.7	0.002	0.1006	2.000
Quail Liver-MS/MSD-Day 4	1.004	0.0500	100.7	0.002	0.1006	2.000

Calculated Concentrations of Standards in the sample matrix

Standard Number	PFOS Std Conc. ng/g	PFDA Std Conc. ng/g
E01-1342-36005	3.99	2001
E01-1342-36006	9.98	2001
E01-1342-36007	20.0	2001
E01-1342-36008	49.9	2001
E01-1342-36009	99.8	2001
E01-1342-36010	200	2001
E01-1342-36011	499	2001
E01-1342-36012	998	2001
E01-1342-36013	1496	2001
E01-1342-36014	1995	2001
E01-1342-36062	1995	2001
E01-1342-36015	4988	2001
E01-1342-36016	9977	2001
E01-1342-36060	14965	2001
E01-1342-36061	19953	2001
QC-10 ppb	200	2001
QC-75 ppb	1496	2001
QC-150 ppb	2993	2001
Quail Liver-MS/MSD-Day 1	499	2001
Quail Liver-MS/MSD-Day 2	499	2001
Quail Liver-MS/MSD-Day 3	499	2001
Quail Liver-MS/MSD-Day 4	499	2001

Calculation sheet added for clarity
LAS 11/26/02

Calculated Concentrations of Standards in the final solvent

Standard Number	PFOS Std Conc. ng/mL	PFDA Std Conc. ng/mL
E01-1342-36005	0.201	101
E01-1342-36006	0.502	101
E01-1342-36007	1.00	101
E01-1342-36008	2.51	101
E01-1342-36009	5.02	101
E01-1342-36010	10.0	101
E01-1342-36011	25.1	101
E01-1342-36012	50.2	101
E01-1342-36013	75.3	101
E01-1342-36014	100	101
E01-1342-36062	100	101
E01-1342-36015	251	101
E01-1342-36016	502	101
E01-1342-36060	753	101
E01-1342-36061	1004	101
QC-10 ppb	10.0	101
QC-75 ppb	75.3	101
QC-150 ppb	151	101
Quail Liver-MS/MSD-Day 1	25.1	101
Quail Liver-MS/MSD-Day 2	25.1	101
Quail Liver-MS/MSD-Day 3	25.1	101
Quail Liver-MS/MSD-Day 4	25.1	101

Verified by: LAS 11/26/02

BOTTLE–TOP DISPENSER DAILY CALIBRATION CHECK

Manufacturer and Model: Ucw 5mL
Date: 02-11-02
Initials: HCJ
Dispenser ID: 0799
Balance ID: 900
Volume: 5mL
Solvent: Acn
ID #: Tw-A-9145

	Mass of solvent (g)	Density[1] (g/ml)	Volume of Solvent
1	3.9525	0.7857	5.0305
2	3.9511		5.0288
3	3.9585		5.0382
4	3.9555		5.0344
5	3.9573		5.0367
		Average Volume:	5.0377
		%Accuracy:	100.7
		Std. Dev.:	0.003987
		%C.V.:	0.0792
		Pass/Fail:	(P)/ F

= SD(Avg × 100

Performance Specifications		
Volume, (mL)	Accuracy, (%)	C.V., (%)
1 to 10	98.0 - 102 %	± 1.0 %

Corrective Actions:

None taken HCJ 2.11.02

[1]Density values taken from the Merck Index, 12th Edition, Copyright 1996
d^{y}_{x} =specific gravity at $y^{degrees\ C}$ referred to water at $x^{degrees\ C}$
n-Hexane d^{20}_{4} =0.660
Acetone d^{25}_{0} =0.788
Methanol d^{25}_{4} =0.7866
Methylene Chloride d^{20}_{4} =1.3255

SPE Columns Extraction Worksheet

(AC) Written over (AC) 08/22/02
Page 1 of 23

Prep Date: 2/12/2002 OK 2.12.02
Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0 1245
Study Number: E01-~~1342/1365~~ (2) MD 2.17.02
Matrix: Liver

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1342-36026	Blank H2O-day 2	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	NA
E01-1342-36027	Blank female liver-day 2	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-36028	Blank male liver-day 2	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-36029	Female liver MS-day 2	40+6	C18, 1g, 6cc, lot W2025B1	50ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36030	Female liver MSD-day 2	40+6	C18, 1g, 6cc, lot W2025B1	50ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-30402	0 ppm a.i. 339 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30411	0 ppm a.i. 340 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30420	0 ppm a.i. 341 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30429	0 ppm a.i. 342 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30438	0 ppm a.i. 343 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30447	0 ppm a.i. 344 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30456	0 ppm a.i. 345 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30465	0 ppm a.i. 346 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30474	0 ppm a.i. 347 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30483	0 ppm a.i. 348 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30492	0 ppm a.i. 349 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30501	0 ppm a.i. 350 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30510	0 ppm a.i. 351 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30519	0 ppm a.i. 352 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30528	0 ppm a.i. 353 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	

Blank Liver TN-A-5143; Amount of liver: 5.0353 g Blank Liver TN-A-5142; Amount of liver: 1.0015 g
Homogenize liver with Kandiyohi water; Amount of water added 45 ml Amount of water added 9 ml
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4145) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker VWR S/N 041694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3M# 769613
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 02001-45, conc. 100.7 ppm, amount added 2 µl
Transfer sample into appripriatelly marked autovial

extraction done by OK. (AC) OK 8.8.02

(1) (Re) OK 82802

SPE Columns Extraction Worksheet (AC) Qual Level 3 LAS 08/22/02

Prep Date: 2/12/2002 OK 2.12.02

Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0 (EV)

Study Number: E01-~~1342/1365~~ 1245 AC 2.12.02

Matrix: Liver

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1342-30537	0 ppm a.i. 354 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	NA
E01-1342-30546	0 ppm a.i. 355 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30555	0 ppm a.i. 356 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30564	0 ppm a.i. 357 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30573	0 ppm a.i. 358 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30582	0 ppm a.i. 359 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30591	0 ppm a.i. 360 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30600	0 ppm a.i. 361 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30609	0 ppm a.i. 362 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30618	0 ppm a.i. 363 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30627	0 ppm a.i. 364 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30636	0 ppm a.i. 365 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30645	0 ppm a.i. 366 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30654	0 ppm a.i. 367 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30663	0 ppm a.i. 368 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
		LAS 08/22/02				

Blank Liver TN-A-5143; Amount of liver: 5.0353 g Blank Liver TN-A-5142; Amount of liver: 1.0015 g

Homogenize liver with Kandiyohi water; Amount of water added 45 ml Amount of water added 9 ml

Aliquot 1ml of liver into 15 ml polypropelene tube

Spike samples accordingly

Add 5ml of ACN (TN-A-4145) to aliquated sample

Shake sample for 20 min @300 rpm (Shaker VWR SHAKER S/N 041694

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3HM769613

Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.

Decant extract into centrifuge tubes with water

shake sample slightly to ensure proper mixing

Condition column with MeOH (TN-A-5754)

Wash Column with Kandiyohi Water

Filter sample through conditioned column, discarding filtrate

Allow column to go completelly dry

Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube

Spike samples with internal standard PFDA standard number 02001.45, conc. 100.7ppm, amount added 2µl

Transfer sample into appripriatelly marked autovial

Extraction done by OK. (AC) (EV) OK 8.8.02

MGD 8/8/02 OK 1

BOTTLE–TOP DISPENSER DAILY CALIBRATION CHECK

Manufacturer and Model:	VWR, 5 ml DISPENSER
Date:	2-12-02
Initials:	OKJ
Dispenser ID:	0799
Balance ID:	900
Volume:	5 ml
Solvent:	ACN
ID #:	TN-A-4145

	Mass of solvent (g)	Density[1] (g/ml)	Volume of Solvent ml
1	3.9198g	0.7857	4.9889
2	3.9526g	↓	5.0307
3	3.9679g	↓	5.0501
4	3.9780g	↓	5.0630
5	3.9618g	↓	5.0424
		Average Volume:	5.0350
		%Accuracy:	100.7
		Std. Dev.:	0.0283
		%C.V.:	0.562G
		Pass/Fail:	(P) / F

Performance Specifications		
Volume, (mL)	Accuracy, (%)	C.V., (%)
1 to 10	98.0 - 102 %	± 1.0 %

Corrective Actions:

none taken!
2-12-02
OKJ

[1]Density values taken from the Merck Index, 12th Edition, Copyright 1996
d^{y}_{x} =specific gravity at $y^{degrees\ C}$ referred to water at $x^{degrees\ C}$
n-Hexane d^{20}_{4} =0.660
Acetone d^{25}_{0} =0.788
Methanol d^{25}_{4} =0.7866
Methylene Chloride d^{20}_{4} =1.3255

SPE Columns Extraction Worksheet

(AC) Quail Sera } LAS 08/21/02
Page 1 of 1

Prep Date: 2/13/2002
Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0
Study Number: E01-1245
Matrix: Sera

Lims Assgned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1245-36153	Blank-H2O	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml of MeOH TN-A-5457	NA
E01-1245-36154	Blank sera	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml of MeOH TN-A-5457	
E01-1245-36155	Sera MS-5ppb in sera extract-1	40+6	C18, 1g, 6cc, lot W2025B1	2ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-36156	Sera MS-5ppb in sera extract-2	40+6	C18, 1g, 6cc, lot W2025B1	2ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-36157	Sera MS-5ppb in sera extract-3	40+6	C18, 1g, 6cc, lot W2025B1	2ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-36158	Sera MS-200ppb in sera extract-1	40+6	C18, 1g, 6cc, lot W2025B1	80ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-36159	Sera MS-200ppb in sera extract-2	40+6	C18, 1g, 6cc, lot W2025B1	80ul of 02001-52	2ml of MeOH TN-A-5457	
E01-1245-36160	Sera MS-200ppb in sera extract-3	40+6	C18, 1g, 6cc, lot W2025B1	80ul of 02001-52	2ml of MeOH TN-A-5457	
		LAS 08/22/02				

