

## **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

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## **Analytical Phase Initiation Date**

January 31, 2002

## **Analytical Phase Completion Date**

April 18, 2003

## **Performing Laboratory**

3M Environmental Laboratory  
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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

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**GLP COMPLIANCE STATEMENT**

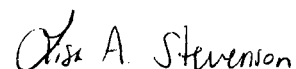
Study Title: Analytical Phase Report for PFOS: A Reproduction Study with The Northern Bobwhite

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:

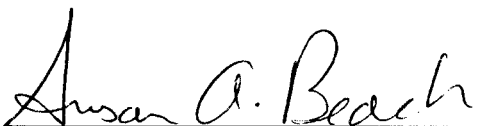
- Automated data collection systems are not validated. The hardcopy printouts are considered as the original raw data.
- The stability of the reference substances and samples has not been determined.
- Not all data generated were recorded directly or promptly, or initiated and dated on date of entry.



Lisa A. Stevenson, Principal Analytical Investigator

04/18/03

Date



Susan A. Beach, Sponsor Representative

4/18/03

Date

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**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           |



Quality Assurance Representative

04/18/03

Date

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## STUDY INFORMATION

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**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.

## SUMMARY

The concentration of the  $\text{C}_8\text{F}_{17}\text{SO}_3^-$  anion of perfluorooctane sulfonate, potassium salt (PFOS,  $\text{C}_8\text{F}_{17}\text{SO}_3^-\text{K}^+$ ) 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  $\text{C}_8\text{F}_{17}\text{SO}_3^-$  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  $\text{C}_8\text{F}_{17}\text{SO}_3^-$ . Concentrations of  $\text{C}_8\text{F}_{17}\text{SO}_3^-$  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,  $\text{C}_8\text{F}_{17}\text{SO}_3^-$  Concentration in Sera and Liver**

| SAMPLING EVENT                 | NUMBER OF SERA SAMPLES | AVERAGE $\text{C}_8\text{F}_{17}\text{SO}_3^-$ CONC. SERA | Range of Results                              | NUMBER OF LIVER SAMPLES | AVERAGE $\text{C}_8\text{F}_{17}\text{SO}_3^-$ 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)*<br>209 ng/g (1 of 20)* |
| Female Control Group           | 17                     | NA                                                        | <400 ng/mL (16 of 17)<br>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 1/2 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%.

**Table 2. Summary Table, C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup> Concentration in Sera and Liver with Outliers Removed**

| SAMPLING EVENT                 | NUMBER OF SERA SAMPLES | AVERAGE C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> CONC. SERA | Range of Results      | NUMBER OF LIVER SAMPLES | AVERAGE C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> 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<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup> 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<sub>10</sub>F<sub>19</sub>O<sub>2</sub>H and is used solely to monitor for gross instrument failure and not as an internal standard for quantitation.

## 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).                                                                            |

## 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     |

| 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, 5 $\mu$ m particle size), a 40°C column compartment temperature, a 10 $\mu$ L injection volume and a 0.300mL/min pump flow rate. The HPLC gradient is listed below:

| TIME,<br>MINUTES | % 2 MM AMMONIUM<br>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  $3.0 \times 10^{-3}$  mBar.

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## 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  $\geq 0.985$ .
- **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  $\pm 25\%$  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:

**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 1/2 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.

- **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.

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## 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.

**Table 10. C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup> Data Summary, Female Quail Sera (Adult and Offspring)**

| 3M SAMPLE NUMBER | SAMPLE DESCRIPTION                         | AMOUNT OF SERA USED (ML) | ADDITIONAL DILUTION | CONC. OF C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> IN SAMPLE (NG/ML OF SERA) | AVERAGE OF DUP. SAMPLES |
|------------------|--------------------------------------------|--------------------------|---------------------|------------------------------------------------------------------------------------------------|-------------------------|
| * E01-1245-28444 | 454-108-330, Oppm female, adult, 01-31-02  | 0.4<br>0.4               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28446 | 454-108-332, Oppm female, adult, 01-31-02  | 0.05<br>0.05             | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28449 | 454-108-336, Oppm female, adult, 01-31-02  | 0.3<br>0.3               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28451 | 454-108-338, Oppm female, adult, 01-31-02  | 0.3<br>0.3               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28453 | 454-108-340, Oppm female, adult, 01-31-02  | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28456 | 454-108-344, Oppm female, adult, 01-31-02  | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28458 | 454-108-346, Oppm female, adult, 02-01-02  | 0.1<br>0.1               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28460 | 454-108-348, Oppm female, adult, 02-01-02  | 0.3<br>0.3               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28462 | 454-108-350, Oppm female, adult, 02-01-02  | 0.4<br>0.4               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28464 | 454-108-352, Oppm female, adult, 02-01-02  | 0.4<br>0.4               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28466 | 454-108-354, Oppm female, adult, 02-01-02  | 0.3<br>0.3               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28468 | 454-108-356, Oppm female, adult, 02-01-02  | 0.3<br>0.3               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28470 | 454-108-358, Oppm female, adult, 02-01-02  | 0.3<br>0.3               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28472 | 454-108-360, Oppm female, adult, 02-01-02  | 0.3<br>0.3               | 1<br>1              | 2050<br>2180                                                                                   | 2110 •                  |
| * E01-1245-28474 | 454-108-362, Oppm female, adult, 02-01-02  | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28477 | 454-108-366, Oppm female, adult, 02-01-02  | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28479 | 454-108-368, Oppm female, adult, 02-01-02  | 0.3<br>0.3               | 1<br>1              | <400<br><400                                                                                   | <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                                                                                           |                         |

| 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<br>0.1               | 1<br>1              | <400<br><400                                         | <400                    |
| * E01-1245-28521 | 454-108-259, 0ppm female, offspring, 02-01-02  | 0.1<br>0.1               | 1<br>1              | <400<br><400                                         | <400                    |
| * E01-1245-28524 | 454-108-273, 0ppm female, offspring, 02-01-02  | 0.08<br>0.08             | 1<br>1              | <400<br><400                                         | <400                    |
| * E01-1245-28525 | 454-108-277, 0ppm female, offspring, 02-01-02  | 0.2<br>0.2               | 1<br>1              | <400<br><400                                         | <400                    |
| * E01-1245-28528 | 454-108-517, 10ppm female, offspring, 02-01-02 | 0.02<br>0.02             | 1<br>1              | 18500<br>16600                                       | 17600                   |
| * E01-1245-28530 | 454-108-525, 10ppm female, offspring, 02-01-02 | 0.03<br>0.03             | 1<br>1              | 12800<br>12100                                       | 12500                   |
| * E01-1245-28532 | 454-108-538, 10ppm female, offspring, 02-01-02 | 0.08<br>0.08             | 1<br>1              | 14600<br>12200                                       | 13400                   |
| * E01-1245-28535 | 454-108-557, 10ppm female, offspring, 02-01-02 | 0.06<br>0.06             | 1<br>1              | 8330<br>7460                                         | 7900                    |
| * E01-1245-28536 | 454-108-561, 10ppm female, offspring, 02-01-02 | 0.05<br>0.05             | 1<br>1              | 11500<br>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.

**Table 11. C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup> Data Summary, Male Quail Sera (Adult and Offspring)**

| 3M SAMPLE NUMBER | SAMPLE DESCRIPTION                      | AMOUNT OF SERA USED (ML) | ADDITIONAL DILUTION | CONC. OF C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> IN SAMPLE (NG/ML OF SERA) | AVERAGE OF DUP. SAMPLES |
|------------------|-----------------------------------------|--------------------------|---------------------|------------------------------------------------------------------------------------------------|-------------------------|
| • E01-1245-28443 | 454-108-329, Oppm male, adult, 01-31-02 | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28445 | 454-108-331, Oppm male, adult, 01-31-02 | 0.4<br>0.4               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| • E01-1245-28447 | 454-108-333, Oppm male, adult, 01-31-02 | 0.4<br>0.4               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| • E01-1245-28448 | 454-108-335, Oppm male, adult, 01-31-02 | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| • E01-1245-28450 | 454-108-337, Oppm male, adult, 01-31-02 | 0.1<br>0.1               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28452 | 454-108-339, Oppm male, adult, 01-31-02 | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28454 | 454-108-341, Oppm male, adult, 01-31-02 | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| • E01-1245-28455 | 454-108-343, Oppm male, adult, 01-31-02 | 0.1<br>0.1               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28457 | 454-108-345, Oppm male, adult, 01-31-02 | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| • E01-1245-28459 | 454-108-347, Oppm male, adult, 02-01-02 | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28461 | 454-108-349, Oppm male, adult, 02-01-02 | 0.02<br>0.02             | 1<br>1              | <400<br><400                                                                                   | <400                    |
| • E01-1245-28465 | 454-108-353, Oppm male, adult, 02-01-02 | 0.3<br>0.3               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28467 | 454-108-355, Oppm male, adult, 02-01-02 | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28469 | 454-108-357, Oppm male, adult, 02-01-02 | 0.07<br>0.07             | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28471 | 454-108-359, Oppm male, adult, 02-01-02 | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28473 | 454-108-361, Oppm male, adult, 02-01-02 | 0.2<br>0.2               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28475 | 454-108-363, Oppm male, adult, 02-01-02 | 0.1<br>0.1               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28476 | 454-108-365, Oppm male, adult, 02-01-02 | 0.4<br>0.4               | 1<br>1              | <400<br><400                                                                                   | <400                    |
| * E01-1245-28478 | 454-108-367, Oppm male, adult, 02-01-02 | 0.1<br>0.1               | 1<br>1              | <400<br><400                                                                                   | <400                    |

| 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<br>0.2               | 100<br>100          | 107000<br>117000                                     | 112000                  |
| * E01-1245-28482 | 454-108-371, 10ppm male, adult, d100,02-01-02 | 0.2<br>0.2               | 100<br>100          | 181000<br>179000                                     | 180000                  |
| * E01-1245-28484 | 454-108-373, 10ppm male, adult, d100,02-01-02 | 0.1<br>0.1               | 100<br>100          | 144000<br>156000                                     | 150000                  |
| * E01-1245-28486 | 454-108-375, 10ppm male, adult, d100,02-01-02 | 0.3<br>0.3               | 100<br>100          | 138000<br>138000                                     | 138000                  |
| * E01-1245-28488 | 454-108-377, 10ppm male, adult, d100,02-01-02 | 0.07<br>0.07             | 100<br>100          | 94400<br>96100                                       | 95300                   |
| * E01-1245-28490 | 454-108-379, 10ppm male, adult, d100,02-01-02 | 0.1<br>0.1               | 100<br>100          | 150000<br>166000                                     | 158000                  |
| * E01-1245-28492 | 454-108-381, 10ppm male, adult, d100,02-01-02 | 0.1<br>0.1               | 100<br>100          | 141000<br>149000                                     | 145000                  |
| * E01-1245-28494 | 454-108-383, 10ppm male, adult, d100,02-01-02 | 0.3<br>0.3               | 100<br>100          | 147000<br>156000                                     | 151000                  |
| * E01-1245-28495 | 454-108-385, 10ppm male, adult, d100,02-01-02 | 0.1<br>0.1               | 100<br>100          | 119000<br>123000                                     | 121000                  |
| * E01-1245-28497 | 454-108-387, 10ppm male, adult, d100,02-01-02 | 0.3<br>0.3               | 100<br>100          | 174000<br>196000                                     | 185000                  |
| * E01-1245-28499 | 454-108-389, 10ppm male, adult, d100,02-01-02 | 0.1<br>0.1               | 100<br>100          | 129000<br>136000                                     | 132000                  |
| * E01-1245-28501 | 454-108-391, 10ppm male, adult, d100,02-01-02 | 0.06<br>0.06             | 100<br>100          | 141000<br>147000                                     | 144000                  |
| * E01-1245-28503 | 454-108-393, 10ppm male, adult, d100,02-01-02 | 0.2<br>0.2               | 100<br>100          | 160000<br>165000                                     | 162000                  |
| * E01-1245-28505 | 454-108-395, 10ppm male, adult, d100,02-01-02 | 0.2<br>0.2               | 100<br>100          | 127000<br>136000                                     | 131000                  |
| * E01-1245-28507 | 454-108-397, 10ppm male, adult, d100,02-01-02 | 0.3<br>0.3               | 100<br>100          | 99600<br>107000                                      | 103000                  |
| * E01-1245-28509 | 454-108-399, 10ppm male, adult, d100,02-01-02 | 0.1<br>0.1               | 100<br>100          | 138000<br>159000                                     | 148000                  |
| * E01-1245-28510 | 454-108-401, 10ppm male, adult, d100,02-01-02 | 0.1<br>0.1               | 100<br>100          | 203000<br>226000                                     | 215000                  |
| * E01-1245-28512 | 454-108-403, 10ppm male, adult, d100,02-01-02 | 0.2<br>0.2               | 100<br>100          | 127000<br>138000                                     | 132000                  |
| * E01-1245-28514 | 454-108-405, 10ppm male, adult, d100,02-01-02 | 0.1<br>0.1               | 100<br>100          | 108000<br>117000                                     | 112000                  |
| * E01-1245-28516 | 454-108-407, 10ppm male, adult, d100,02-01-02 | 0.1<br>0.1               | 100<br>100          | 99200<br>112000                                      | 105000                  |

| 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<br>0.02             | 1<br>1              | <400<br><400                                         | <400                    |
| * E01-1245-28520 | 454-108-256, 0ppm male, offspring, 02-01-02  | 0.02<br>0.02             | 1<br>1              | <400<br><400                                         | <400                    |
| * E01-1245-28522 | 454-108-266, 0ppm male, offspring, 02-01-02  | 0.07<br>0.07             | 1<br>1              | <400<br><400                                         | <400                    |
| * E01-1245-28523 | 454-108-270, 0ppm male, offspring, 02-01-02  | 0.2<br>0.2               | 1<br>1              | <400<br><400                                         | <400                    |
| * E01-1245-28526 | 454-108-283, 0ppm male, offspring, 02-01-02  | 0.02<br>0.02             | 1<br>1              | <400<br><400                                         | <400                    |
| * E01-1245-28527 | 454-108-291, 0ppm male, offspring, 02-01-02  | 0.05<br>0.05             | 1<br>1              | <400<br><400                                         | <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<br>0.07             | 1<br>1              | 9310<br>8220                                         | 8760                    |
| * E01-1245-28533 | 454-108-545, 10ppm male, offspring, 02-01-02 | 0.04<br>0.04             | 1<br>1              | 15500<br>13600                                       | 14600                   |
| * E01-1245-28534 | 454-108-552, 10ppm male, offspring, 02-01-02 | 0.07<br>0.07             | 1<br>1              | 13600<br>11500                                       | 12500                   |
| * E01-1245-28537 | 454-108-568, 10ppm male, offspring, 02-01-02 | 0.1<br>0.1               | 1<br>1              | 12100<br>10400                                       | 11200                   |

\* 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.

**Table 12. C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup> Data Summary, Female Quail Liver (Adult and Offspring)**

| 3M SAMPLE NUMBER | SAMPLE DESCRIPTION      | AMOUNT OF LIVER WEIGHED (G) | ADDITIONAL DILUTION | CONC. OF C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> 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*                                                                                          |

| 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%.

**Table 13. C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup> Data Summary, Male Quail Liver (Adult and Offspring)**

| 3M SAMPLE NUMBER | SAMPLE DESCRIPTION      | AMOUNT OF LIVER WEIGHED (G) | ADDITIONAL DILUTION | CONC. OF C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> 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*                                                                                        |

| 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).

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)*<br>209 ng/g (1 of 20)* |
| Female Control Group           | 17                     | NA                                   | <400 ng/mL (16 of 17)<br>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%.

**Table 15. Summary Table, C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup> Concentration in Sera and Liver with Outliers Removed**

| SAMPLING EVENT                 | NUMBER OF SERA SAMPLES | AVERAGE C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> CONC. SERA | Range of Results      | NUMBER OF LIVER SAMPLES | AVERAGE C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> 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%.

The protocol and method state that 2/3 of all QC and a minimum of 1/2 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.

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## 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 from Biological Matrices".

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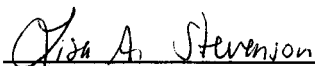
## LIST OF ATTACHMENTS

- 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

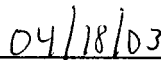
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## SIGNATURE PAGE

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



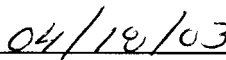
Lisa A. Stevenson  
Principal Analytical Investigator



Date



William K. Reagen  
Laboratory Management



Date

---

**ATTACHMENT A: EXTRACTION AND ANALYTICAL METHOD, ETS-8-231.1,  
“SOLID PHASE EXTRACTION AND ANALYSIS OF FLUORO-CHEMICAL  
COMPOUNDS FROM BIOLOGICAL MATRICES”**

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## 3M Environmental Laboratory

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### Method

#### *Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices*

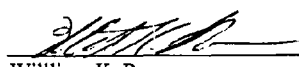
**Method Number: ETS-8-231.1**

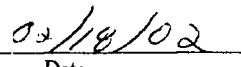
**Adoption Date: 11/13/01**

**Revision Date: 2/18/02**

**Effective Date: 2/18/02**

Approved By:

  
\_\_\_\_\_  
William K. Reagen  
Laboratory Manager

  
\_\_\_\_\_  
Date

ETS-8-231.1  
Solid Phase Extraction and Analysis of Fluorochemical  
Compounds from Biological Matrices

Page 1 of 19

## 1 Scope and Application

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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

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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<sub>18</sub> 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

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### 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

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### 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.

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

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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

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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 sample 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<sub>18</sub> (Waters)

75 mL sample reservoir (or other appropriate size)

Crimp cap glass autovials and caps

Crimpers

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

## 7 Reagents and Standards

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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:  $1000 \mu\text{g/mL} \times 5 \text{ mL}/100 \text{ mL} = 50 \mu\text{g/mL}$ .

Dilute working standard 1 with methanol to produce a working standard 2 solution of approx. 5.0 ppm. Example calculation:  $50 \mu\text{g/mL} \times 10 \text{ mL}/100 \text{ mL} = 5.0 \mu\text{g/mL}$ .

Dilute working standard 1 with methanol to produce a working standard 3 solution of approx. 0.50 ppm. Example calculation:  $50 \mu\text{g/mL} \times 1.0 \text{ mL}/100 \text{ mL} = 0.5 \mu\text{g/mL}$ .

### 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.

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

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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

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### 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

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

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### 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.

| TABLE I<br>APPROXIMATE SPIKING AMOUNTS FOR STANDARDS AND SPIKES<br>USING 1.0 mL OF MATRIX |               |                                                                        |                                                                         |
|-------------------------------------------------------------------------------------------|---------------|------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Working standard<br>(approximate concentration)                                           | $\mu\text{L}$ | Approximate final<br>concentration of analyte<br>in Matrix diluted 1:5 | Approximate final<br>concentration of analyte<br>in Final 2.0 mL volume |
| -                                                                                         | -             | Blank                                                                  | Blank                                                                   |
| 0.500 $\mu\text{g/mL}$                                                                    | 1.5           | 5.00 ng/g or ng/mL                                                     | 0.375 ng/mL                                                             |
| 0.500 $\mu\text{g/mL}$                                                                    | 3.0           | 10.0 ng/g or ng/mL                                                     | 0.750 ng/mL                                                             |
| 0.500 $\mu\text{g/mL}$                                                                    | 8.0           | 25.0 ng/g or ng/mL                                                     | 2.00 ng/mL                                                              |
| 0.500 $\mu\text{g/mL}$                                                                    | 16            | 50.0 ng/g or ng/mL                                                     | 4.00 ng/mL                                                              |
| 0.500 $\mu\text{g/mL}$                                                                    | 32            | 100 ng/g or ng/mL                                                      | 8.00 ng/mL                                                              |
| 5.00 $\mu\text{g/mL}$                                                                     | 5.6           | 175 ng/g or ng/mL                                                      | 14.0 ng/mL                                                              |
| 5.00 $\mu\text{g/mL}$                                                                     | 8.0           | 250 ng/g or ng/mL                                                      | 20.0 ng/mL                                                              |
| 5.00 $\mu\text{g/mL}$                                                                     | 16            | 500 ng/g or ng/mL                                                      | 40.0 ng/mL                                                              |
| 5.00 $\mu\text{g/mL}$                                                                     | 24            | 750 ng/g or ng/mL                                                      | 60.0 ng/mL                                                              |
| 5.00 $\mu\text{g/mL}$                                                                     | 32            | 1000 ng/g or ng/mL                                                     | 80.0 ng/mL                                                              |
| 5.00 $\mu\text{g/mL}$                                                                     | 40            | 1250 ng/g or ng/mL                                                     | 100 ng/mL                                                               |
| 50.0 $\mu\text{g/mL}$                                                                     | 5.0           | 1500 ng/g or ng/mL                                                     | 125 ng/mL                                                               |
| 50.0 $\mu\text{g/mL}$                                                                     | 6.0           | 1750 ng/g or ng/mL                                                     | 150 ng/mL                                                               |
| Surrogate Std 100 $\mu\text{g/mL}$                                                        | 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 ( $\pm$  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 sample 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 sample 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  $\pm$  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.

## 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 sample 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.

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  $\mu$ L injection

Inject/sample = 1

Cycle time = 10.0 minutes

Flow rate = 300  $\mu$ L/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 10–500  $\mu$ L/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

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:

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

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:

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

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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 ( $\mu\text{g/mL}$ ):

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

Calculate actual concentration of analyte in tissue ( $\mu\text{g/g}$ ):

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

## 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  $0.5 < \text{AF} < 2.0$ , 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%.

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

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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

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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

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Attachment A: Extraction Worksheet

Attachment B: Sample Weight/Volume Worksheet

Attachment C, Calibration Standard Concentration Worksheet

Attachment D, Dilutions Summary Worksheet

## 17 References

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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

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None

## 19 Revisions

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| <u>Revision<br/>Number</u> | <u>Revision Description</u>                                                                                                                                                         | <u>Revision<br/>Date</u> |
|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| 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                 |

## Attachment A – Extraction Worksheet

Study Number:  
Prep Date:  
Analysts initials:  
Box#:

Method Revision: ETS-8-231.1  
Matrix:  
Sample Timepoint:

[illegible]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 polypropylene 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.

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## Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices

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

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

[illegible]

Form Completion Verified By: \_\_\_\_\_

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Solid Phase Extraction and Analysis of Fluorochemical  
Compounds from Biological Matrices

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## 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<br>Std conc<br>ug/mL |  | All<br>Am't spiked<br>mL | All<br>Final<br>Volume:<br>mL | All<br>Initial<br>Fluid<br>Dilution<br>mL/mL | All<br>Initial<br>Tissue<br>Density<br>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<br>Final<br>conc.<br>ng/mL | Surrogate<br>Std conc<br>ng/mL | Analyte<br>Final<br>conc.<br>ng/mL | Surrogate<br>Std conc<br>ng/mL | Analyte<br>Final<br>conc.<br>ng/g | Surrogate<br>Std conc<br>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                              |                                |

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Solid Phase Extraction and Analysis of Fluorochemical  
Compounds from Biological Matrices

Attachment D: Dilutions Summary Worksheet

Study:  
Dilution Date/Analyst:  
Box Number:

Solvent/TN Number:  
Extraction Date/Analyst:  
Matrix/Timepoint:

| Sample Number<br>or Description | Dilutions |    |    |    |    |    |    | Comments | Verified By: |
|---------------------------------|-----------|----|----|----|----|----|----|----------|--------------|
|                                 | 1/        | 1/ | 1/ | 1/ | 1/ | 1/ | 1/ |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |
|                                 |           |    |    |    |    |    |    |          |              |

Notes:  
1/10 dilution = \_\_\_\_\_ of sample + \_\_\_\_\_ of solvent

Form Completion Verified By: \_\_\_\_\_

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**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) | log 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |

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) | log 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |

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                                    | 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                                 | 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                                     | 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

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 all in rerun analysis (recovery)

|                |
|----------------|
| avg %rec 95.57 |
| std dev 14.18  |

## 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) | log 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm female, adult, 02-01-02 | d020201059      | 0.3                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       | 400  |
|                  |              |                                           | d020207103      | 0.3                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       |      |
| * E01-1245-28472 | 06/15/2001   | 454-108-360, Oppm female, adult, 02-01-02 | d020201061      | 0.3                      | 40                      | 1                   | 51.27                          | 400                             | 2051                                    |                         | 2114 |
|                  |              |                                           | d020207105      | 0.3                      | 40                      | 1                   | 54.43                          | 400                             | 2177                                    |                         |      |
| * E01-1245-28474 | 06/15/2001   | 454-108-362, Oppm female, adult, 02-01-02 | d020201063      | 0.2                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       | 400  |
|                  |              |                                           | d020207107      | 0.2                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       |      |
| * E01-1245-28477 | 06/15/2001   | 454-108-366, Oppm female, adult, 02-01-02 | d020201066      | 0.2                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       | 400  |
|                  |              |                                           | d020207110      | 0.2                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       |      |
| * E01-1245-28479 | 06/15/2001   | 454-108-368, Oppm female, adult, 02-01-02 | d020201073      | 0.3                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       | 400  |
|                  |              |                                           | d020207117      | 0.3                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       |      |

+ Concentration &lt;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

With outliers  
 avg 500.8  
 std dev 409.7  
 %RSD 81.80  
 rejected  
 rejected  
 Without outliers  
 avg 400  
 std dev 0  
 %RSD -

## 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) | log 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                                    |                         |
| E01-1245-28502   | 06/15/2001   | 454-108-392, 10ppm female, adult, 02-01-02     | d020203039      | 0.3                      | 40                      | 1                   | 235.08                         | 400                             | 9403                                    |                         |
| E01-1245-28504   | 06/15/2001   | 454-108-394, 10ppm female, adult, 02-01-02     | d020203040      | 0.3                      | 40                      | 1                   | 284.92                         | 400                             | 11397                                   |                         |
| E01-1245-28506   | 06/15/2001   | 454-108-396, 10ppm female, adult, 02-01-02     | d020203041      | 0.09                     | 40                      | 1                   | 117.84                         | 400                             | 4714                                    |                         |
| 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                                    |                         |
| E01-1245-28513   | 06/15/2001   | 454-108-404, 10ppm female, adult, 02-01-02     | d020203044      | 0.1                      | 40                      | 1                   | 226.40                         | 400                             | 9056                                    |                         |
| E01-1245-28515   | 06/15/2001   | 454-108-406, 10ppm female, adult, 02-01-02     | d020203045      | 0.2                      | 40                      | 1                   | 224.49                         | 400                             | 8980                                    |                         |
| E01-1245-28517   | 06/15/2001   | 454-108-408, 10ppm female, adult, 02-01-02     | d020203046      | 0.2                      | 40                      | 1                   | 196.03                         | 400                             | 7841                                    |                         |
| * E01-1245-28518 | 07/23/2001   | 454-108-246, 0ppm female, offspring, 02-01-02  | d020201132      | 0.1                      | 40                      | 1                   | 10.0                           | 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                                     | +                       |
|                  |              |                                                | 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                                     | +                       |
|                  |              |                                                | d020207159      | 0.08                     | 40                      | 1                   | 10.0                           | 400                             | 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                                     | +                       |
|                  |              |                                                | d020207160      | 0.2                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       |
| * E01-1245-28528 | 07/23/2001   | 454-108-517, 10ppm female, offspring, 02-01-02 | d020201147      | 0.02                     | 100                     | 1                   | 185.25                         | 400                             | 18525                                   |                         |
|                  |              |                                                | 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                                   |                         |
|                  |              |                                                | 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                                   |                         |
|                  |              |                                                | 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                                    |                         |
|                  |              |                                                | d020207175      | 0.06                     | 40                      | 1                   | 186.59                         | 400                             | 7464                                    |                         |
| • E01-1245-28536 | 07/23/2001   | 454-108-561, 10ppm female, offspring, 02-01-02 | d020201155      | 0.05                     | 40                      | 1                   | 287.26                         | 400                             | 11490                                   |                         |
|                  |              |                                                | d020207176      | 0.05                     | 40                      | 1                   | 252.41                         | 400                             | 10096                                   |                         |

+ Concentration &lt;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) | log 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm 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, Oppm male, adult, 02-01-02 | d020201067      | 0.1                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       | 400 |
|                      |              |                                         | d020207111      | 0.1                      | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       |     |
| + Concentration <LOQ |              |                                         |                 |                          |                         |                     |                                |                                 |                                         |                         |     |
|                      |              |                                         |                 |                          |                         |                     |                                |                                 |                                         | avg 400                 |     |
|                      |              |                                         |                 |                          |                         |                     |                                |                                 |                                         | std dev 0               |     |
|                      |              |                                         |                 |                          |                         |                     |                                |                                 |                                         | %RSD -                  |     |