Blank Sera TN-A-5992; Amount of sera aliquated: 2 ml
Dilute Sera with Kandiyohi water; Amount of water added 38 ml
Aliquot 1ml of sera into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4145) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker VWR S/N 041694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3M# 769613)
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 02001-115, conc. 100.7ppm ; added 2ul
Transfer sample into appripriatelly marked autovial
extraction done by OK.
OK. 8/20

Bottle–Top Dispenser Daily Calibration Check

EXACT COPY OF THE ORIGINAL 38.02 OK

Manufacturer and Model:	VWR 5ml dispenser
Date:	2/13/2002
Initials:	OK
Dispenser ID:	0799
Balance ID:	900
Volume (mL):	5
Solvent:	ACN
ID #:	TN-A-4145

OK 2-13-02

	Mass of solvent (g)	Density[1] (g/ml)	Volume of Solvent	
1	3.965	0.7857	5.046	
2	3.9715	0.7857	5.055	
3	3.9818	0.7857	5.068	
4	3.9484	0.7857	5.025	
5	3.9788	0.7857	5.064	
		AverageVolume:	5.052	
		%Accuracy:	101.03	**PASS**
		Std. Dev.:	0.0169	
		%C.V.:	0.33	**PASS**
		Pass/Fail:	**PASS**	

Performance Specifications		
Volume, (mL)	Accuracy, (%)	C.V., (%)
1 to 10	98.0 - 102 %	± 1.0 %

Corrective Actions:

NONE TAKEN! OK 2-13-02

[1]Density values taken from the Merck Index, 12th Edition, Copyright 1996

d^{y}_{x} =specific gravity at $y^{degrees\ C}$ referred to water at $x^{degrees\ C}$

n-Hexane d^{20}_{4} =0.660

Acetone d^{25}_{0} =0.788

Methanol d^{25}_{4} =0.7866

Methylene Chloride d^{20}_{4} =1.3255

SPE Columns Extraction Worksheet

(AC) Quality Check } LAS 08/22/02
Page 1 of 2 }

Prep Date: 2/13/2002
Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0
Study Number: E01-~~1342/1365~~ 1245 (2) HOJ 2.13.02
Matrix: Liver

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1342-36031	Blank H2O-day 3	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	NA
E01-1342-36032	Blank female liver-day 3	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-36033	Blank male liver-day 3	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-36034	Female liver MS-day 3	40+6	C18, 1g, 6cc, lot W2025B1	50ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36035	Female liver MSD-day 3	40+6	C18, 1g, 6cc, lot W2025B1	50ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-30672	10 ppm a.i. 369 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30681	10 ppm a.i. 370 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30690	10 ppm a.i. 371 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30699	10 ppm a.i. 372 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30708	10 ppm a.i. 373 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30717	10 ppm a.i. 374 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30726	10 ppm a.i. 375 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30735	10 ppm a.i. 376 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30744	10 ppm a.i. 377 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30753	10 ppm a.i. 378 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30762	10 ppm a.i. 379 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30771	10 ppm a.i. 380 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30780	10 ppm a.i. 381 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30789	10 ppm a.i. 382 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30798	10 ppm a.i. 383 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	

Blank Liver TN-A-__5143__; Amount of liver: __5.0353g

Blank Liver TN-A-__5142__; Amount of liver: __1.0015g

Homogenize liver with Kandiyohi water; Amount of water added __45__ (AC) 45 ml OK 2.13.02

Amount of water added _9ml

Aliquot 1ml of liver into 15 ml polypropelene tube

Spike samples accordingly

Add 5ml of ACN (TN-A-_4145_) to aliquated sample

Shake sample for 20 min @300 rpm (Shaker VWR s/N 041694

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3M# 769613)

Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.

Decant extract into centrifuge tubes with water

shake sample slightly to ensure proper mixing

Condition column with MeOH (TN-A-_5754_)

Wash Column with Kandiyohi Water

Filter sample through conditioned column, discarding filtrate

Allow column to go completelly dry

Elute column with indicated amount of solvent (_MeOH_ TN-A-_5754_) into appropriate 15 ml centrifuge tube

Spike samples with internal standard PFDA standard number _02001-45_, conc._100.7ppm_, amount added_2ul_

Transfer sample into appripriatelly marked autovial

EXTRACTION DONE BY OK. (AC)(LP) OK 8.8.02

(1)(RO) OK 8.28.02

SPE Columns Extraction Worksheet

AL LAS 08/22/02
Page 2 of 23

Prep Date: 2/13/2002
Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0
Study Number: E01-1342/1365
Matrix: Liver

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1342-30820	10 ppm a.i. 384 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	NA
E01-1342-30829	10 ppm a.i. 385 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30838	10 ppm a.i. 386 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30847	10 ppm a.i. 387 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30856	10 ppm a.i. 388 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30865	10 ppm a.i. 389 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30874	10 ppm a.i. 390 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30883	10 ppm a.i. 391 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30892	10 ppm a.i. 392 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30901	10 ppm a.i. 393 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30910	10 ppm a.i. 394 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30919	10 ppm a.i. 395 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30928	10 ppm a.i. 396 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30937	10 ppm a.i. 397 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30946	10 ppm a.i. 398 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
		LAS 08/22/02				

Blank Liver TN-A-__5143__; Amount of liver: __5.0353g
Homogenize liver with Kandiyohi water; Amount of water added __45ml
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-_4145_) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker VWR s/N 941694
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 34#769813
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-_5754_)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (_MeOH_ TN-A-_5754_) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number _02001.45_, conc._100.7 ppm_, amount added_2ul_
Transfer sample into appripriatelly marked autovial

OK 2.13.02

Blank Liver TN-A-__5142__; Amount of liver: __1.0015g
Amount of water added _9ml

Extraction done by OKJ. OK 02.18.02

Bottle–Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR 5ml dispenser
Date:	2/13/2002
Initials:	OK
Dispenser ID:	0799
Balance ID:	900
Volume (mL):	5
Solvent:	ACN
ID #:	TN-A-4145

OK 2.13.02

	Mass of solvent (g)	Density[1] (g/ml)	Volume of Solvent	
1	3.965	0.7857	5.046	
2	3.9715	0.7857	5.055	
3	3.9818	0.7857	5.068	
4	3.9484	0.7857	5.025	
5	3.9788	0.7857	5.064	
		AverageVolume:	5.052	
		%Accuracy:	101.03	**PASS**
		Std. Dev.:	0.0169	
		%C.V.:	0.33	**PASS**
		Pass/Fail:	**PASS**	

Performance Specifications		
Volume, (mL)	Accuracy, (%)	C.V., (%)
1 to 10	98.0 - 102 %	± 1.0 %

Corrective Actions:

NONE TAKEN! OK 2.13.02

[1]Density values taken from the Merck Index, 12th Edition, Copyright 1996

d^{y}_{x} =specific gravity at $y^{degrees\ C}$ referred to water at $x^{degrees\ C}$

n-Hexane d^{20}_{4} =0.660

Acetone d^{25}_{0} =0.788

Methanol d^{25}_{4} =0.7866

Methylene Chloride d^{20}_{4} =1.3255

Attachment D: Dilutions Summary Worksheet

Study: E01-1245
Dilution Date/Analyst: (1)
Box Number: E01-1245

Solvent/TN Number: MeOH/5457 W/IS PFDA
Extraction Date/Analyst: ~~2/13/02~~ OK 4802 2.1.02
Matrix/Timepoint: Quail sera

Sample Number or Description	Dilutions								
	1/10	1/100	1/	1/	1/	1/	1/	Comments	Verified By:
E01-1245-28480	NA	x							MJ 4.4.02
E01-1245-28482		x							
E01-1245-28484		x							
E01-1245-28486		x							
E01-1245-28488		x							
E01-1245-28490		x							
E01-1245-28492		x							
E01-1245-28494		x						LAS 08/21/02	
E01-1245-28495		x							
E01-1245-28497		x							
E01-1245-28499		x							
E01-1245-28501		x							
E01-1245-28503		x							
E01-1245-28505		x							
E01-1245-28507		x							
E01-1245-28509		x							
E01-1245-28510		x							
E01-1245-28512		x							
E01-1245-28514		x							
E01-1245-28516		x							

Notes:

1/100 dilution= 10ul of sample +990ul of solvent

(1) **All dilutions were made on day of extraction or the day after analysis.** OK 4802 QC. OK 8.28.02

Form Completion Verified By: MJ 4.4.02

Attachment D: Dilutions Summary Worksheet

Study: E01-1342 E01-1245 (AC) OBJ 4.11.02 **Solvent/TN Number:** MeOH/5457 W/IS PFDA
Dilution Date/Analyst: (1) **Extraction Date/Analyst:** 2/13/02
Box Number: E01-1342 **Matrix/Timepoint:** Quail liver

Sample Number or Description	Dilutions								
	1/10	1/100	1/	1/	1/	1/	1/	Comments	Verified By:
E01-1342-30672	x								OBJ 4.11.02
E01-1342-30690	x								
E01-1342-30708	x								
E01-1342-30726	x								
E01-1342-30744	x								
E01-1342-30762	x								
E01-1342-30780	x								
E01-1342-30798	x								
E01-1342-30829	x					LAS 08/21/02			
E01-1342-30847	x								
E01-1342-30865	x								
E01-1342-30883	x								
E01-1342-30901	x								
E01-1342-30919	x								
E01-1342-30937	x								
E01-1342-30955	x								
E01-1342-30973	x								
E01-1342-30991	x								
E01-1342-31009	x								
E01-1342-31027	x								

Notes:
1/10 dilution = 100ul of sample + 900ul of solvent
1/100 dilution= 10ul of sample +990ul of solvent

(1) **All dilutions were made on day of extraction or the day after analysis.** OK 4.8.02 (AC) OK 8.28.02

Form Completion Verified By: OBJ 4.11.02

(AC) Page 1 of 2 (AS) 05/22/02

Prep Date: 2/15/2002 OK 2.15.02
Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0
Study Number: E01-~~1342/1365~~(23)/1245 (AS) 2.15.02
Matrix: Liver