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                                  |                         |
| • 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                  |
|                  |              |                                               | d020207147      | 0.1                      | 40                      | 100                 | 27.90                          | 400                             | 111600                                  |                         |

avg of dups 141112  
std dev 30225  
%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) | log 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                                     | +                       |       |
| * 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   |
|                  |              |                                              | d020207162      | 0.05                     | 40                      | 1                   | 10.0                           | 400                             | 400                                     | +                       |       |
| 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 |
|                  |              |                                              | d020201150      | 0.07                     | 40                      | 1                   | 232.67                         | 400                             | 9307                                    |                         |       |
| * E01-1245-28531 | 07/23/2001   | 454-108-533, 10ppm male, offspring, 02-01-02 | d020207171      | 0.07                     | 40                      | 1                   | 205.40                         | 400                             | 8216                                    |                         | 8761  |
|                  |              |                                              | d020201152      | 0.04                     | 50                      | 1                   | 309.79                         | 400                             | 15490                                   |                         |       |
| * E01-1245-28533 | 07/23/2001   | 454-108-545, 10ppm male, offspring, 02-01-02 | d020207173      | 0.04                     | 50                      | 1                   | 272.51                         | 400                             | 13626                                   |                         | 14558 |
|                  |              |                                              | d020201153      | 0.07                     | 40                      | 1                   | 339.60                         | 400                             | 13584                                   |                         |       |
| * E01-1245-28534 | 07/23/2001   | 454-108-552, 10ppm male, offspring, 02-01-02 | d020207174      | 0.07                     | 40                      | 1                   | 286.47                         | 400                             | 11459                                   |                         | 12521 |
|                  |              |                                              | d020201156      | 0.1                      | 40                      | 1                   | 301.89                         | 400                             | 12076                                   |                         |       |
| * E01-1245-28537 | 07/23/2001   | 454-108-568, 10ppm male, offspring, 02-01-02 | d020207177      | 0.1                      | 40                      | 1                   | 260.08                         | 400                             | 10403                                   |                         | 11239 |
|                  |              |                                              |                 |                          |                         |                     |                                |                                 |                                         |                         |       |

avg 400  
std dev 0  
%RSD -

avg 12601  
std dev 3374  
%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 Homogenizing (mL) | Final Volume (mL) | Amount of liver in diluted sample (g) or water (mL) | additional dilution | Inst Conc. (ng/mL of Methanol) | log 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 Homogenizing (mL) | Final Volume (mL) | Amount of liver in diluted sample (g) | additional dilution | Inst Conc. (ng/mL of Methanol) | log 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 &lt;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 Homogenizing (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 Homogenizing (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 Homogenizing (mL) | Final Volume (mL) | Amount of liver in diluted sample (g) | additional dilution | Inst Conc. (ng/mL of Methanol) | log 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 Homogenizing (mL) | Final Volume (mL) | Amount of liver in diluted sample (g) | additional dilution | Inst Conc. (ng/mL of Methanol) | log 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

$$\text{Conc of PFOS in Sample (ng/g)} = (\text{Inst conc(ng/mL)}) \times (\text{additional dilution}) \times (\text{final volume (mL)}) / \text{amount of liver in diluted sample (g)}$$

$$\text{amount of liver in diluted sample (g)} = \text{amount of liver weighed (g)} / (\text{water added for homogenizing (mL=g)}) + \text{amount of liver weighed(g)}$$

## 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 Homogenizing (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              | 0.108993                              | 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                                    | * |
| 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                                    | * |
| 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                                    | * |
| 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                                    |   |
| 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                                    |   |
| 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                                    |   |
| 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                                    | * |
| 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                                    | * |
| 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                                    | * |
| 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                                    |   |
| 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                                   |   |
| 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                                    |   |
| 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                                    |   |

avg 50.2  
std dev 0  
%RSD -

avg 4898  
std dev 1035  
%RSD 21.1

avg 20.0  
std dev 0  
%RSD -

rejected

Without outliers  
avg 5494  
std dev 1137  
%RSD 20.7

With outliers  
avg 7182  
std dev 3900  
%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 Homogenizing (mL) | Final Volume (mL) | Amount of liver in diluted sample (g) | additional dilution | Inst Conc. (ng/mL of Methanol) | log 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                                    | *        |
| 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                                    | *        |
| 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                                    | *        |
| 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                                    | *        |
| 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                                    | *        |
| 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                                    | *        |
| 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                                    | *        |
| 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                                    | *        |
| 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                                  |          |
| 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                                  |          |
| 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                                  |          |
| 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                                    | *        |
| 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                                    | *        |
| 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                                    | *        |
| 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                                    |          |
| 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                                    |          |
| 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                                    |          |
| 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                                    |          |

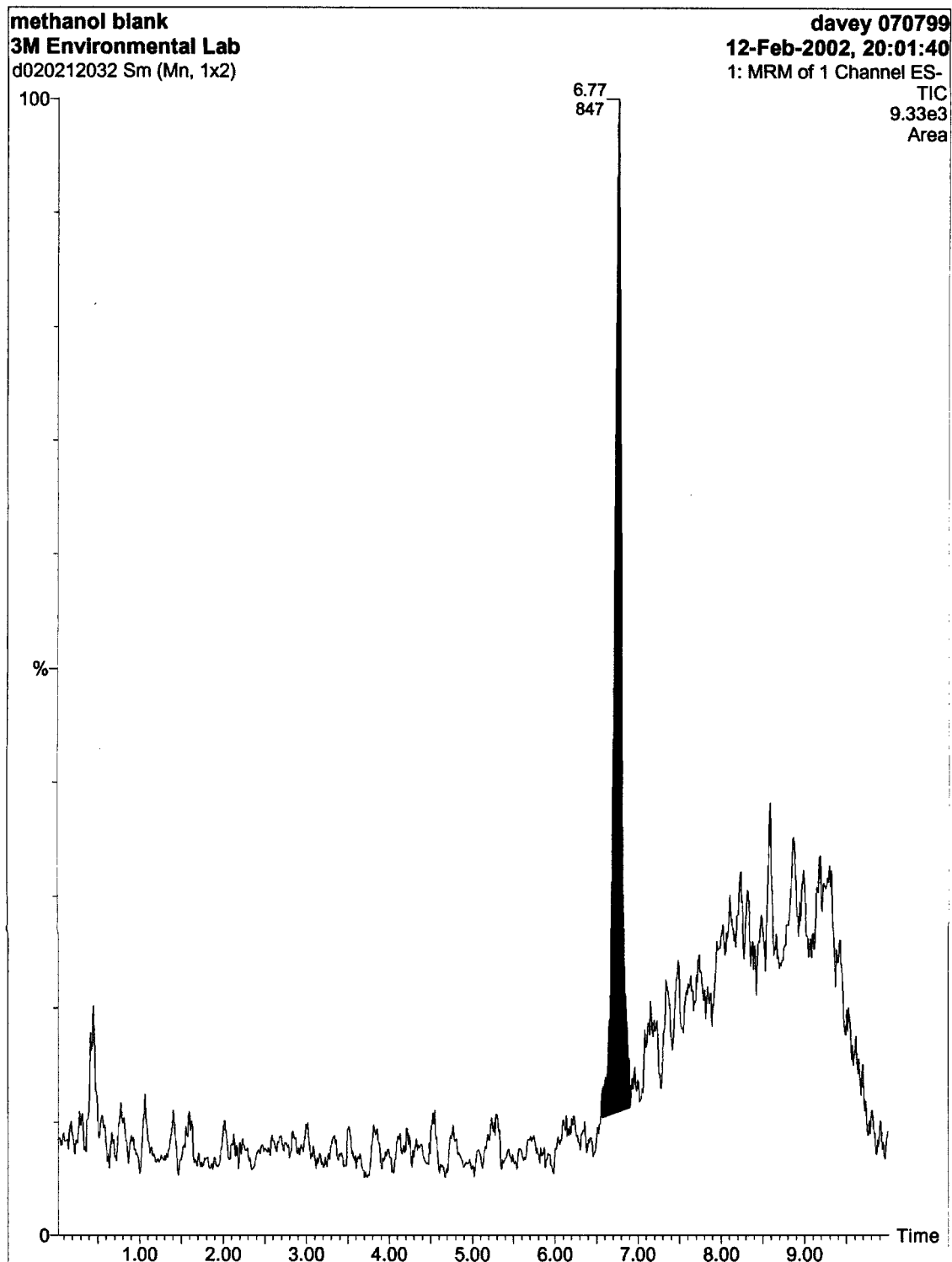
\* Concentration &lt;LOQ

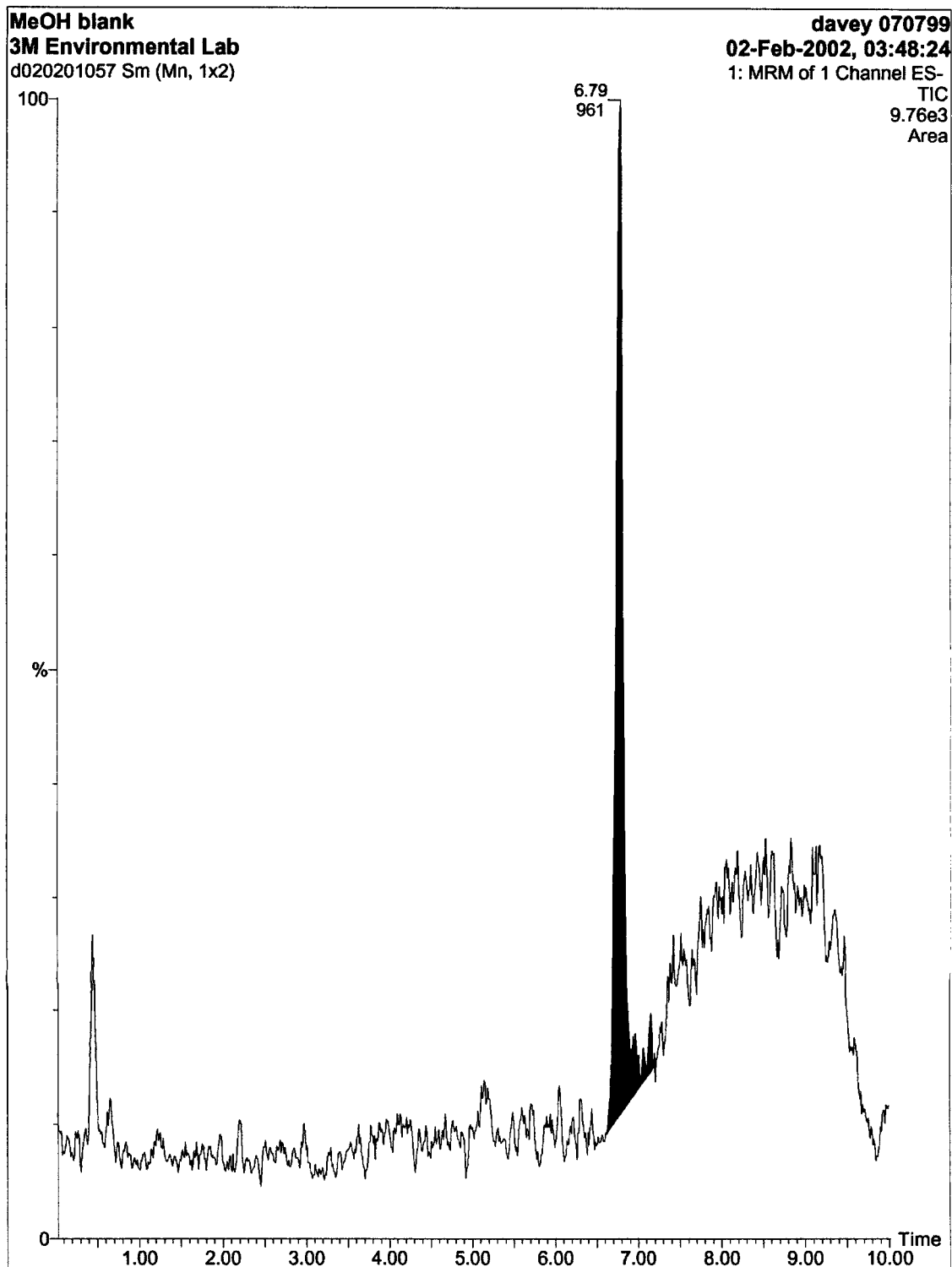
Conc of PFOS in Sample (ng/g) = (Inst conc(ng/mL)) x (additional dilution) x (final volume (mL))/ amount of liver in diluted sample (g)

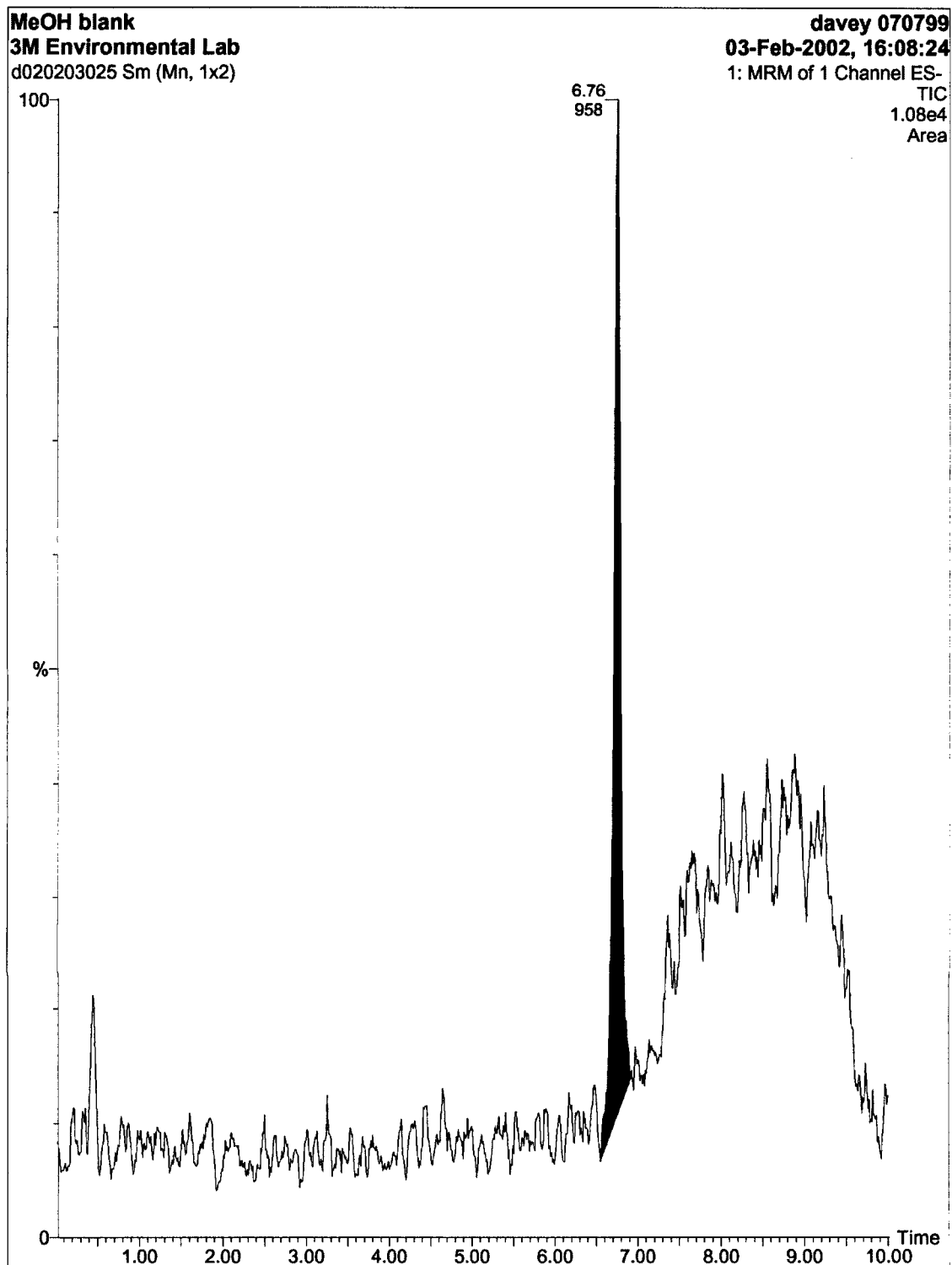
amount of liver in diluted sample (g) = amount of liver weighed (g)/ (water added for homogenizing (mL=g)) + amount of liver weighed(g))