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1342-36036	Blank H2O-day 4	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	NA
E01-1342-36037	Blank female liver-day 4	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-36038	Blank male liver-day 4	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-36039	Female liver MS-day 4	40+6	C18, 1g, 6cc, lot W2025B1	50ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-36040	Female liver MSD-day 4	40+6	C18, 1g, 6cc, lot W2025B1	50ul of 02001-66	2ml MeOH TN-A-5754	
E01-1342-30955	10 ppm a.i. 399 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30964	10 ppm a.i. 400 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30973	10 ppm a.i. 401 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30982	10 ppm a.i. 402 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-30991	10 ppm a.i. 403 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-31000	10 ppm a.i. 404 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-31009	10 ppm a.i. 405 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-31018	10 ppm a.i. 406 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-31027	10 ppm a.i. 407 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1342-31036	10 ppm a.i. 408 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31184	0 ppm a.i. 246 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31194	0 ppm a.i. 253 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31199	0 ppm a.i. 256 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31204	0 ppm a.i. 259 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31209	0 ppm a.i. 266 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	

Blank Liver TN-A-__5143__; Amount of liver: __5.0353g
Blank Liver TN-A-__5142__; Amount of liver: __1.0015g
Homogenize liver with Kandiyohi water; Amount of water added__45ml_
Amount of water added _9ml
Aliquot 1ml of liver into 15 ml polypropelene tube
Spike samples accordingly
Add 5ml of ACN (TN-A-4145) to aliquated sample
Shake sample for 20 min @300 rpm (Shaker VWR S/N 041694)
Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3M# 769613)
Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.
Decant extract into centrifuge tubes with water
shake sample slightly to ensure proper mixing
Condition column with MeOH (TN-A-5754)
Wash Column with Kandiyohi Water
Filter sample through conditioned column, discarding filtrate
Allow column to go completelly dry
Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube
Spike samples with internal standard PFDA standard number 02001-45, conc. 100 ppm, amount added 2ml
Transfer sample into appripriatelly marked autovial

Extraction done by OK. (AC) (AS) OK 2.15.02

SPE Columns Extraction Worksheet (AC) (Quality Review) LAS 08/22/02 Page 2 of 2

Prep Date: 2/15/2002 2.15.02 OK

Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0 (RO)

Study Number: E01-~~1342/1365~~ 1245 adj 2.15.02

Matrix:

Lims Assigned sample number	Sample Number or description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1365-31214	0 ppm a.i. 270 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	NA
E01-1365-31219	0 ppm a.i. 273 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31224	0 ppm a.i. 277 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31229	0 ppm a.i. 283 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31234	0 ppm a.i. 291 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31240	10 ppm a.i. 517 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31245	10 ppm a.i. 522 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31250	10 ppm a.i. 525 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31255	10 ppm a.i. 533 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31260	10 ppm a.i. 538 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31265	10 ppm a.i. 545 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31270	10 ppm a.i. 552 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31275	10 ppm a.i. 557 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31280	10 ppm a.i. 561 F Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
E01-1365-31285	10 ppm a.i. 568 M Liver	40+6	C18, 1g, 6cc, lot W2025B1	NA	2ml MeOH TN-A-5754	
			LAS 08/22/02			

Blank Liver TN-A-__5143__; Amount of liver: __5.0353g

Homogenize liver with Kandiyohi water; Amount of water added__45ml_

Blank Liver TN-A-__5142__; Amount of liver: __1.0015g

Amount of water added _9ml

Aliquot 1ml of liver into 15 ml polypropelene tube

Spike samples accordingly

Add 5ml of ACN (TN-A-4145) to aliquated sample

Shake sample for 20 min @300 rpm (Shaker VWR S/N 041694)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3M# 769613)

Add 40 ml of Kandiyohi water to 50 ml polypropelene centrifuge tube.

Decant extract into centrifuge tubes with water

shake sample slightly to ensure proper mixing

Condition column with MeOH (TN-A-5754)

Wash Column with Kandiyohi Water

Filter sample through conditioned column, discarding filtrate

Allow column to go completelly dry

Elute column with indicated amount of solvent (MeOH TN-A-5754) into appropriate 15 ml centrifuge tube

Spike samples with internal standard PFDA standard number 02001-45, conc. 100.7ppm, amount added 2ul

Transfer sample into appripriatelly marked autovial

Extraction done by OK (AC) (RO) OK 82802

OK 8802

Bottle–Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR 5ml
Date:	2/15/2002
Initials:	OK
Dispenser ID:	0799
Balance ID:	900
Volume (mL):	5
Solvent:	ACN
ID #:	TN-A-4145

	Mass of solvent (g)	Density[1] (g/ml)	Volume of Solvent	
1	3.8904	0.7857	4.9515	
2	3.9526	0.7857	5.0307	
3	3.9724	0.7857	5.0559	
4	3.961	0.7857	5.0414	
5	3.964	0.7857	5.0452	
		AverageVolume:	5.0249	
		%Accuracy:	100.50	**PASS**
		Std. Dev.:	0.04202	
		%C.V.:	0.84	**PASS**
		Pass/Fail:	**PASS**	

Performance Specifications		
Volume, (mL)	Accuracy, (%)	C.V., (%)
1 to 10	98.0 - 102 %	± 1.0 %

Corrective Actions:

[1]Density values taken from the Merck Index, 12th Edition, Copyright 1996

d^{y}_{x} =specific gravity at $y^{degrees\ C}$ referred to water at $x^{degrees\ C}$

n-Hexane d^{20}_{4} =0.660

Acetone d^{25}_{0} =0.788

Methanol d^{25}_{4} =0.7866

Methylene Chloride d^{20}_{4} =1.3255

(AC) info entered into form directly since computer was available in prep lab. D.D.

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

ATTACHMENT E: STUDY PROTOCOL, PROTOCOL AMENDMENTS, AND DEVIATIONS

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-108, E01-1245

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE NORTHERN BOBWHITE

PROTOCOL NO.: 454/120700/QR/SUB454

AMENDMENT NO.: 9

SPONSOR: 3M Corporation

PROJECT NO.: 454-108, E01-1245

EFFECTIVE DATE: January 24, 2002

AMENDMENT:

This amendment includes an attachment (Attachment A) which provides the information necessary to complete the analytical phase of this study.

REASON:

The protocol did not include information for completing the analyses required by the sponsor.

AMENDMENT:

Harold Johnson is the Principal Analytical Investigator

REASON:

The sponsor requested that a principal analytical investigator be added to the protocol.

AMENDMENT:

The 3M Environmental Laboratory Project Identification Number to be included with the study is E01-1245.

REASON:

The sponsor requested that the 3M Project Identification Number be included in the protocol.

PROJECT NO.: 454-108,
E01-1245

WILDLIFE INTERNATIONAL, LTD

Sean P. Gallagher, Study Director — Date 1/29/02

Joann B. Beavers, Wildlife International, LTD Laboratory Management — Date 1/29/02

Harold O. Johnson, Principal Analytical Investigator — Date 4.4.02

William K. Reagen, 3M Environmental Laboratory, Laboratory Management — Date 04/04/02

Rochelle R. Robideau, Sponsor's Representative — Date 1/31/02

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-108, E01-1245

Sean P. Gallagher, Study Director	Date
Joann B. Beavers, Wildlife International, LTD Laboratory Management	Date
Harold O. Johnson, Principal Analytical Investigator	Date
William K. Reagen, 3M Environmental Laboratory, Laboratory Management	Date
Rochelle R. Robideau, Sponsor's Representative	Date

Protocol Amendment # 9 - Attachment A

Wildlife International Ltd. # 454-108

STUDY TITLE

PFOS: A Reproduction Study with the Northern Bobwhite

SPONSOR

3M Environmental Laboratory
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

PERFORMING ANALYTICAL LABORATORY

3M Environmental Laboratory
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

LABORATORY PROJECT IDENTIFICATION

3M Environmental Laboratory: E01-1245
Wildlife International Ltd.: 454-108

Study Identification

PFOS: A Reproduction Study with the Northern Bobwhite

Sponsor	3M Environmental Laboratory Building 2-3E-09 PO Box 33331 St. Paul, MN 55133-3331
Principal Analytical Investigator	Harold Johnson 3M Environmental Laboratory Building 2-3E-09 PO Box 33331 St. Paul, MN 55133-3331 651-778-6479
Study Locations	
ANALYTICAL TESTING LABORATORY	3M Environmental Laboratory Building 2-3E-09 935 Bush Ave. PO Box 33331 St. Paul, MN 55133-3331
ANALYTICAL TESTING LABORATORY	PACE Tier 2 1700 Elm Street, Suite 200 Minneapolis, MN 55414
Proposed Study Timetable	
ANALYTICAL EXPERIMENTAL START DATE	01/24/02
ANALYTICAL EXPERIMENTAL TERMINATION DATE	03/24/02

1. PURPOSE/OBJECTIVES

In the in-life phase of this study, Northern Bobwhite quail were regularly exposed to nominal doses of perfluorooctanesulfonate, potassium salt (PFOS) in their feed in order to evaluate the effects of dietary exposure upon adults over a five-month period. The purpose of this analytical phase of the study is to quantify PFOS in the liver and sera of both control and dosed Northern Bobwhite quail. The analytical information may be used to compare with toxicological/histopathological results from the in-life portion of this bird study. The in-life portion of the study was completed at Wildlife International, Ltd. in study 454-108.

2. REGULATORY COMPLIANCE

This analytical phase study will be conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR 792.

3. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit will audit analytical phase study conduct, data, and the final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures. The Quality Assurance Unit will report all findings to the Sponsor Representative and the Study Director.

4. REFERENCE SUBSTANCE

4.1 *Physical Description-* Perfluorooctanesulfonate, potassium salt from Lot 217 is a white crystalline material.

4.2 *Purity and Stability*—The purity of Lot #217 has been determined to be 86.9%. PFOS has been shown to be stable in methanol solutions for up to 6 months at ambient temperature in the validation report from study PS013, "Method Validation and Instrument Detection Limit Determination for LC/MS and GC/MS Analysis of FC807 Compounds in Methanol." Refer to the Certificate of Analysis (stored in the Test, Control, and Reference database in the 3M Environmental Laboratory) for further purity and stability information.

4.3 *Storage Conditions*—The test article may be stored at ambient temperature or lower.