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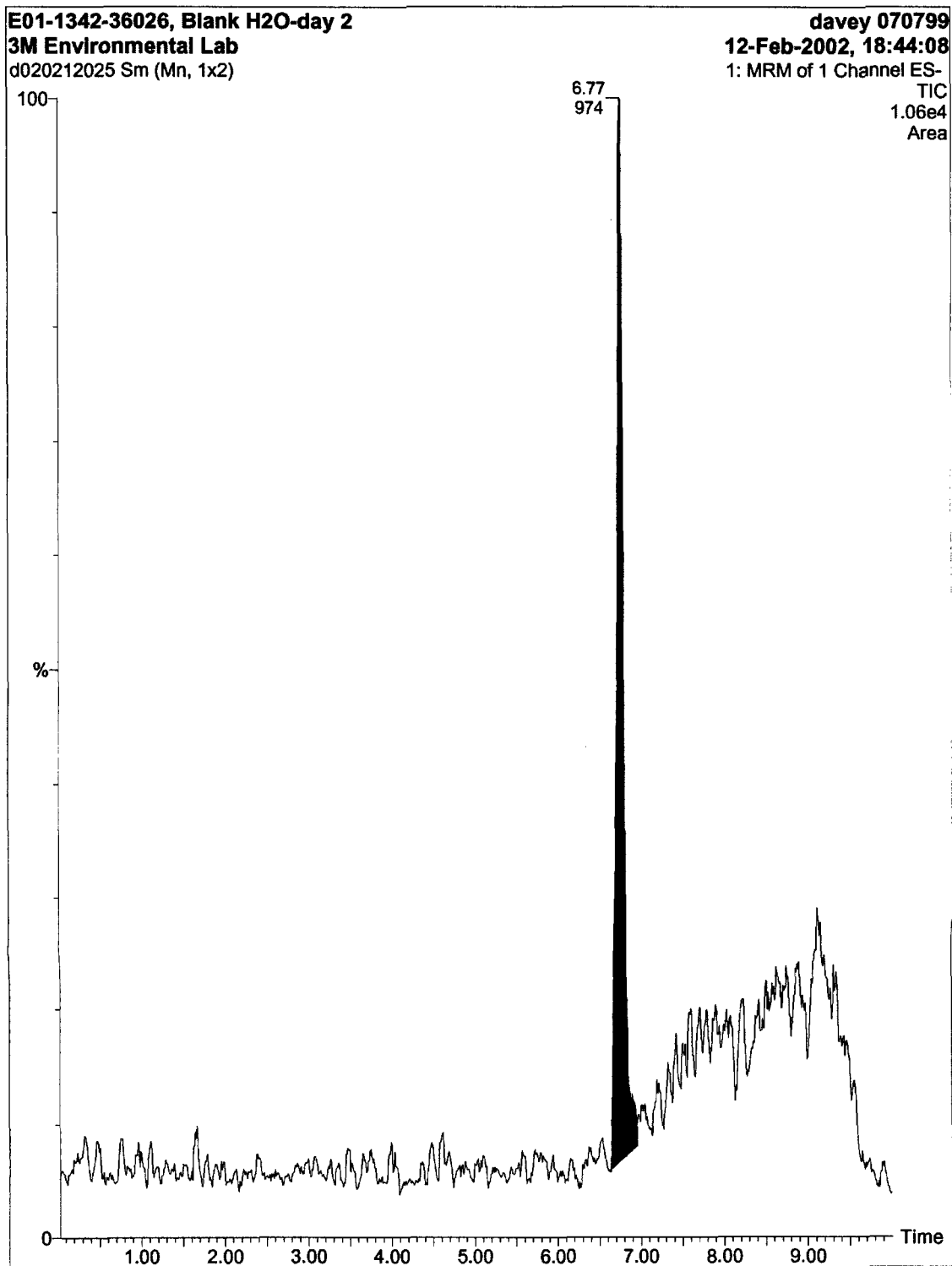
**ATTACHMENT C: SAMPLE CHROMATOGRAMS, CALIBRATION INFORMATION,  
AND INSTRUMENT INFORMATION**