4.4 *Reserve Samples*—A reserve sample of PFOS from Lot #217 has been frozen at -20°C ±5°C or colder and is kept in the 3M Environmental Laboratory.

4.5 *CAS Number*—The CAS number is: 2795-39-3.

4.6 *Molecular Weight*—The molecular weight is 538 ($C_8F_{17}SO_3K$). Note: The molecular weight of the anion (PFOS) is 499 ($C_8F_{17}SO_3^-$).

5. CONTROL MATRICES

Types of control matrices and their source, physical description, storage requirements, and traceability numbers will be recorded in the raw data and included in the final report.

6. TEST SYSTEM

The test system is Northern Bobwhite quail. The test system was dosed (at Wildlife International Ltd. during the in-life phase of this study) with food prepared by mixing PFOS with feed in a large mixer in appropriate portions to achieve the nominal 10 ppm level. A food dose verification validation study was performed by Wildlife International under study number 454C-110 (3M #U2723), "Analytical Method Verification for the Determination of PFOS in Avian Diet." Wildlife International verified the actual dosed food for this study and the results will be contained in the in-life Wildlife phase report. Northern Bobwhite serum and liver samples were collected by Wildlife International, Ltd. and sent to 3M Environmental Laboratory for analysis.

6.1 Justification of the Selection of the Test System

Northern Bobwhite quail represent wild bird populations and they are an EPA recommended species because they do well in a laboratory environment. The use of these matrices for the study is to determine if PFOS was deposited in the liver and serum of mating adults that were fed PFOS. Furthermore, analysis of these matrices from hatchlings will determine if PFOS was passed on to mating adults' offspring.

7. SOURCE OF SUPPLY FOR THE TEST SYSTEM

The birds were obtained by Wildlife International, Ltd. See their protocol (study 454-108) for information about bird origination location.

8. SPECIMEN AND SAMPLE RECEIPT AND MAINTENANCE

Sera and liver were collected by Wildlife International, Ltd. and shipped to the 3M Environmental Laboratory for analysis. The 20-week endpoint sera and liver samples received by the 3M Environmental Laboratory were from the following birds:

- 40 control adult birds (20 mating pairs) that ingested food containing 0 ppm PFOS
- 40 experimental adult birds (20 mating pairs) that ingested food containing 10 ppm PFOS
- 20 first generation offspring birds that ingested food containing 0 ppm PFOS

Samples will be maintained frozen except when removed for extraction and analysis as described in the method. The samples will be kept isolated from the test substance during storage.

9. SUB-CONTRACTED ANALYSIS

9.1 All sera and liver extractions as detailed in this protocol amendment will be performed at Pace Tier 2, 1700 Elm Street, Suite 200, Minneapolis, MN 55414.

9.2 All sera and liver analyses as detailed in this protocol amendment will be performed at 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106.

10. EXPERIMENTAL CONDUCT

The 21-week endpoint liver and sera samples received (see section 8) will be extracted and analyzed for PFOS quantities using the methods listed below.

10.1 Preparatory Methods

10.1.1 ETS-8-6, Extraction of Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.1.2 ETS-8-4, Extraction of Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.2 Analytical Methods

10.2.1 ETS-8-7, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray / Mass Spectrometry

10.2.2 ETS-8-5, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry

11. METHOD VALIDATION

Method validation for PFOS quantitation in quail sera and liver was performed at Centre Analytical Inc. in study 023-054. The method will be applied to sample analyses without additional validation.

12. DATA QUALITY OBJECTIVES DURING SAMPLE ANALYSIS

The following criteria will be met using the validated calibration levels and fortification levels determined in section 11:

12.1 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. The peak area precision and retention time precision 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 2.0% RSD, the precision of the retention time must be equal to or less than 2.5% RSD and the peak asymmetry (fronting or tailing) must be $0.5<AF<2.0$, where AF is the asymmetry factor. If any item of the

system suitability fails, system maintenance must be completed prior to running a second set of system suitability samples and the system suitability must pass before starting the calibration.

12.2 Calibration

It will consist of a minimum of nine (9) levels, including a blank with surrogate and a blank without surrogate, the lowest standard will be at approximately 50% of the LLOQ and the highest standard will be approximately 50% higher than the ULOQ.

The standard curve equation will be determined by regression analysis using the peak areas of the analyte. The accuracy of each level will be verified. Any level outside 80% - 120% accuracy must be deactivated, and regression re-calculated. The curve must have a correlation of determination (r^2) greater than or equal to 0.985. All levels must show a response greater than two times that of the blank. The total number of standards excluded my not exceed 20%. If the preceding criterion are not met, the curve and sample set will be reanalyzed.

12.3 Limits of Quantitation (LOQ)

The lower limit of quantitation (LLOQ) was determined to be 10 ng/g in liver and 10 ng/mL serum during the method validation as described above in section 11. The LLOQ may be determined to be higher than that found in the validation study during a screening to be done for endogenous levels of PFOS in the blank matrices available at 3M Environmental Laboratory. If endogenous levels of PFOS are found to be higher than the LLOQ or if the levels are found to be highly variable, the LLOQ will be adjusted to accommodate this. The screening procedures and LLOQ determination will be recorded in a note to file and kept with the raw study data as well as being reported in the final report. Recovery efficiency at the LLOQ will be determined during each run using an extracted quality control sample spiked at the LLOQ level. If the quality control sample at the LLOQ level fails during a run, the LLOQ for that specific run would be considered to be the next lowest passing quality control sample and any samples below the new LLOQ must be reanalyzed.

The upper limit of quantitation (ULOQ) was determined to be 250 ng/g in liver and 250 ng/mL in serum during the method validation as described above in section 11. An extracted quality control sample spiked at the ULOQ will be used to measure recovery efficiency at the ULOQ. If the quality control sample at the ULOQ fails during a run the ULOQ for that specific run would be considered to be the next highest passing quality control sample and any samples above the new ULOQ must be reanalyzed.

Any sample with an area greater than that of the highest acceptable standard will need to be diluted into the range of the calibration curve. If samples are diluted into the range of the curve during analyses and enough sample remains, a post-run dilution validation will be performed to verify sample values. To perform the

dilution validation, one sample will be separated into two representative samples (i.e. two 1 mL aliquots for fluid samples or two 1 gram amounts for tissue samples) then diluted using two procedures. The first procedure consists of diluting the sample with additional matrix prior to extraction (sera adding sera), while the second procedure consists of diluting the extract with solvent post-extraction (methanol extract adding additional methanol solvent).

If the values are not within 15% of each other additional testing will be required to determine which value is a correct representation of the sample concentration.

12.4 Continuing Calibration Verifications

An unextracted midlevel standard (continuing calibration verification – CCV) will be analyzed after every 10 injections (starting after the last injection of the calibration curve). The accuracy of the CCV must be within 20%. Data may only be reported if bracketed by passing CCV's (or a CCV and the same level calibration curve point). Data bracketed by one or more failing CCV's must be reanalyzed.

12.5 Quality Control Samples

A minimum of three (3) quality control samples (QC) will be prepared with each set of samples. They will consist of blank matrix spiked with three levels of analyte. The levels are:

- Low level: at the LLOQ (10 ng/mL in serum and 10 ng/g in liver),
- Mid-level: 5 times the LLOQ,
- High level: at the ULOQ (25 times the LLOQ)

12.6 QC Performance Criteria

Each QC is expected to show an accuracy of 80-120% of expected. A minimum of 2/3 of all QC must meet these criteria. If not, the entire sample set must either be re-analyzed or re-extracted (as decided by the Study Director).

12.7 Use of Confirmatory Methods

Confirmatory methods are typically not needed with LC/MS/MS analysis.

12.8 Demonstration of Specificity

PFOS specificity will be substantiated by chromatographic retention time, by the characteristic primary ion (499), and the characteristic product ion (99) using LC/MS/MS.

13. SUB-CONTRACTED ANALYSIS

13.1 All fluid and tissue extractions as detailed in this protocol amendment will be performed at Pace Tier 2, 1700 Elm Street, Suite 200, Minneapolis, MN 55414.

13.2 All fluid and tissue analyses as detailed in this protocol amendment will be performed at 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106.

13.3 Pace Analytical Services, Inc. will follow all applicable 3M Environmental Laboratory Standard Operating Procedures and the following Pace Analytical Inc. Standard Operating Procedures.

PSS-Admin-01	Quality System
PSS-Admin-04	Laboratory Facilities
PSS-Admin-06	Training Record Files Maintained by QAU
PSS-ARC-02	Disposition of Archive Materials
PSS-ARC-04	Archiving Data, Specimen and Logbooks
PSS-DC-05	Lab Notebooks, Logbooks, and Phone Logs
PSS-DM-03	Statistical Evaluation of Data
PSS-OPS-01	Use and Maintenance of Ultra-Turrax T25 Homogenizer
PSS-OPS-02	Raw Data
PSS-OPS-03	Hazardous Waste Disposal
PSS-OPS-04	Use and Maintenance of N-Evap Analytical Evaporator
PSS-OPS-05	Use of Compressed Gases in the Laboratory
PSS-OPS-06	Use and Maintenance of NANOpure II Water Purification System
PSS-OPS-07	Cleaning Non-disposable Volumetric Pipettes
PSS-OPS-08	Laboratory Calculations
PSS-OPS-09	Deviations from Established Procedures
PSS-OPS-10	Analytical Definitions
PSS-OPS-11	Use and Calibration of a Bottle-Top Dispenser
PSS-OPS-13	Use and Maintenance of Lab Refrigerators, Freezers, and Ovens
PSS-OPS-14	Use and Calibration of Syringes with a Hamilton Chaney Adapter
PSS-OPS-15	Glassware Cleaning
PSS-OPS-16	Use and Maintenance of the Fisher Scientific Isotemp Freezer
PSS-OPS-17	Use and Maintenance of Ultrasonic Water Baths (Sonicators)
PSS-OPS-18	Use and Maintenance of Vortex Shakers
PSS-OPS-24	Use and Maintenance of a Desiccator
PSS-OPS-31	Use and Maintenance of Shakers
PSS-OPS-32	Operation, Maintenance and Calibration of Laboratory
PSS-OPS-37	Operation, Maintenance, and Calibration of pH Meters and pH Electrodes
PSS-MC-01	Purchasing of Laboratory Supplies
PSS-MC-02	Receipt of Laboratory Supplies
PSS-MTR-01	Calibration of Certified Weight Set
PSS-MTR-02	Calibration of Certified Thermometers/Thermocouples
PSS-MTR-05	Calibration of Eppendorf Pipettes
PSS-MTR-06	Verification of Calibration of the Eppendorf Pipettor
PSS-MTR-07	New Equipment Qualification (IQ, PQ, OQ)
PSS-MTR-08	Verification of Calibration and Use of Analytical Balances

PSS-MTR-09	Use and Maintenance of Fisher Scientific Electronic Temperature Monitoring Devices
PSS-Safety-01	General Laboratory Safety
PSS-Safety-02	Use and Maintenance of Biological Safety Cabinets
PSS-Safety-04	Emergency Evacuation Procedures
PSS-Safety-05	Use and Maintenance of Laboratory Fume Hoods
PSS-TS-01	Training Procedures

13.4 An amendment to the protocol will be written if extractions and analyses are performed at laboratories other than the Pace Tier 2 or 3M Environmental Laboratory, respectively.

14. STATISTICAL ANALYSIS

Statistical methods will be limited to the calculation of means and standard deviations. Examples of the calculations used in the analyses will be included in the analytical phase report.

15. REPORT

A final report will be prepared by the 3M Environmental Laboratory. The final report will follow all GLP requirements, and will include a description of all materials and methods used, including a narrative discussion with a tabular presentation of all analyses results.

16. LOCATION OF RAW DATA, RECORDS, AND FINAL REPORT

When the final report is completed, all original paper data, including protocol, phase report, study correspondence, sample receipt and tracking, sample preparation, and analytical data, will be retained in the archives of 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained either at Pace Tier 2 or the 3M Environmental Laboratory, as applicable.

17. SPECIMEN RETENTION

Samples of the test substance (any leftover control sera or liver and samples) and the analytical standards will be maintained in the laboratory for a period of time as specified by regulation or as long as the quality of the preparation affords evaluation. However, samples will not be maintained more than 10 years after the effective date of the final test rule (if applicable). 3M Environmental Laboratory Standard Operating Procedures also apply to sample retention times.

18. REFERENCES

18.1 Wildlife International, Ltd. Protocol No. 454-108, "PFOS: A Reproduction Study with the Northern Bobwhite".

18.2 Centre Analytical Laboratories, Inc. Study 023-054, "Validation of Analytical Methods "Extraction of Potassium Perfluoroocatanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC Electrospry/Mass Spectrometry" and "Extraction of Potassium

Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC Electrospray/Mass Spectrometry in Quail Serum and Liver."

18.3 3M Study U2723, Wildlife Study 454C-110, "Analytical Method Verification for the Determination of PFOS in Avian Diet."

19. PROTOCOL AMENDMENTS AND DEVIATIONS

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor 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 Sponsor Representative and filed with the raw data. All changes to the protocol will be indicated in the final report.

PROJECT NO.: 454-108,
E01-1245

WILDLIFE INTERNATIONAL, LTD

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE NORTHERN BOBWHITE

PROTOCOL NO.: 454/120700/QR/SUB454 AMENDMENT NO.: 10

SPONSOR: 3M Corporation PROJECT NO.: 454-108, E01-1245

EFFECTIVE DATE: January 30, 2002

AMENDMENT:

This amendment includes an attachment (Attachment A) which provides updated information necessary to complete the analytical phase of this study. This attachment A replaces the attachment A in amendment 9.

REASON:

The amendment 9 attachment A was updated to include a different extraction and analytical method, to update the data quality objectives, and to change the liver and sera extraction location.

Sean P. Gallagher, Study Director — Date 1/30/02

Joann B. Beavers, Wildlife International, LTD, Laboratory Management — Date 1/30/02

Harold O. Johnson, Principal Analytical Investigator — Date 01/31/02

William K. Reagen, 3M Environmental Laboratory, Laboratory Management — Date 01/31/02

Rochelle R. Robideau, Sponsor's Representative — Date 01/31/02

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-108, E01-1245

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE NORTHERN BOBWHITE

PROTOCOL NO.: 454/120700/QR/SUB454

AMENDMENT NO.: 10

SPONSOR: 3M Corporation

PROJECT NO.: 454-108, E01-1245

EFFECTIVE DATE: January 30, 2002

AMENDMENT:

This amendment includes an attachment (Attachment A) which provides updated information necessary to complete the analytical phase of this study. This attachment A replaces the attachment A in amendment 9.

REASON:

The amendment 9 attachment A was updated to include a different extraction and analytical method, to update the data quality objectives, and to change the liver and sera extraction location.

Sean P. Gallagher, Study Director	Date
Joann B. Beavers, Wildlife International, LTD, Laboratory Management	Date
Harold O. Johnson, Principal Analytical Investigator	Date
William K. Reagen, 3M Environmental Laboratory, Laboratory Management	Date
Rochelle R. Robideau, Sponsor's Representative	Date

Protocol Amendment # 10 - Attachment A

Wildlife International Ltd. # 454-108

STUDY TITLE

PFOS: A Reproduction Study with the Northern Bobwhite

SPONSOR

3M Environmental Laboratory
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

PERFORMING ANALYTICAL LABORATORY

3M Environmental Laboratory
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

LABORATORY PROJECT IDENTIFICATION

3M Environmental Laboratory: E01-1245
Wildlife International Ltd.: 454-108

Study Identification

PFOS: A Reproduction Study with the Northern Bobwhite

Sponsor

3M Environmental Laboratory
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

Principal Analytical Investigator

Harold Johnson
3M Environmental Laboratory
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331
651-778-6479

Study Locations

ANALYTICAL TESTING LABORATORY

3M Environmental Laboratory
Building 2-3E-09
935 Bush Ave.
PO Box 33331
St. Paul, MN 55133-3331

Proposed Study Timetable

ANALYTICAL EXPERIMENTAL START DATE	01/31/02
ANALYTICAL EXPERIMENTAL TERMINATION DATE	03/31/02

1. PURPOSE/OBJECTIVES

In the in-life phase of this study, Northern Bobwhite quail were regularly exposed to nominal doses of perfluorooctanesulfonate, potassium salt (PFOS) in their feed in order to evaluate the effects of dietary exposure upon adults over a five month period. The purpose of this analytical phase of the study is to quantify PFOS in the liver and sera of both control and dosed Northern Bobwhite quail. The analytical information may be used to compare with toxicological/histopathological results from the in-life portion of this bird study. The in-life portion of the study was completed at Wildlife International, Ltd. in study 454-108.

2. REGULATORY COMPLIANCE

This analytical phase study will be conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR 792.

3. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit will audit analytical phase study conduct, data, and the final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures. The Quality Assurance Unit will report all findings to the Sponsor Representative and the Study Director.

4. REFERENCE SUBSTANCE

4.1 *Physical Description-* Perfluorooctanesulfonate, potassium salt from Lot 217 is a white crystalline material.

4.2 *Purity and Stability*—The purity of Lot #217 has been determined to be 86.9%. PFOS has been shown to be stable in methanol solutions for up to 6 months at ambient temperature in the validation report from study PS013, "Method Validation and Instrument Detection Limit Determination for LC/MS and GC/MS Analysis of FC807 Compounds in Methanol." Refer to the Certificate of Analysis (stored in the Test, Control, and Reference database in the 3M Environmental Laboratory) for further purity and stability information.

4.3 *Storage Conditions*—The test article may be stored at ambient temperature or lower.

4.4 *Reserve Samples*—A reserve sample of PFOS from Lot #217 has been frozen at -20°C ±5°C or colder and is kept in the 3M Environmental Laboratory.

4.5 *CAS Number*—The CAS number is: 2795-39-3.

4.6 *Molecular Weight*—The molecular weight is 538 ($C_8F_{17}SO_3K$). Note: The molecular weight of the anion (PFOS) is 499 ($C_8F_{17}SO_3^-$).

5. CONTROL MATRICES

Types of control matrices and their source, physical description, storage requirements, and traceability numbers will be recorded in the raw data and included in the final report.

6. TEST SYSTEM

The test system is the Northern Bobwhite quail. The test system was dosed (at Wildlife International Ltd. during the in-life phase of this study) with food prepared by mixing PFOS with feed in a large mixer in appropriate portions to achieve the nominal 10 ppm level. A food dose verification validation study was performed by Wildlife International under study number 454C-110 (3M #U2723), "Analytical Method Verification for the Determination of PFOS in Avian Diet." Wildlife International verified the actual dosed food for this study and the results will be contained in the in-life Wildlife phase report. Northern Bobwhite serum and liver samples were collected by Wildlife International, Ltd. and sent to 3M Environmental Laboratory for analysis.

6.1 Justification of the Selection of the Test System

Northern Bobwhite quail represent wild bird populations and they are an EPA recommended species because they do well in a laboratory environment. The use of these matrices for the study is to determine if PFOS was deposited in the liver and serum of mating adults that were fed PFOS. Furthermore, analysis of these matrices from hatchlings will determine if PFOS was passed on to mating adults' offspring.

7. SOURCE OF SUPPLY FOR THE TEST SYSTEM

The birds were obtained by Wildlife International, Ltd. See their protocol (study 454-108) for information about bird origination location.

8. SPECIMEN AND SAMPLE RECEIPT AND MAINTENANCE

Sera and liver were collected by Wildlife International, Ltd. and shipped to the 3M Environmental Laboratory for analysis. The 21-week endpoint sera and liver samples received by the 3M Environmental Laboratory were from the following birds:

- 40 control adult birds (20 mating pairs) that ingested food containing 0 ppm PFOS
- 40 experimental adult birds (20 mating pairs) that ingested food containing 10 ppm PFOS
- 20 first generation offspring birds that ingested food containing 0 ppm PFOS

Samples will be maintained frozen except when removed for extraction and analysis as described in the method. The samples will be kept isolated from the test substance during storage.