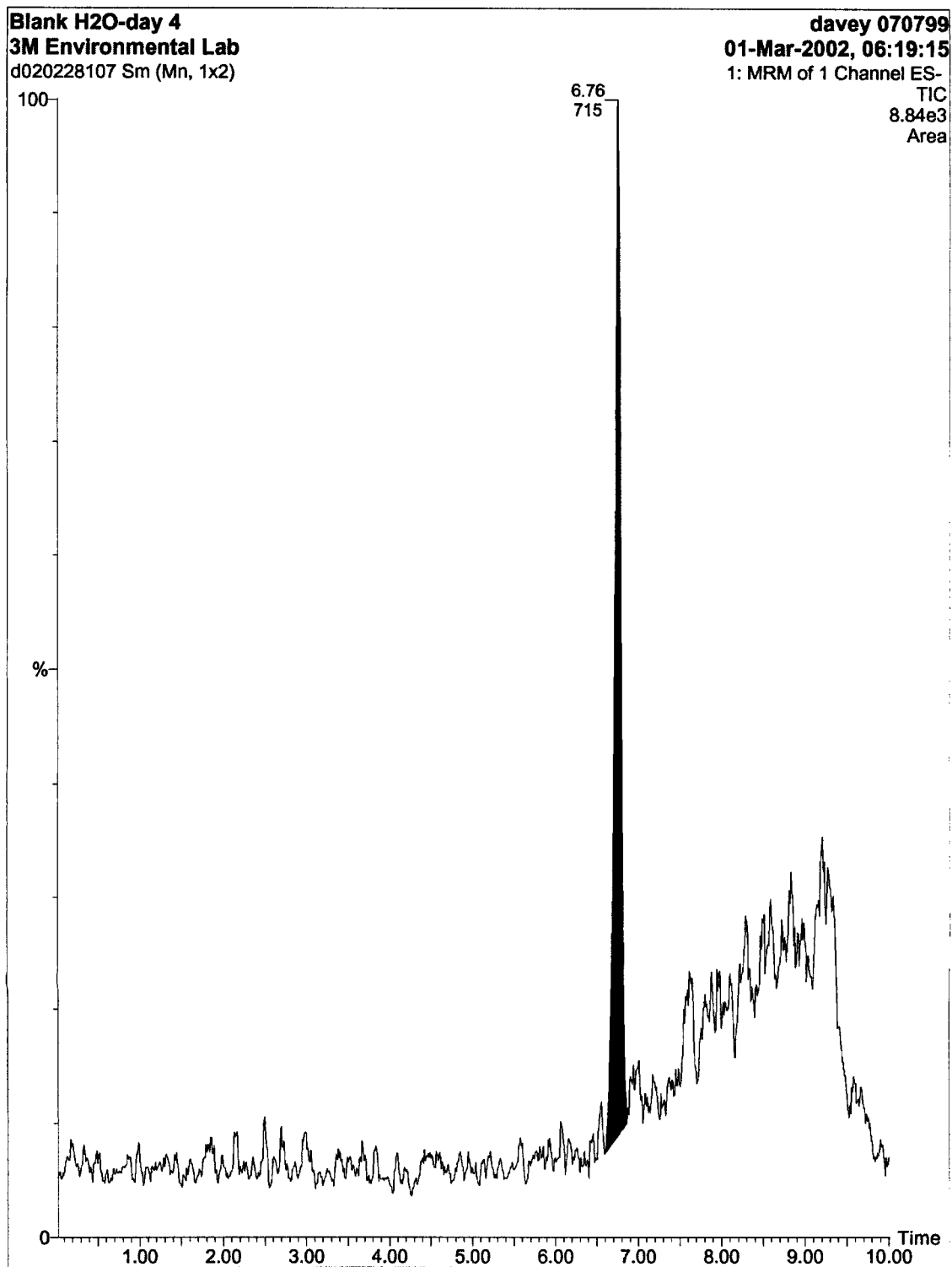
**Methanol Solvent Blanks**

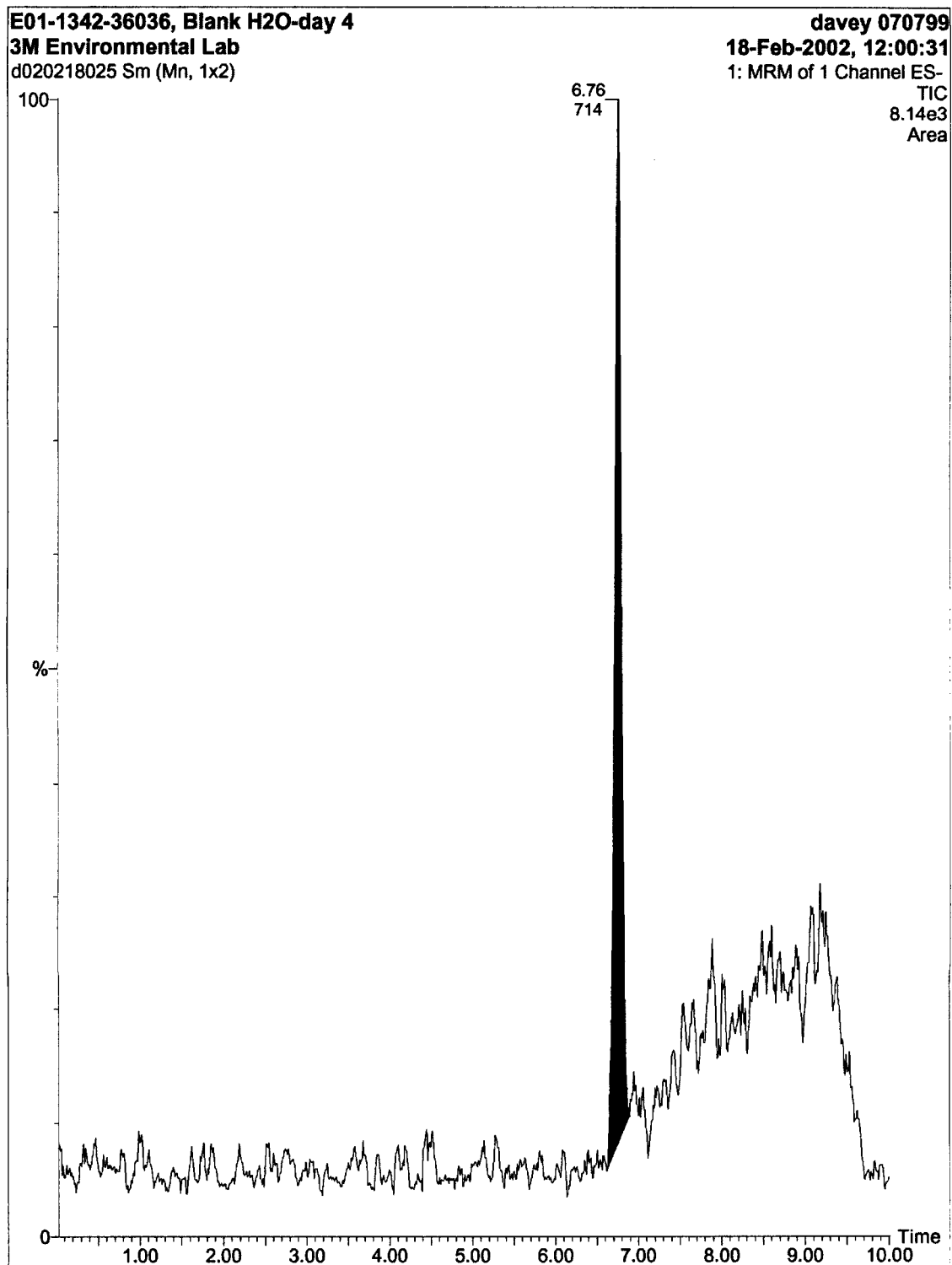




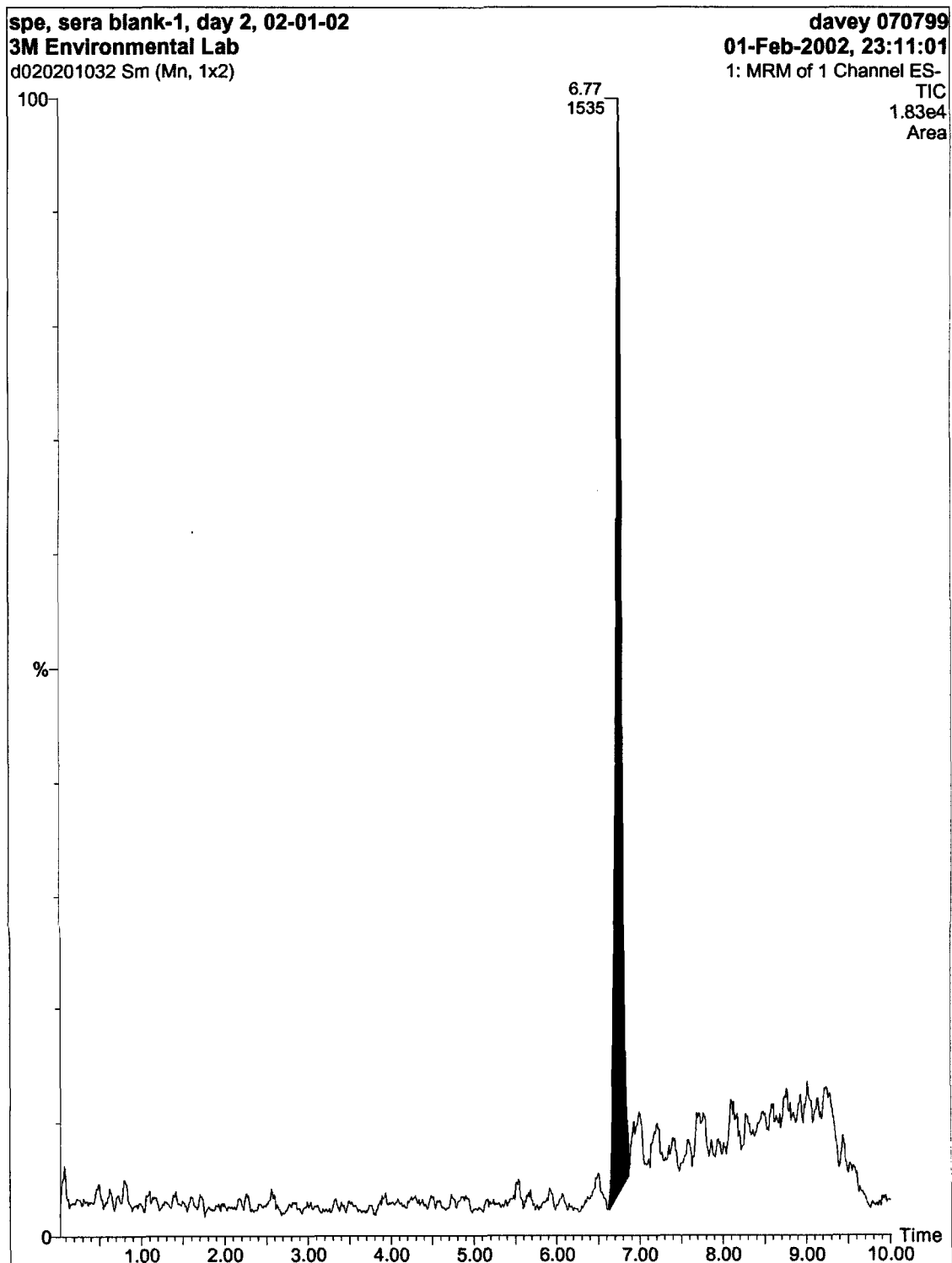
### Water Blank Samples





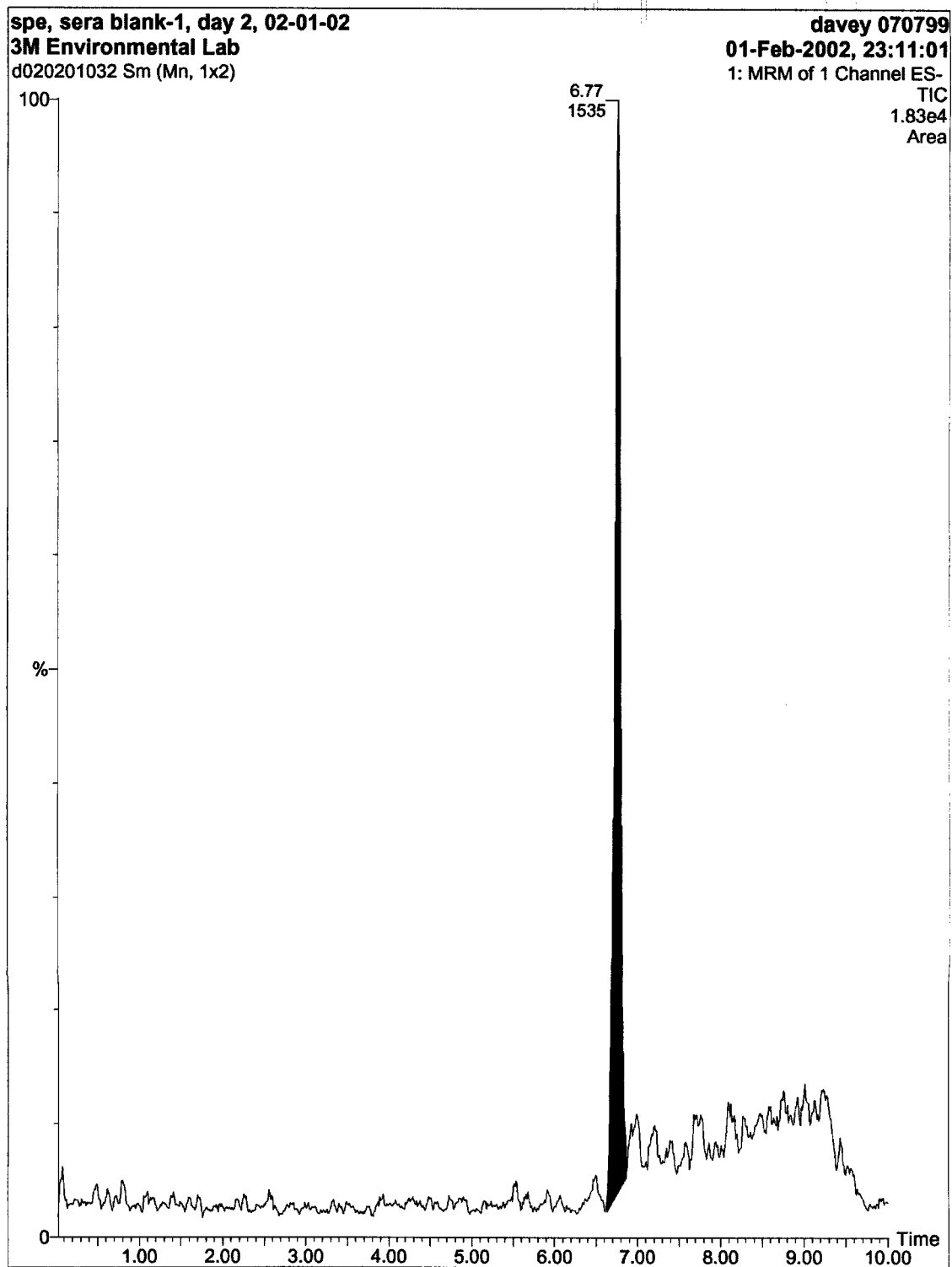


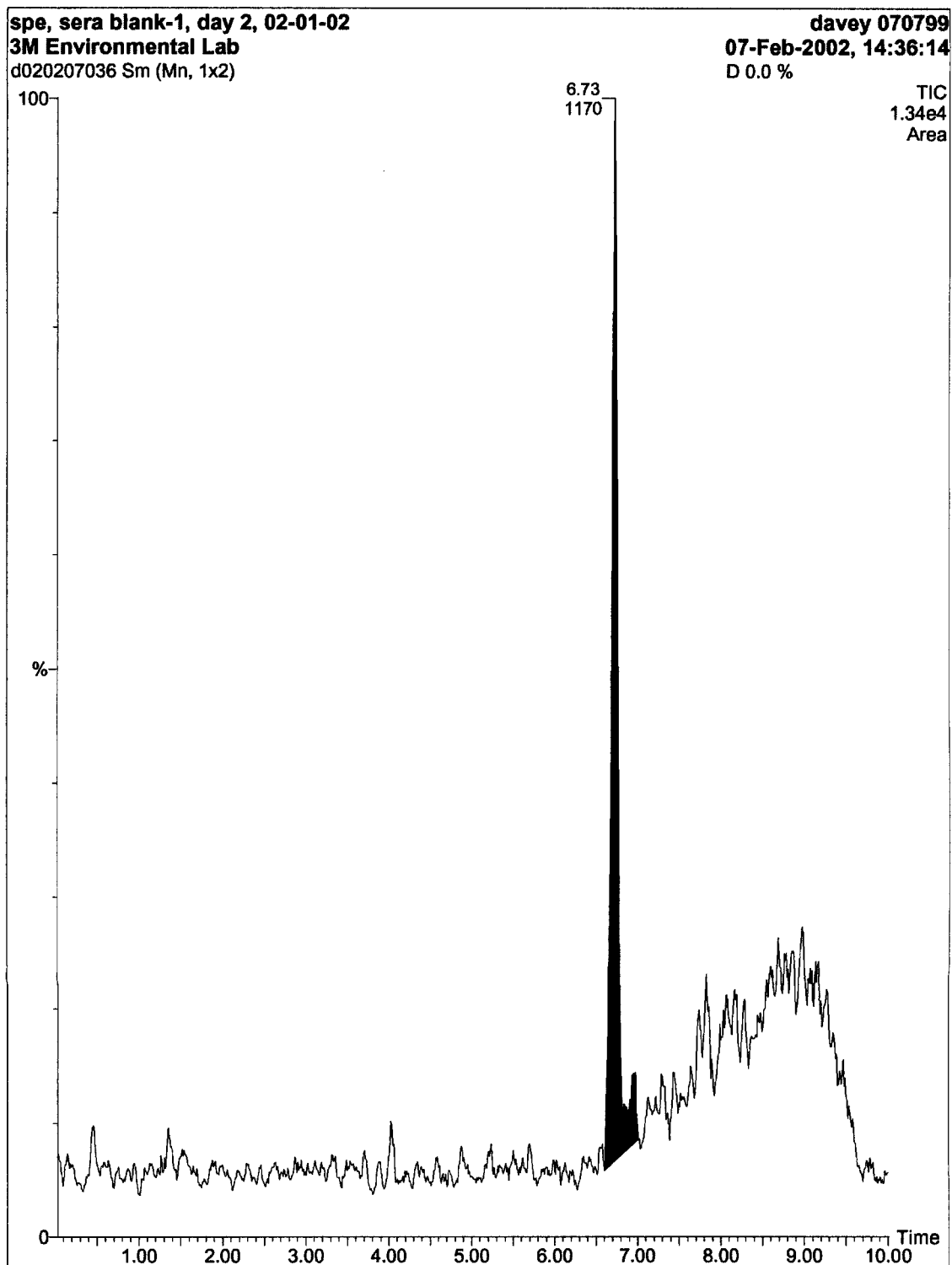