9. SUB-CONTRACTED ANALYSIS

All sera and liver extractions and analyses as detailed in this protocol amendment will be performed at 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106.55414.

10. EXPERIMENTAL CONDUCT

The 21-week endpoint liver and sera samples received (see section 8) will be extracted and analyzed for PFOS quantities using the methods listed below.

10.1 Preparatory and Analytical Method

10.1.1 ETS-8-231 Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices

11. DATA QUALITY OBJECTIVES DURING SAMPLE ANALYSIS

Method validation for PFOS quantitation in rat and mouse sera and liver is in process in the 3M Environmental Laboratory study E01-1277. Limited Northern Bobwhite control matrix preclude a cross validation, however, the E01-1277 study methods will be applied to sample analyses of Northern Bobwhite sera and liver samples using the following performance based criteria:

11.1 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. The peak area precision and retention time precision will be monitored at the beginning and the end of the run separately. The peak area precision must be equal to or less than 2.0% RSD, the precision of the retention time must be equal to or less than 2.5% RSD. If any item of the system suitability fails, system maintenance must be completed prior to running a second set of system suitability samples and the system suitability must pass before starting the calibration.

11.2 Absolute Recovery

The absolute recovery of the method will be evaluated one time in both liver and serum. For each matrix a minimum of triplicate samples will be fortified at a minimum of two levels of PFOS that bracket the range of analyte determined for the samples (i.e., at near the highest level determined in samples and at ~150% of LOQ for samples). The samples will be extracted through the method, and analyzed by comparison with an external calibration of non-extracted standards. The non-extracted standard calibration curves will be prepared in methanol, and will consist of a minimum of nine (9) levels, including a methanol blank. The best appropriate regression will be used to describe this curve (for best accuracy at all levels of the standards).

The accuracy (% absolute recovery) and precision (%CV of the recoveries) will be determined at each level using the external calibration curve for quantitation. There is no control limit for accuracy; precision must be better than 20% at each level. Should a level (high or low) not meet this precision, the method will only be considered valid for levels between the two valid levels. Additional testing may be conducted at other levels to extend the acceptable range of the method.

11.3 Calibration

A calibration curve should be prepared in each matrix by spiking the matrix with known concentrations of the analyte and surrogate. A calibration curve should consist of at least nine standard curve points covering the expected range, including the LLOQ. The simplest model that adequately describes the concentration-response relationship should be used. The accuracy of each level will be verified. Any level outside 75% - 125% of nominal must be deactivated, and regression re-calculated, except the LLOQ which must be within 30% of nominal. All levels must show a response greater than twice that of the matrix blank. A maximum of four (4) levels may be deactivated in any one set, or the set will be re-analyzed.

11.4 Limits of Quantitation (LOQ)

The lower limit of quantitation (LLOQ) will be defined in liver (ng/g) and in serum (ng/mL) as the lowest acceptable extracted calibration curve point that is within the 70% -130% nominal criteria and with an analyte peak area at least 2 times the blank. The LLOQ determination will be recorded in a note to file and kept with the raw study data as well as being reported in the final report.

The upper limit of quantitation (ULOQ) will be defined in liver and in serum as the highest acceptable extracted curve point that is within the 75% - 125% nominal criteria and includes applicable dilution factor corrections.

11.5 Quality Control Samples

A minimum of one set of nine (9) quality control samples (QC) will be prepared one time in each matrix. They will consist of three levels of analyte, each in triplicate. The quality control samples should be prepared in each matrix by spiking the control matrix with known concentrations of the analyte. The levels bracket the sample concentrations determined in the study:

- Low level: equivalent to an analyte concentration near or below the lowest level of analyte determined in samples
- Mid-level: equivalent to an analyte concentration near the middle of the sample range
- High level: equivalent to an analyte concentration near or above the highest level of analyte determined in samples

11.6 Quality Control Sample Acceptable Recoveries

Each QC is expected to show an accuracy of 75-125% of expected. A minimum of 2/3 (6 out of 9) of all QC must meet these criteria and a minimum of one half (2 out of 3) of the QC at each level must meet these criteria. If not, the entire sample set must either be re-analyzed or re-extracted (as decided by the Principal Analytical Investigator).

11.7 Matrix Spikes

Two matrix spikes will be extracted with each sample preparation. The matrix spikes will consist of one fortification level in the range of the samples, extracted in duplicate. If the matrix spike and/or matrix spike duplicate fall outside of 25% accuracy, the curve, CCV's, matrix spikes and samples will be reanalyzed.

11.8 Blanks

One control matrix and one water method blank will be extracted with each sample preparation.

11.9 Continuing Calibration Verification

Two extracted standards (continuing calibration verification – CCV) will be analyzed after every 10 injections (starting after the last injection of the calibration curve). The accuracy of the CCV must be within 25%. Data may only be reported if bracketed by passing CCV's (or a CCV and the same level calibration curve point). Data bracketed by one or more failing CCV must be reanalyzed.

11.10 Use of Confirmatory Methods

Confirmatory methods are typically not needed with LC/MS/MS analysis.

11.11 Demonstration of Specificity

PFOS specificity will be substantiated by chromatographic retention time, by the characteristic primary ion (499), and the characteristic product ion (99) using LC/MS/MS.

12. SUB-CONTRACTED ANALYSIS

12.1 All fluid and tissue extractions and analyses as detailed in this protocol amendment will be performed at the 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106.

12.2 An amendment to the protocol will be written if extractions and analyses are performed at laboratories other than the 3M Environmental Laboratory.

13. STATISTICAL ANALYSIS

Statistical methods will be limited to the calculation of means and standard deviations. Examples of the calculations used in the analyses will be included in the analytical phase report.

14. REPORT

A final report will be prepared by the 3M Environmental Laboratory. The final report will follow all GLP requirements, and will include a description of all materials and methods used, including a narrative discussion with a tabular presentation of all analyses results.

15. LOCATION OF RAW DATA, RECORDS, AND FINAL REPORT

When the final report is completed, all original paper data, including protocol, phase report, study correspondence, sample receipt and tracking, sample preparation, and analytical data, will be retained in the archives of 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained at the 3M Environmental Laboratory, as applicable.

16. SPECIMEN RETENTION

Samples of the test substance (any leftover control sera or liver and samples) and the analytical standards will be maintained in the laboratory for a period of time as specified by regulation or as long as the quality of the preparation affords evaluation. However, samples will not be maintained more than 10 years after the effective date of the final test rule (if applicable). 3M Environmental Laboratory Standard Operating Procedures also apply to sample retention times.

17. REFERENCES

18.1 Wildlife International, Ltd. Protocol No. 454-108, "PFOS: A Reproduction Study with the Northern Bobwhite".

18.2 3M Study U2723, Wildlife Study 454C-110, "Analytical Method Verification for the Determination of PFOS in Avian Diet."

18. PROTOCOL AMENDMENTS AND DEVIATIONS

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor 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 Sponsor Representative and filed with the raw data. All changes to the protocol will be indicated in the final report.

PROJECT NO.: 454-108,
WILDLIFE INTERNATIONAL, LTD E01-1245

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE NORTHERN BOBWHITE

PROTOCOL NO.: 454/120700/QR/SUB454 AMENDMENT NO.: 11

SPONSOR: 3M Corporation PROJECT NO.: 454-108, E01-1245

EFFECTIVE DATE: March 27, 2002

AMENDMENT: Section 11.1, Sentence 4

Change: The peak area precision must be equal to or less than 2.0% RSD, the precision of the retention time must be equal to or less than 2.5% RSD.

To: 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.

REASON: Information received from our instrument supplier indicates that a 2.0 % RSD for the area count is common to UV detector specifications. Mass spectrometers, which we used for this study, are more sensitive, in general and the fact that analyte ionization can be suppressed by other species present in the sample matrix and mobile phase make area reproducibility more challenging to maintain. Sample and source cleanliness are the determining factors, but $\leq$ 2.0% is still a bit aggressive for mass spec. The application chemists at Micromass, the instrument vendor, suggest a value of $\leq$ 5.0%.

AMENDMENT: Section 11.3, Sentence 5.

Change: Any level outside 75% - 125% of nominal value must be deactivated, and regression recalculated, except the LLOQ which must be within 30% of nominal.

To: In some cases the lowest curve point, although outside the 30% nominal requirement, may be included in the linear range to influence the lower end of the curve. Only one point, the lowest level point, can be left in for this purpose. It will not be used to determine the limit of quantitation. The limit of quantitation will be the next point on the curve meeting the 30% nominal requirement.

REASON: The removal of the lowest calibration curve point in some cases causes the next lowest calibration point to deviate by greater than 30%. Removal of this point in turn causes the next calibration point to deviate by greater than 30%. This continued deletion of calibration points in some cases could continue until there are very few points

remaining in the curve. The inclusion of a curve point with a deviation greater than 30% is used to influence the lower end of the curve and is not used for determination of the LOQ.

AMENDMENT: Section 11.3, the last sentence.

Change: A maximum of four (4) levels may be deactivated in any one set, or the set will be reanalyzed.

To: The calibration curve will contain a minimum of six (6) calibration curve points.

REASON: In most cases the calibration curves contain greater than nine (9) calibration points. The large number of points in the curve is used to select a range representative to the samples. Limiting the removal of only four (4) points in some cases could cause a calibration range not representative of the samples.

AMENDMENT: Section 11.3

Add: The coefficient of determination for the calibration curve should be ≥ 0.985.

REASON: No coefficient of determination is included in the protocol. It was inadvertently removed prior to printing of the final copy.