**Sera Blank Samples**



Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite

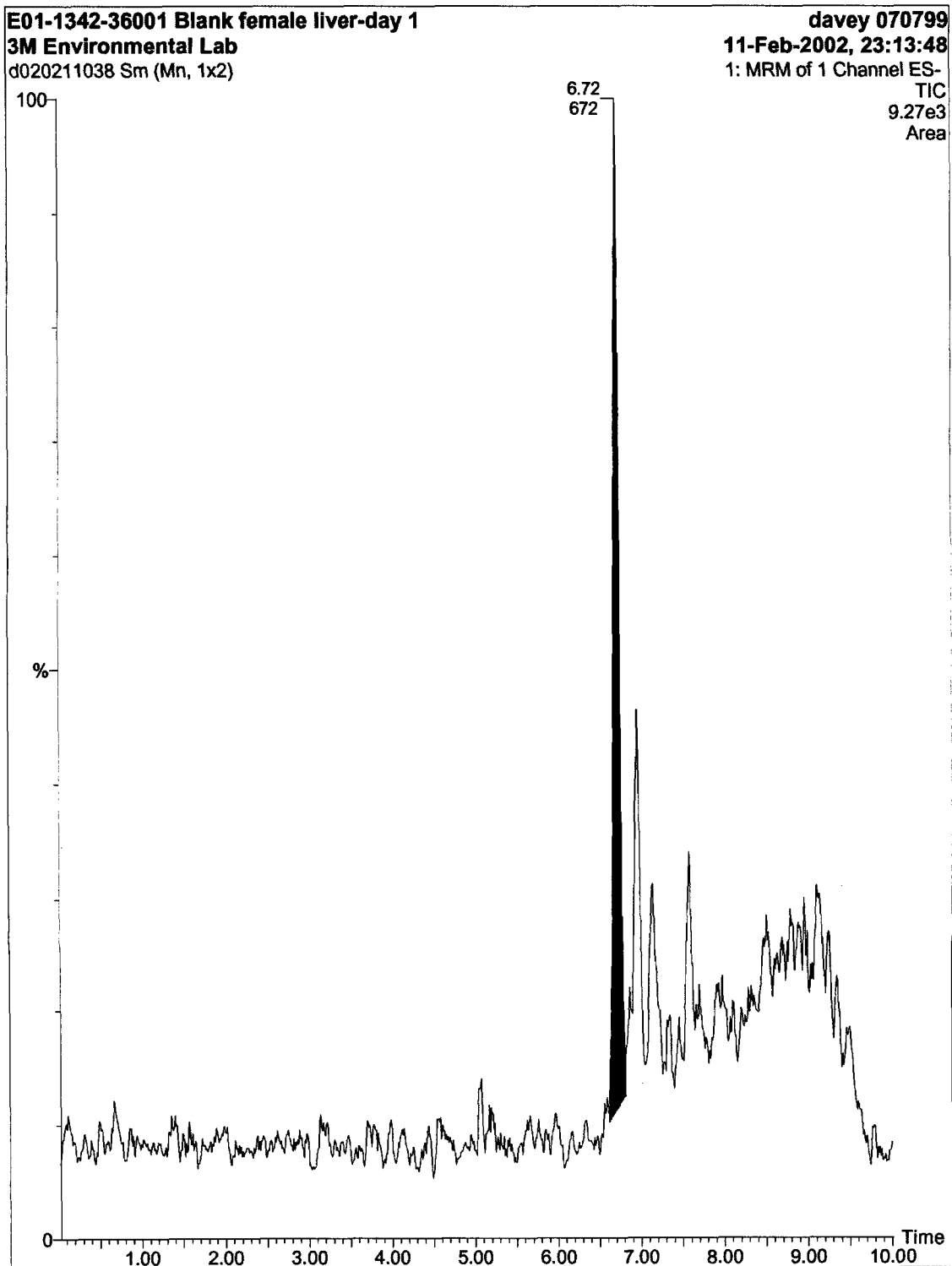




Report E01-1245

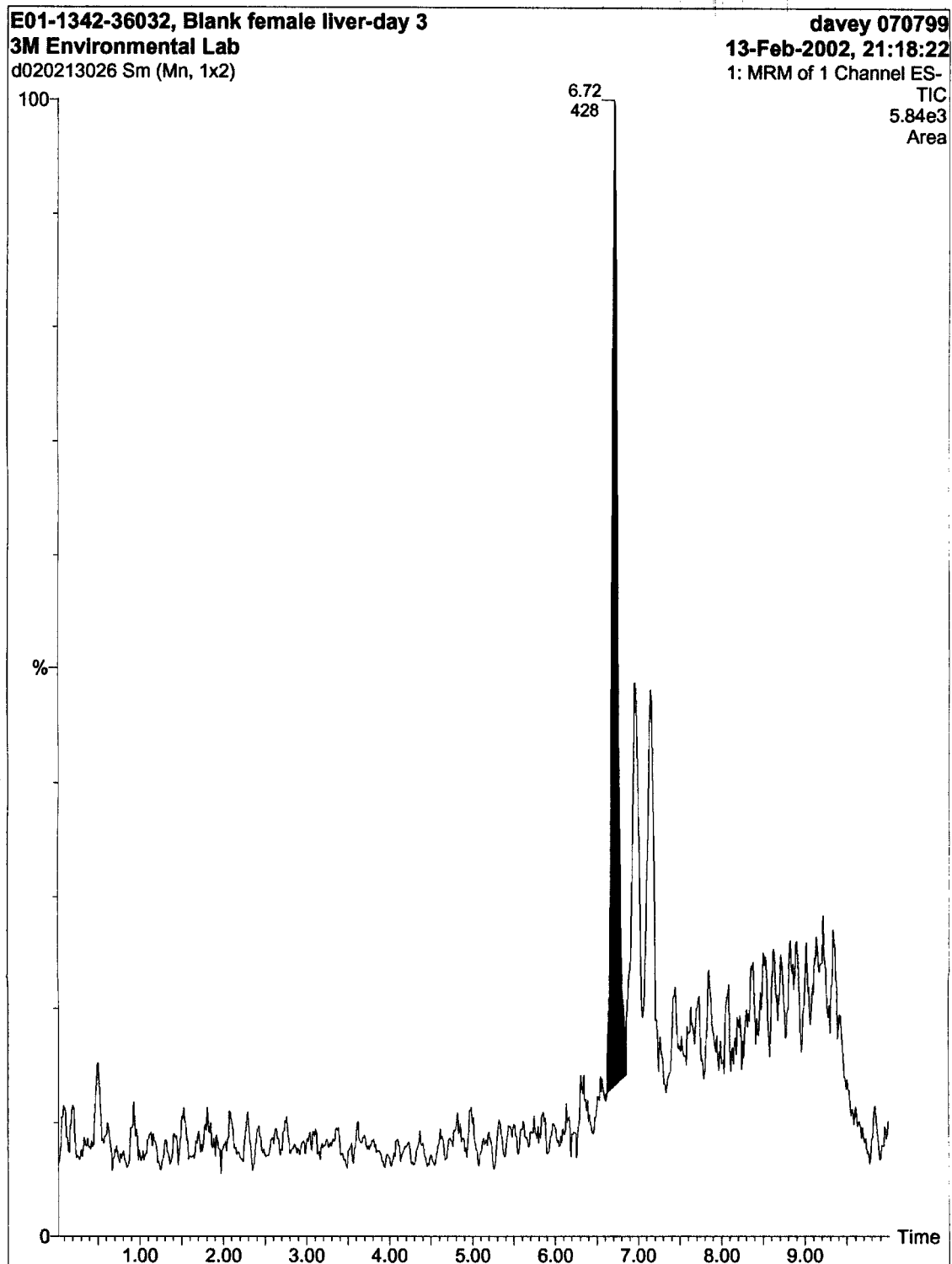
PFOS: A Reproduction Study with the Northern Bobwhite

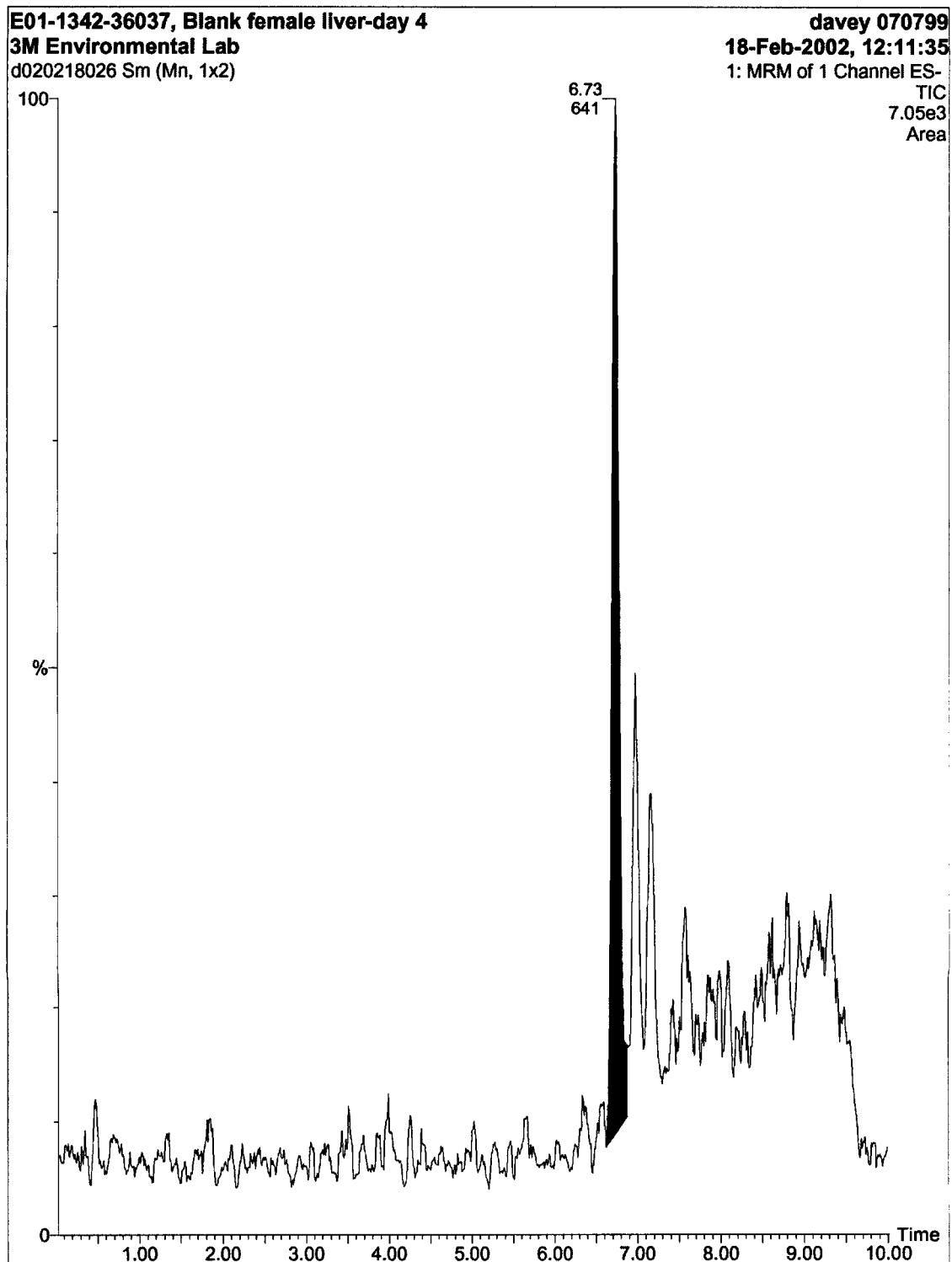
### Female Liver Blank Samples

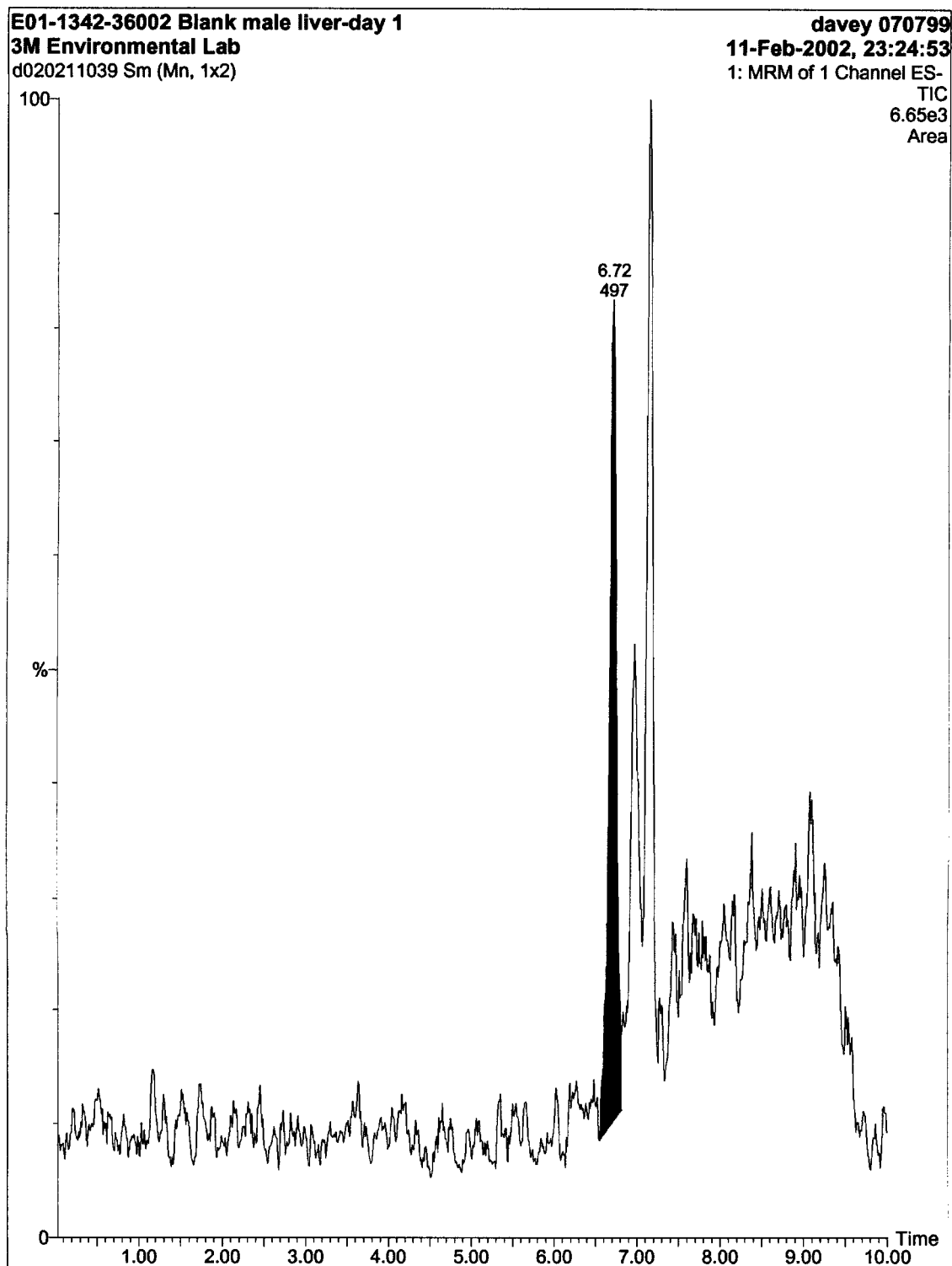


Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite

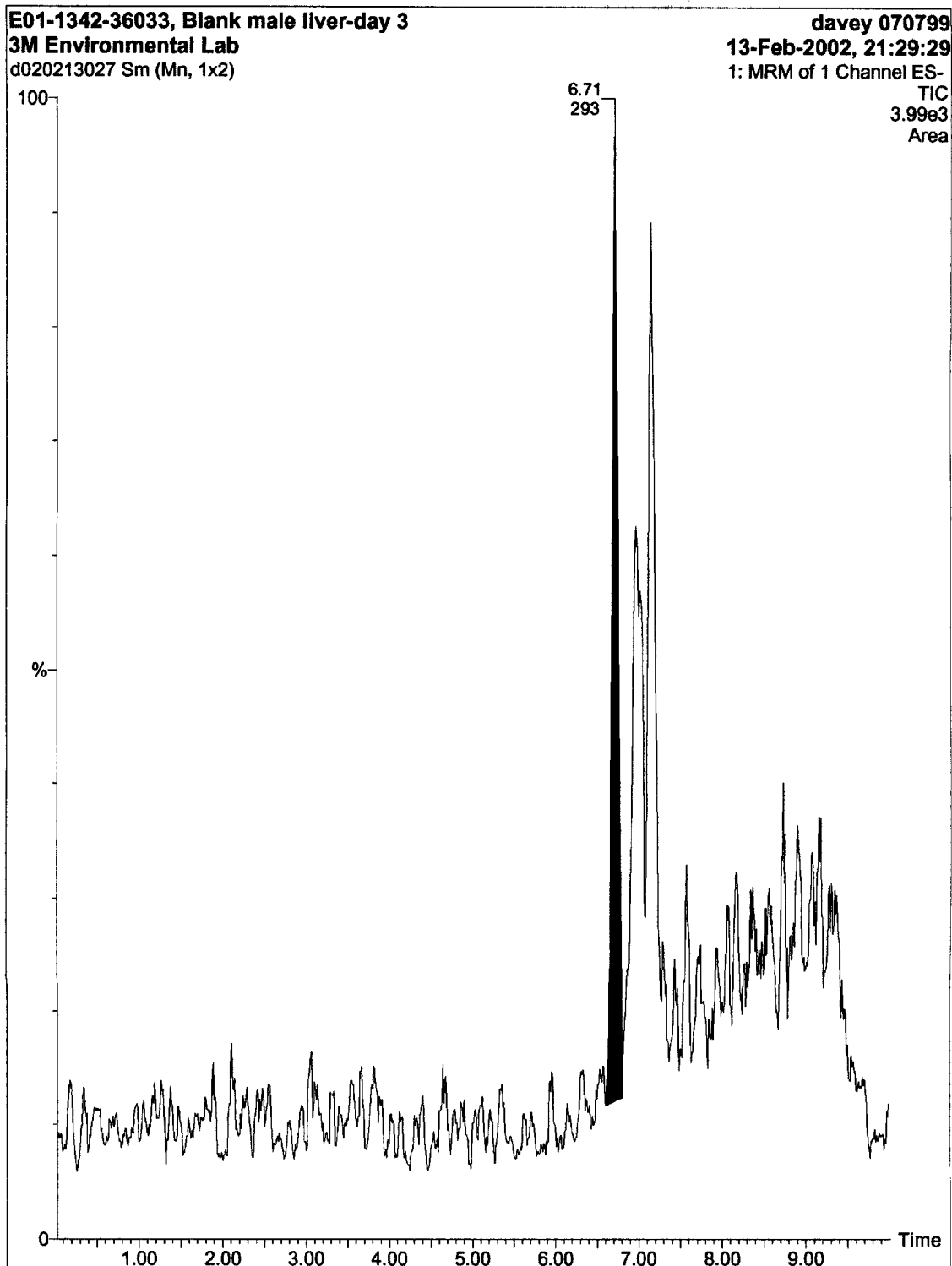


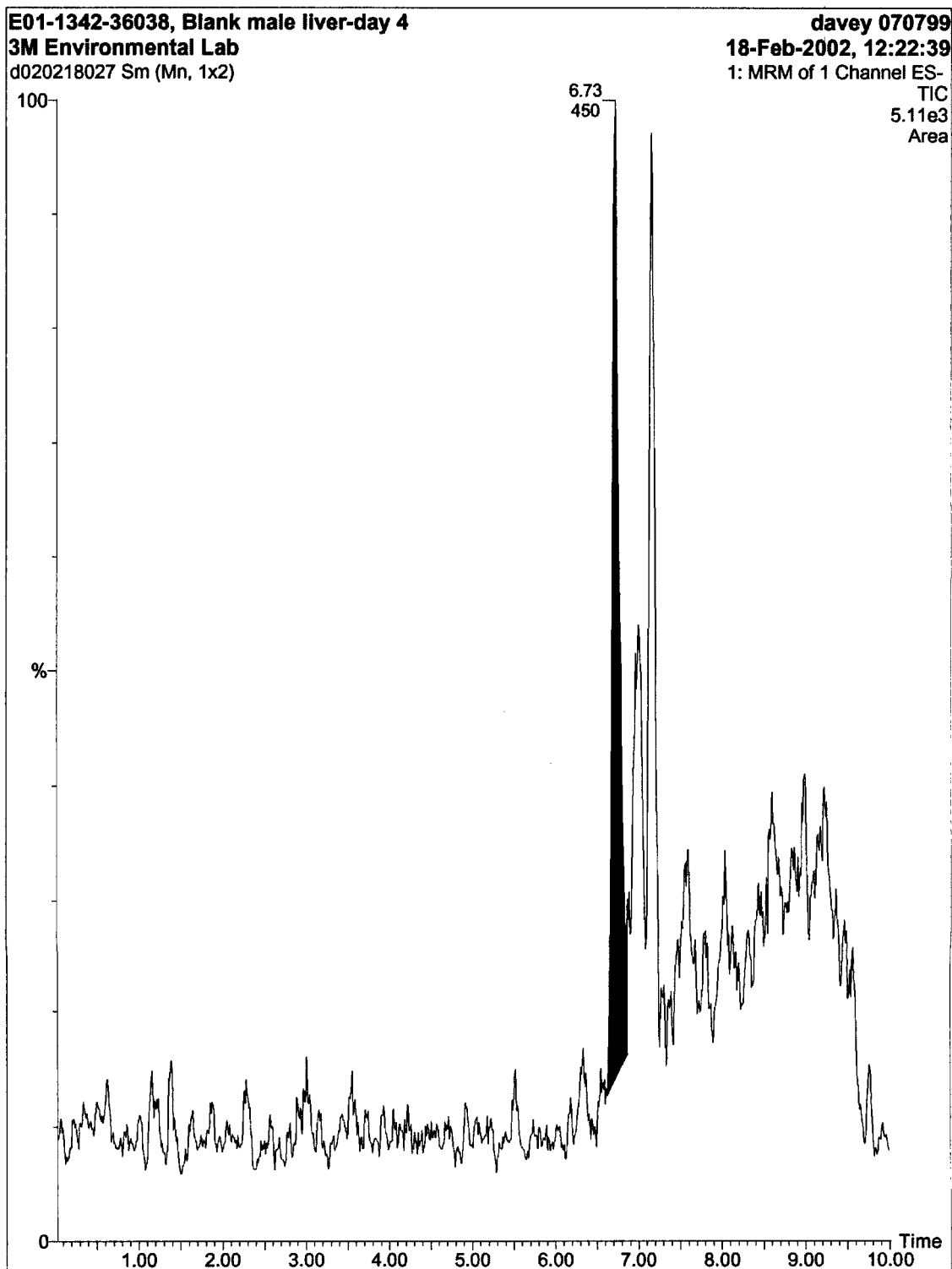


**Male Liver Blank Samples**

Report E01-1245

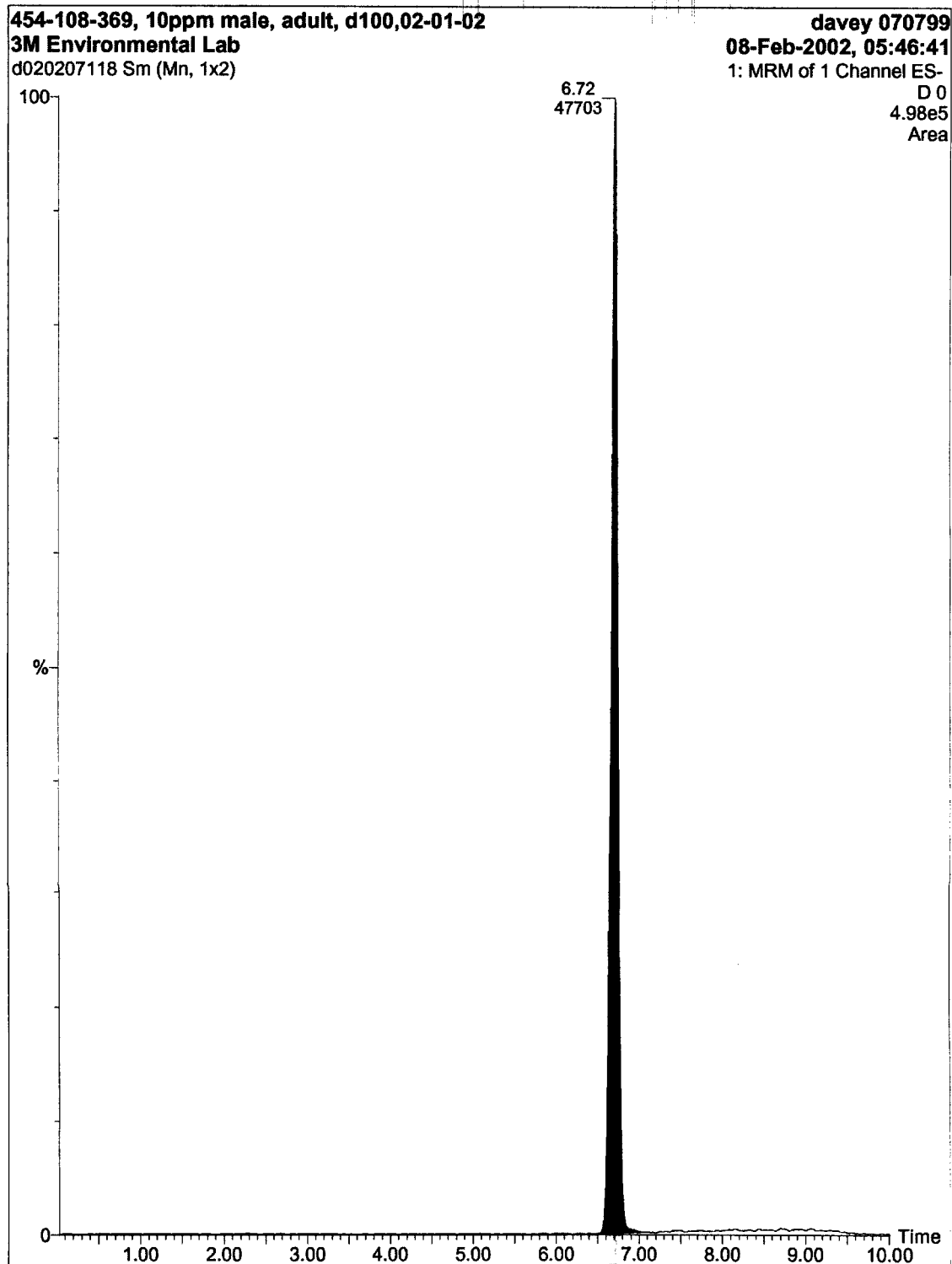
PFOS: A Reproduction Study with the Northern Bobwhite