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-108, E01-1245

Sean P. Gallagher, Study Director — Date 4/2/02

Joann B. Beavers, Wildlife International, LTD Laboratory Management — Date 4/2/02

Harold O. Johnson, Principal Analytical Investigator — Date

William K. Reagen, 3M Environmental Laboratory, Laboratory Management — Date

Rochelle K. Robideau, Sponsor's Representative — Date 4/1/02

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-108, E01-1245

Sean P. Gallagher, Study Director — Date

Joann B. Beavers, Wildlife International, LTD Laboratory Management — Date

Harold O. Johnson, Principal Analytical Investigator — Date 4-2-02

William K. Reagen, 3M Environmental Laboratory, Laboratory Management — Date 04-02-02

Rochelle R. Robideau, Sponsor's Representative — Date 4/1/02

PROJECT NO.: 454-108,
WILDLIFE INTERNATIONAL, LTD E01-1245

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE NORTHERN BOBWHITE

PROTOCOL NO.: 454/120700/QR/SUB454 AMENDMENT NO.: 12

SPONSOR: 3M Corporation PROJECT NO.: 454-108, E01-1245

EFFECTIVE DATE: August 16, 2002

AMENDMENT:

Change: The 3M Environmental Laboratory Project Identification Number (LIMS) to be included with the study is E01-1245.

To: The main 3M Environmental Laboratory Project Identification Number (LIMS) to be included with the study is E01-1245. Additional tracking numbers associated with this study are U2723, E01-1342, and E01-1365.

REASON:

Bobwhite samples were received in the 3M Environmental at several time points. Upon arrival, samples were assigned a LIMS tracking number. Not aware of the scope of this or any other particular study pertaining to these samples, they were checked in to different 3M LIMS projects. LIMS project numbers assigned to this study include the following: U2723, E01-1245, E01-1342, and E01-1365. Samples in each of these projects pertaining to this study were analyzed under one main study number E01-1245.

AMENDMENT:

Change: Harold Johnson is the Principal Analytical Investigator.

To: Lisa A. Stevenson is the Principal Analytical Investigator.

REASON:

To change role of the Principal Analytical Investigator.

PROJECT NO.: 454-108,
E01-1245

WILDLIFE INTERNATIONAL, LTD

Sean P. Gallagher, Study Director — Date 9/25/02

Joann B. Beavers, Wildlife International, LTD, Laboratory Management — Date 9/25/02

Lisa A. Stevenson, Principal Analytical Investigator — Date 08/20/02

William K. Reagen, 3M Environmental Laboratory, Laboratory Management — Date 08/20/02

Rochelle R. Robideau, Sponsor's Representative — Date 8/20/02

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-108, E01-1245

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE NORTHERN BOBWHITE

PROTOCOL NO.: 454/120700/QR/SUB454

AMENDMENT NO.: 13

SPONSOR: 3M Corporation

PROJECT NO.: 454-108, E01-1245

EFFECTIVE DATE: September 27, 2002

AMENDMENT:

Change: Rochelle R. Robideau is the Sponsor Representative.

To: Susan A. Beach is the Sponsor Representative.

REASON:

To change role of the Sponsor Representative.

Sean P. Gallagher, Study Director — Date: 10/1/02

Joann B. Beavers, Wildlife International, LTD, Laboratory Management — Date: 10/1/02

Lisa A. Stevenson, Principal Analytical Investigator — Date: 09/27/02

William K. Reagen, 3M Environmental Laboratory, Laboratory Management — Date: 09/27/02

Susan A. Beach, Sponsor's Representative — Date: 9/27/02

3M Confidential

Record of Deviation

I. Identification

Study / Project No.
E01-1245

Deviation type (Check one): [X] SOP [ ] Method [ ] Equipment Procedure [ ] Protocol [ ] Other:

Document number ETS-4-4.0

Date(s) of occurrence
1/31/02, 2/1/02, 2/13/02, 2/7/02, 2/8/02, 2/11/02, 2/12/02, 2/13/02, 2/15/02

II. Description

Required procedure/process:
ETS-4-4.0 Internal chain of custody
The internal chain of custody form to be used to track all movement of samples
Within the lab. The form should be filed with study data or other relevant sample documents.

Actual procedure/process:
Internal chains of custodies were not used to track samples and sample extracts. Since receipt samples were Stored in freezer F1. After homogenizing liver and aliquating sera samples were stored in Freezer F8 Until extraction date. Samples remained frozen until extraction.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

Deviation to SOP written.

Recorded by Wojciech Kuklian Date 9-9-02

Authorized by [signature] Date 9.10.02

Deviation No. 1
(assigned by Study Director or Project Lead at the end of study or project)

Study Director: Sean P. Gallagher 9/24/02

Laboratory Management: [signature] 09/20/02

Record of Deviation

I. Identification

Study / Project No.E01-1245

Deviation Type (*Check one*): ☐ SOP ☐ Method ☐ Equipment Procedure √ Protocol ☐ Other:

Document Number:Protocol #: 454/120700/QR/SUB454, amendment #: 10

Date(s) of occurrence: :1/31/02, 2/1/02, 2/2/02, 2/7/02, 2/11/02, 2/12/02, 2/13/02, 2/14/02, 2/18/02, 4/11/02, 4/16/02

II. Description:

Required Procedure/process: Section 11.1, System Suitability, second sentence says that a mid-level unextracted calibration standard will be analyzed.

Actual Procedure/process:
On occasion through the dropping of calibration points, the system suitability samples were on the high end of the curve or outside the working range of the curve. Although these were unextracted curve points their area counts were at the high end of the calibration curve range, they do pass the system suitability criteria and these data packet will be considered acceptable. The data from 2/7/02 was just above the calibration range. These data will also be considered acceptable.

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

Issuing of this protocol deviation.

Recorded By Harold O. Johnston III Date 4.19.02

IV. Impact on Study / Project

There will be no impact on the study as the injections involved were used for system suitability and were within the acceptable precision ranges even though above the extracted calibration curve.

Authorized By *(Study Director // Project Lead)* Harold O. Johnston III Date 4.19.02

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 2
(assigned by Study Director or Project Lead at the end of study or project)

Lab Director: Dean P. Dallmuller 9/24/02

Record of Deviation

I. Identification

Study / Project No.E01-1245

Deviation Type (*Check one*): √ SOP ☐ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:

Document Number:ETS-8-231.1

Date(s) of occurrence:1/31/02, 2/1/02, 2/2/02, 2/7/02, 2/11/02, 2/12/02, 2/13/02, 2/14/02, 2/18/02, 4/11/02, 4/16/02

II. Description:

Required Procedure/process: Section 12.1, Calculations: If other calculations are used than those listed, they will be documented in the raw data.

Actual Procedure/process:

Standard E01-1342-36010, Prep sheet description "Curve Point – 10ppb in liver", was prepped by spiking 20uL of 02001-66 (1.00ug/mL) into 1 mL of liver homogenate. This homogenate consists of 5g liver plus 45mL water. Since 1mL out of 50mL homogenate is used to prep each extracted standard, 1/50 of the 5g (0.1g) of liver is "spiked". After this homogenate is spiked and prepared using SPE, the final eluate is 2.0mL of MeOH. THEREFORE…

(20ul) (1.00ugPFOS$^-$/mL MeOH) (1.0mL/1000uL) (1000ng/1.0ug) = 20ng PFOS$^-$ onto SPE column

20ng PFOS$^-$ / 2.0mL MeOH = 10ng PFOS$^-$/mL MeOH.
(Since this standard is recorded as 10ppb, the correct units are 10ngPFOS$^-$/mL of MeOH.)
These are therefore the units being measured and reported on the chromatographic system.

Similarly, the following statement is true.

(20ng PFOS$^-$ / 1.0mL homogenate) (1.0mL homogenate / 0.1g liver) = 200ng PFOS$^-$/ g liver

This relationship shows the factor of 20 used to "correct" the units when calculating the final result in ng/g from the chromatographic result that is in ng/mL.

Sample Calculation:
"E01-1342-30348, 0ppm a.i. 333 M liver"

Chromatographic result is 10.76 ng/mL; reported value is 208.4 ng/g. Since the 1.0333g initial sample weight of liver is homogenized with 9.0mL water, and 1mL (10% of total liver weighed, since a liver density of 1.00 is assumed) is used for the entire SPE procedure, the calculation is as follows:

(10.76ng PFOS-/ mL MeOH) (2mL MeOH / 0.1033 g liver) = 208.4 ng PFOS- / g liver

Sample Calculation:
Sample "E01-1342-36017, QC@10ppb-1": This QC sample is prepared by adding 4uL of 02001-53 (5.020ug PFOS$^-$ / mL MeOH) spiked into 1mL liver homogenate.

((4ul) (5.020ug/mL) (1mL/1000uL) (1000ng/ug)) / (0.1g liver) = 200.8ng PFOS$^-$ / g liver

Using the earlier calculation, the analytical result of 13.59ng/mL calculates to 271.8ng/g liver. Since the spike level is 200.8ng PFOS$^-$/ g liver, the recovery is (271.8/200.8)(100)=135.4%.

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 3
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

Issuing of this SOP deviation and a note to file.

Recorded By

Date 4.19.02

IV. Impact on Study / Project

There will be no impact on the study as this is for clarification purposes.

Authorized By *(Study Director / Project Lead)*

Date 4.19.02

Study Director: 9/24/02

Laboratory Management: 09/20/02

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 3
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No. E01-1245

Deviation Type (*Check one*): ☐ SOP ☐ Method ☐ Equipment Procedure √ Protocol ☐ Other:

Document Number: ETS-8-231.1

Date(s) of occurrence: Throughout the study

II. Description:

Required Procedure/process:

Section 11.3, Sentence 5 states: Any level outside 75% - 125% of nominal value must be deactivated, and regression recalculated…

Actual Procedure/process:

Many of the high calibration points in this study are kept in the curve despite being above the apex of the curve. When a calibration point lies above the apex, it is considered indeterminant (flagged with an I) and no concentration is calculated for the standard. If no concentration is calculated, no percent difference is calculated for evaluating whether or not that standard is within the stated criteria.

Removal of these points in most cases causes the next calibration point to then be above the apex of the curve. This continued deletion of points could continue until there are no points remaining on the curve. Therefore, calibration points above the apex were included in the range of the curve with no evaluation of percent deviation criteria.

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written, a note to file was written, and data points which were above the next highest calibration point were reanalyzed.

Recorded By: Lisa A. Stevenson — Lisa A. Stevenson — Date: 08/16/02 08/16/02

Authorized By *(Study Director / Project Lead)*: Study Director: Lisa P. Hallagan — 9/24/02

Sponsor Representative: [signature] — 8/20/02

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 4
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No. E01-1245

Deviation Type (*Check one*): √ SOP ☐ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:

Document Number: ETS-8-231.1 | Date(s) of occurrence: Entire Study

II. Description:

Required Procedure/process:

Section 12.1, Calculations: If other calculations are used than those listed, they will be documented in the raw data.

Actual Procedure/process:

Standard E01-1345-35771, Prep sheet description "10 ppb sera curve point", was prepped by spiking 4uL of 02001-52 (5.020ug/mL) into 2 mL of pooled sera. This diluted sera consists of 2mL sera plus 38mL water. Since 1mL out of 40mL diluted sera is used to prepare each extracted standard, 1/40 of the 2mL of sera is "spiked". After 1.0 mL of diluted sera is spiked and prepared using SPE, the final eluate is 2.0mL of MeOH. THEREFORE...

(5.020ug $PFOS^-$/mL MeOH) * (4ul) * (1.0mL/1000uL) * (1000ng/1.0ug) = 20ng $PFOS^-$ onto SPE column

20ng PFOS / 2.0mL MeOH = 10ng PFOS /mL MeOH.
(Since this standard is recorded as 10ppb, the correct units are 10ng $PFOS^-$/mL MeOH.)
These are therefore the units being measured and reported on the chromatographic system.

Similarly, the following statement is true.
(20ng PFOS / 1.0mL diluted sera) * (40.0mL diluted sera/ 2.0mL initial sera) = 400ng $PFOS^-$/ mL Sera

This relationship shows the factor of 40 (or 2.0mL MeOH/ 0.05mL Sera) used to "correct" the units when calculating the final result in ng PFOS/mL Sera from the chromatographic result that is in ng PFOS/mL MeOH.

Sample Calculation:
"E01-1245-28481, 454-108-370, 10 ppm female, adult, 02-01-02
Chromatographic result is 229.2 ng/mL MeOH; reported value is 9170 ng/mL Sera. Since the 1.0 mL initial sample volume of sera is diluted with ~~38.0~~ 19.0, or a 1/20 dilution mL water, and 1mL is used for the entire SPE procedure, the calculation is as follows: RE LAS 11/20/02
(229.2ng PFOS-/ 1.0mL MeOH) * (2.0mL MeOH/ 0.05mL Sera) = 9170ng PFOS- / mL Sera

Sample Calculation:
Sample "E01-1245-36155, QC@100ppb-1": This QC sample is prepared by adding 2uL of 02001-52 (5.02ug $PFOS^-$ / mL MeOH) spiked into 1mL diluted sera (or 0.05 mL of initial sera).

((5.02ug/mL) * (2ul) * (1mL/1000uL) * (1000ng/ug)) / (0.05 mL Sera) = 200.8 ng $PFOS^-$ / mL Sera

Using the earlier calculation, the analytical result of 6.17 ng/mL calculates to 246.8ng/mL Sera. Since the spike level is 200.8ng $PFOS^-$/ mL Sera, the recovery is (246.8/200.8)*(100)=122.9%.

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written and a note to file was written.

Recorded By: Lisa A. Stevenson Lisa A. Stevenson — Date: 08/16/02 08/16/02

Authorized By *(Study Director / Project Lead)* — Date

Study Director: Sean P. Gallagher 9/24/02 11/25/02 11/20/02

Laboratory Management: 11/20/02

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 5
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No. E01-1245

Deviation Type (*Check one*): ☐ SOP √ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:

Document Number: ETS-8-231.1

Date(s) of occurrence: Throughout the study

II. Description:

Required Procedure/process:

Section 13.3 of the method states: Two thirds of all quality control samples and 1/2 of each quality control sample at each level are expected to show an accuracy of 75-125%.

Actual Procedure/process:

All 25 ppb quail liver quality control sample recoveries were >125%.

10 (RE) LAS 03/11/03

03/12/03

DJ 3/14/03

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written, liver data reported at the low range of the curve may be biased high, and quality control data not within criteria are flagged in the raw data.

Recorded By: Lisa A. Stevenson — Date: 08/26/02 8/26/02

Authorized By *(Study Director / Project Lead)* — Date

Study Director: 10/1/02

Laboratory Management: 08/26/02

(RE) LAS 11/26/02

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 16
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No.E01-1245

Deviation Type (*Check one*): √ SOP ☐ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:

Document Number:ETS-8-231.1

Date(s) of occurrence:1/31/02, 2/1/02, 2/2/02, 2/7/02, 2/11/02, 2/12/02, 2/13/02, 2/14/02, 2/18/02, 4/11/02, 4/16/02

II. Description:

Required Procedure/process: Section 12.1, Calculations: If other calculations are used than those listed, they will be documented in the raw data.

Actual Procedure/process:

This deviation supersedes deviation #3 explaining the liver calculation:

Standard E01-1342-36010, Prep sheet description "Curve Point – 10ppb in liver", was prepped by spiking 20uL of 02001-66 (1.00ug/mL) into 1 mL of liver homogenate. This homogenate consists of 5.0353g liver plus 45.0mL water. Since 1mL out of 50.0353mL homogenate is used to prep each extracted standard, 1/50 of the 5.0353g (0.100635g = 5.0353 g/(9.0 of water + 5.0353 g of liver) is "spiked". After this homogenate is spiked and prepared using SPE, the final eluate is 2.0mL of MeOH. THEREFORE…

(20 uL) * (1.000 ug $PFOS^-$/mL MeOH) * (1.0 mL/1000 uL) * (1000 ng/1.0 ug) = 20.0 ng $PFOS^-$ onto SPE column

20.0 ng $PFOS^-$ / 2.0 mL MeOH = 10.0 ng $PFOS^-$/mL MeOH.
(Since this standard is recorded as 10ppb, the correct units are 10.0 ng $PFOS^-$/mL of MeOH.)
These are the units being measured and reported on the chromatographic system.

Similarly, the following statement is true.

(20.0ng $PFOS^-$ / 1.0mL homogenate) * (1.0mL homogenate / 0.100635 g liver) = 199ng $PFOS^-$/ g liver

Sample Calculation:
"E01-1342-30348, 0ppm a.i. 333 M liver"

Chromatographic result is 10.76 ng/mL; reported value is 209 ng/g. Since the 1.0338g initial sample weight of liver is homogenized with 9.0mL water, and 1.0 mL is used for the entire SPE procedure, the calculation is as follows:

1.0338 g / (9.0 mL water + 1.0338 g liver) = 0.103032 g of liver in 1.0 mL of homogenate removed for extraction.

(10.76 ng PFOS-/ mL MeOH) * (2.0 mL MeOH / 0.103032 g liver) = 209 ng PFOS- / g liver

Sample Calculation:
Sample "E01-1342-36017, QC@10ppb-1": This QC sample is prepared by adding 4 uL of 02001-52 (5.020ug $PFOS^-$ / mL MeOH) spiked into 1mL liver homogenate.

((4ul) * (5.020ug/mL) * (1mL/1000uL) * (1000ng/ug)) / (0.100635g liver) = 200 ng $PFOS^-$ / g liver

Using the earlier calculation, the analytical result of 13.59ng/mL calculates to 270 ng/g liver. Since the spike level is 200ng $PFOS^-$/ g liver, the recovery is (270/200) * (100)=135.0%.

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

Issuing of this SOP deviation.

Recorded By
Lisa A. Stevenson Lisa A. Stevenson

Date
12/01/02 12/01/02

Authorized By *(Study Director / Project Lead)*
Study Director: Sean Gallagher Sean P. Gallagher

Date
12/3/02

Laboratory Management: William Reagen 12/05/02
3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 7
(assigned by Study Director or Project Lead at the end of study or project)

Report E01-1245 PFOS: A Reproduction Study with the Northern Bobwhite

ATTACHMENT F: EXAMPLE CALCULATIONS

FORMULA USED FOR SERA ANALYSES IN STUDY E01-1245

AR (ng/mL MeOH) x DF x Dilution(diluted sera) = ng/mL Sera

Calculation Used for E01-1245-28481, 454-108-370, 10 ppm Female, adult (initial volume = 0.3 mL)

229.24 ng/mL MeOH x 1 x 40 = 9170 ng/mL Sera

FORMULA USED FOR LIVER ANALYSES IN STUDY E01-1245

$$\text{AR (ng/mL MeOH)} \times \text{DF} \times \frac{\text{Final Volume (mL)}}{\text{amount of liver in diluted sample (g)}} = \text{ng/g Liver}$$

Calculation Used for E01-1342-30672, 10 ppm, a.i. 369 M Liver (initial weight = 1.0010 g)

$$393.21 \text{ ng/mL MeOH} \times 10 \times \frac{2.0 \text{ mL MeOH}}{0.100090 \text{ g Liver}} = 78600 \text{ ng/g Liver}$$

AR = Analytical result from MassLynx summary
DF = Dilution factor
Dilution(diluted sera) = Final volume(mL)/amount of sera in diluted sample(mL)

Amount of sera in diluted sample(mL) = amount of sera used(mL)/total amount of sera + water(mL)

Amount of liver in diluted sample(g) = amount of liver weighed(g)/ amount of liver weighed(g) + water added for homogenizing(mL=g)

Examples:

Amount of sera in diluted sample(mL) = amount of sera used (0.3 mL)/total amount of sera + water (6 mL) = 0.05 mL

Dilution(diluted sera)(mL) = final volume (2.0 mL)/amount of sera in diluted sample (0.05 mL) = 40

Amount of liver in diluted sample(g) = amount of liver weighed (1.0010 g)/amount of liver weighed (1.0010 g) + water added for homogenizing (9.00 mL) = 1.0010 g / 10.0010 g = 1.00090 g

Note: For sera samples with less than 0.05 mL sera received, additional water was added in order to remove 1.0 mL of diluted sera for extraction. For these samples, the dilution(diluted sera) value was updated to reflect the correct dilution.

For example, if a sample was received with 0.02mL sera available, 0.98 mL of water was added. the amount of sera in diluted sample = 0.02 mL/1.0 mL = 0.02 mL.

The dilution(diluted sera)(mL) = 2.0 mL/0.02 mL = 50