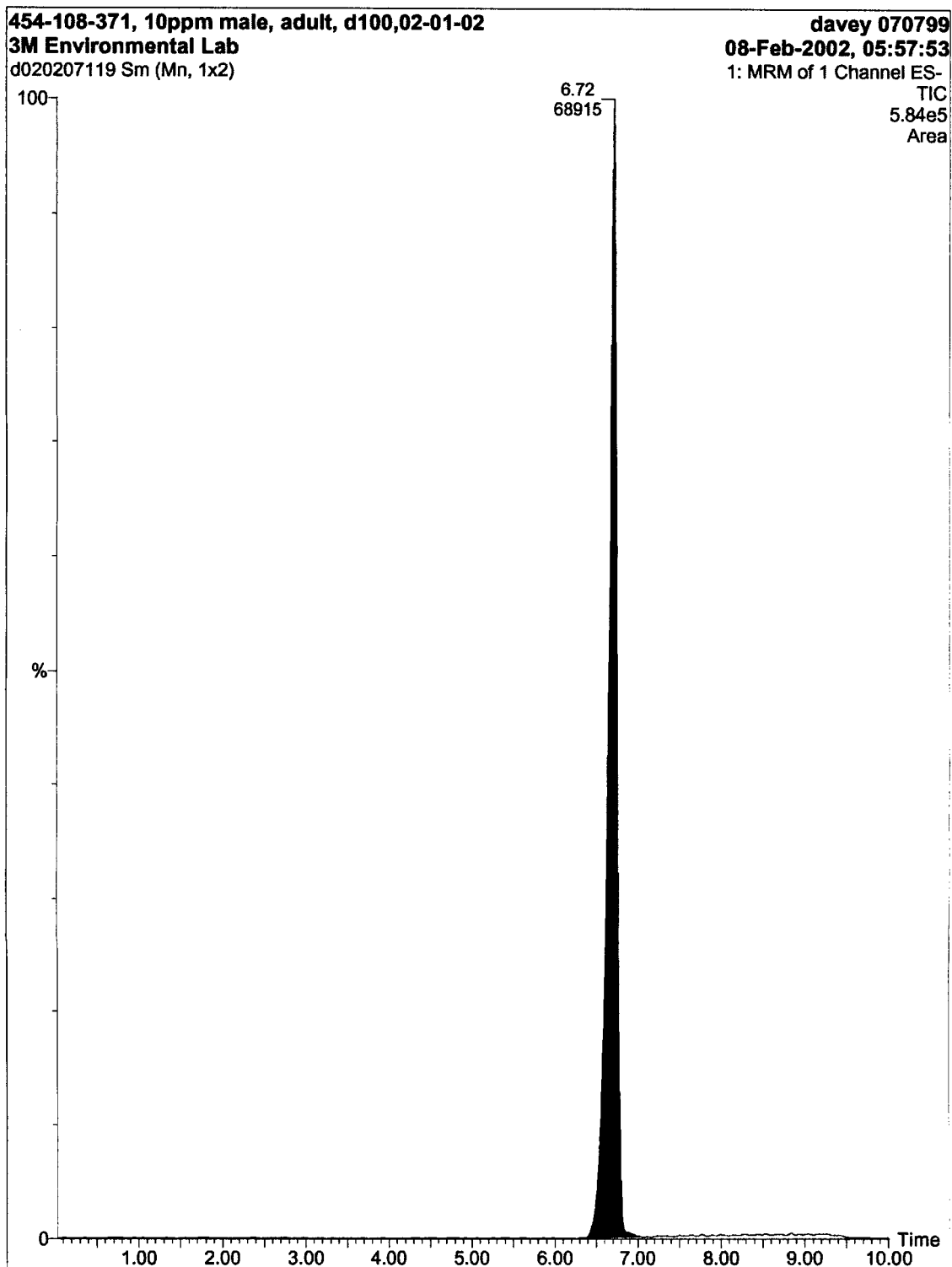


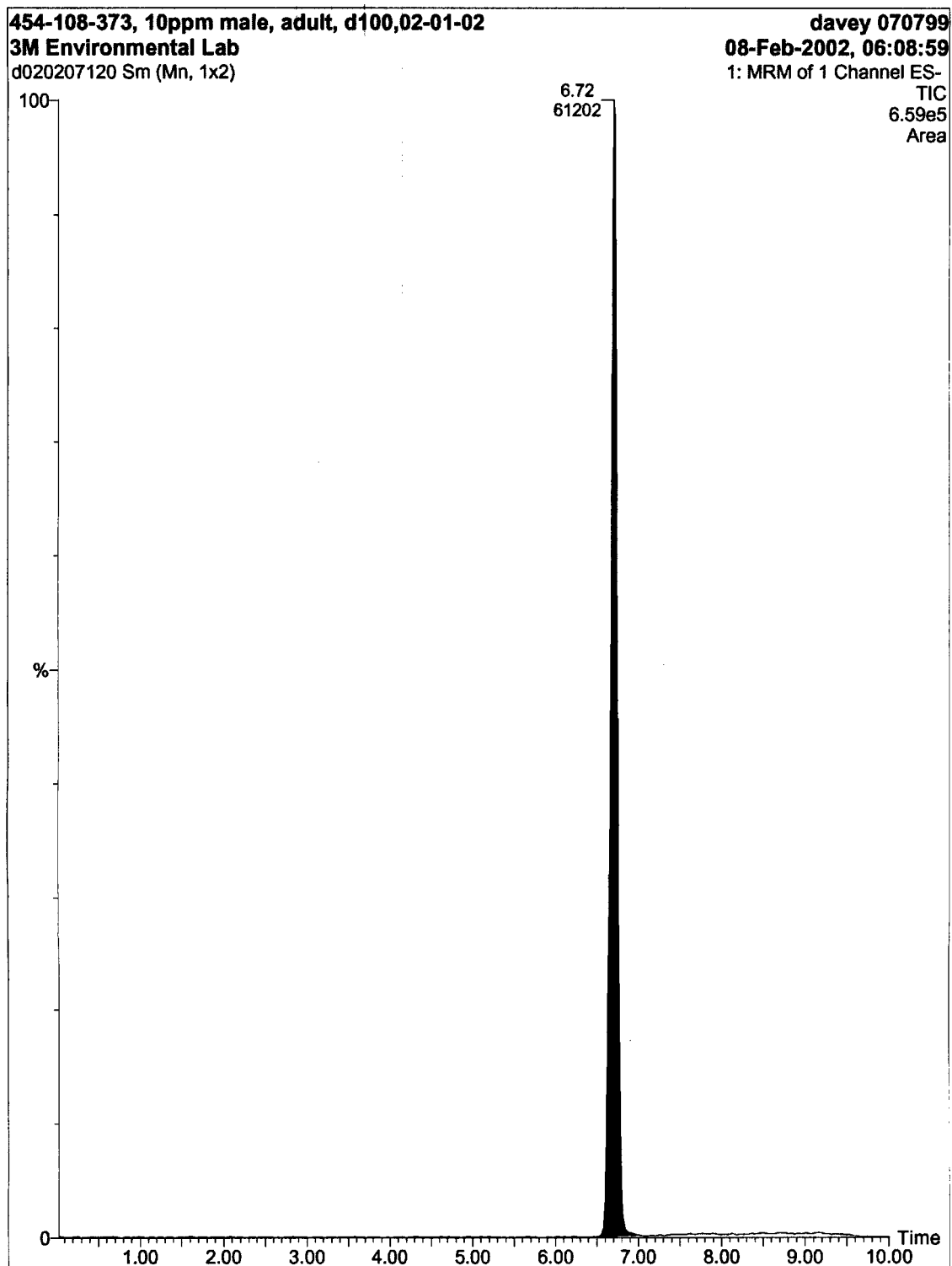


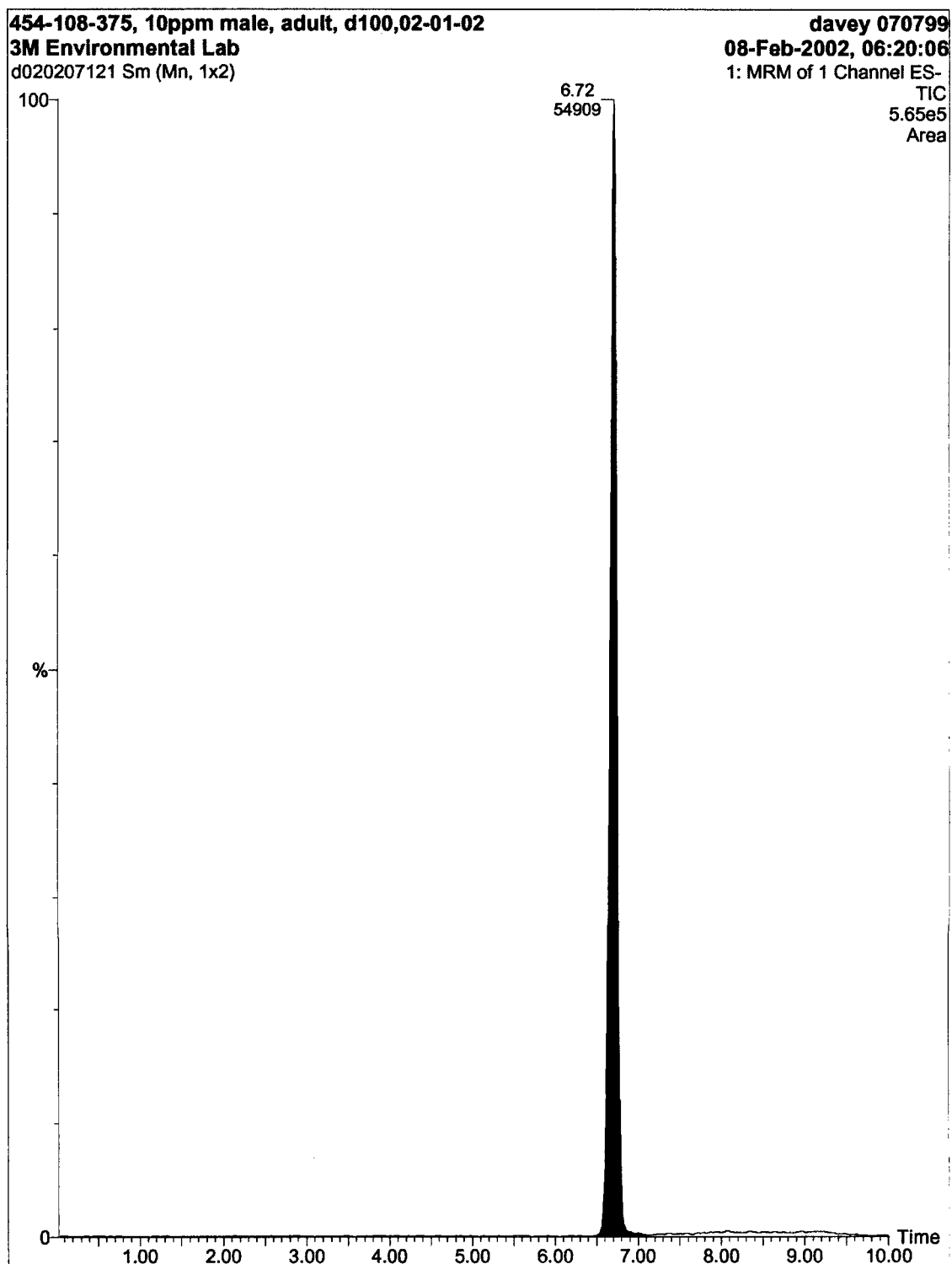
Report E01-1245

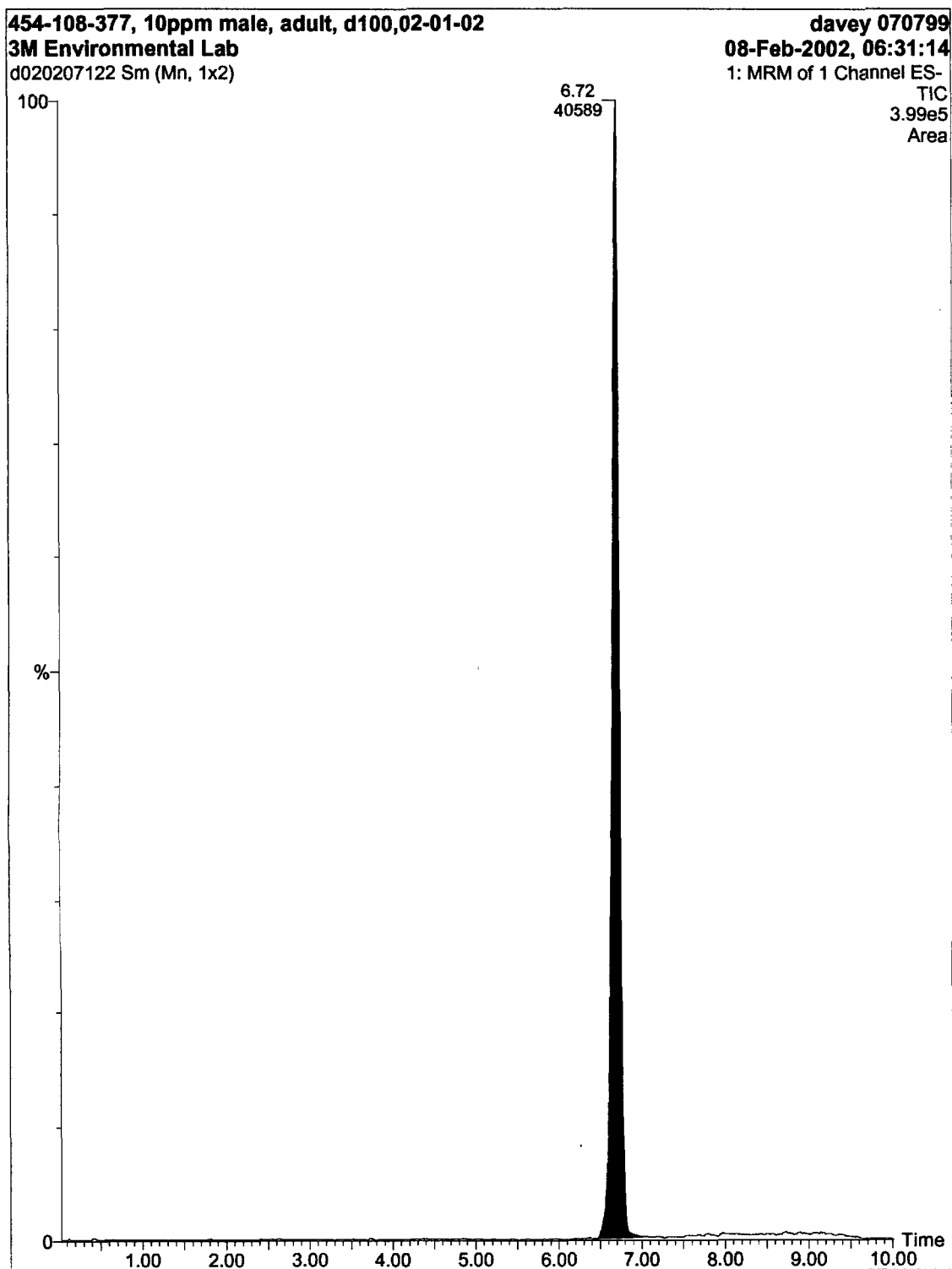
PFOS: A Reproduction Study with the Northern Bobwhite

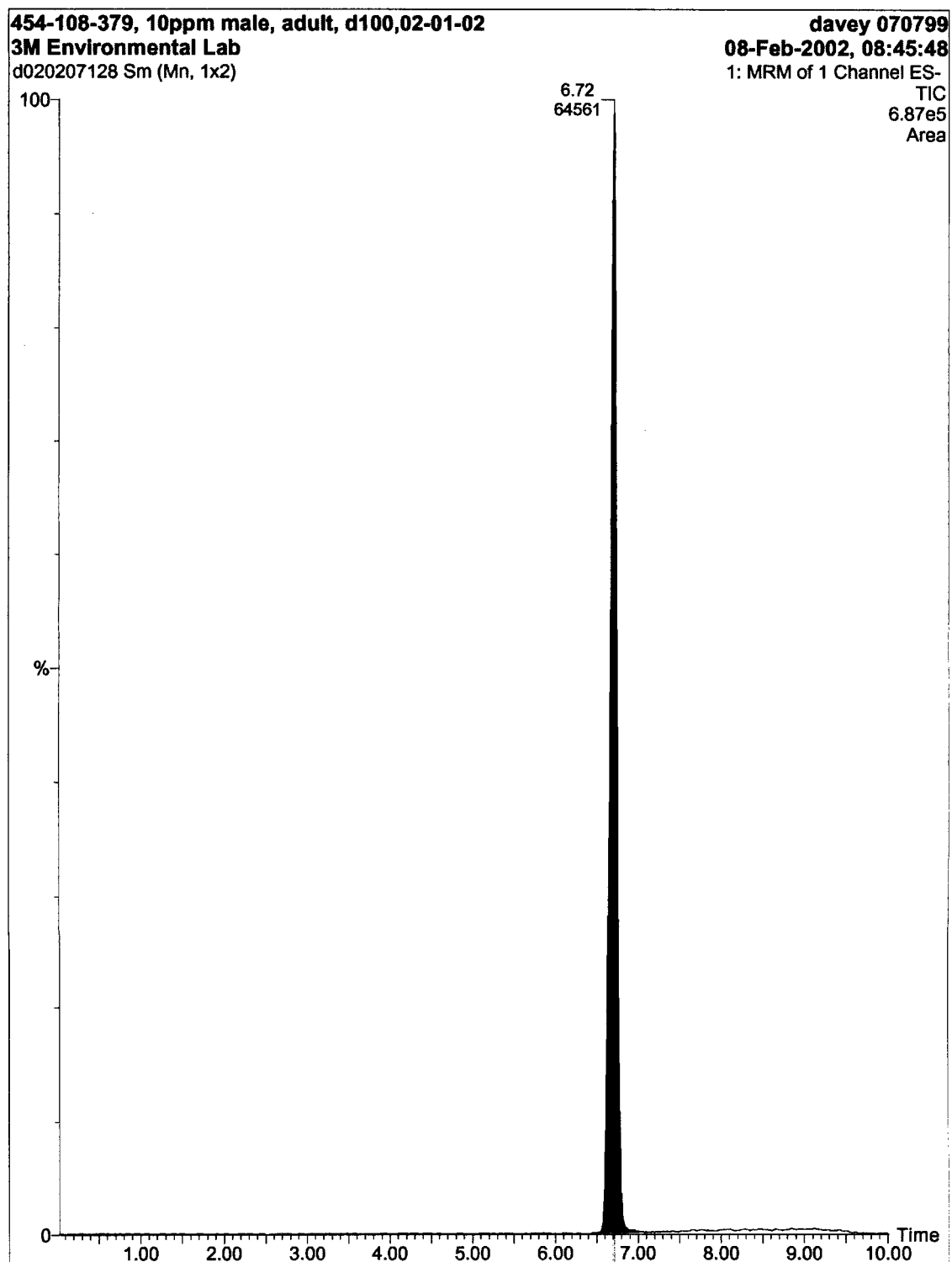
**Dosed Group Adult Male Sera Samples, Diluted 1:10**





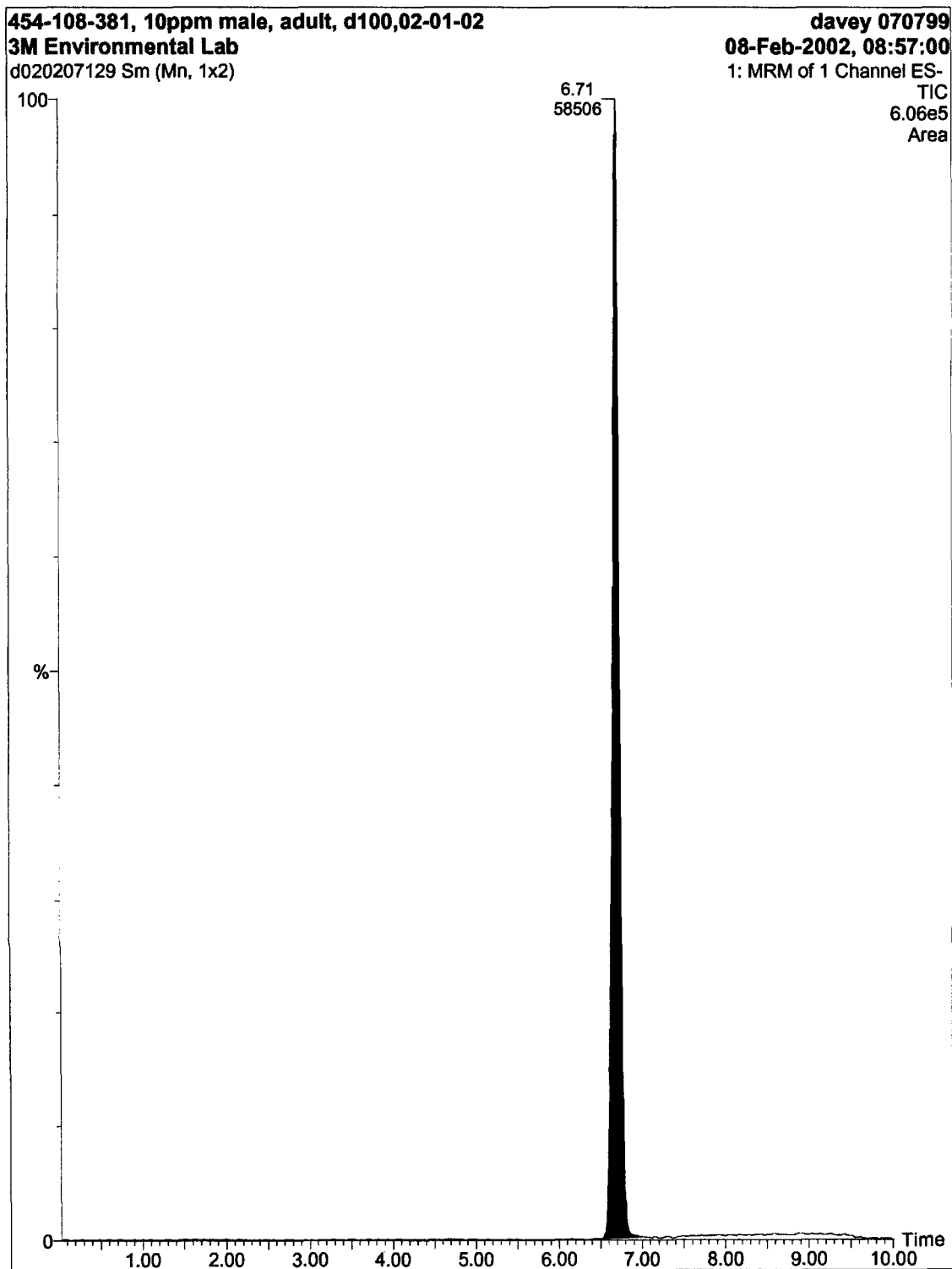


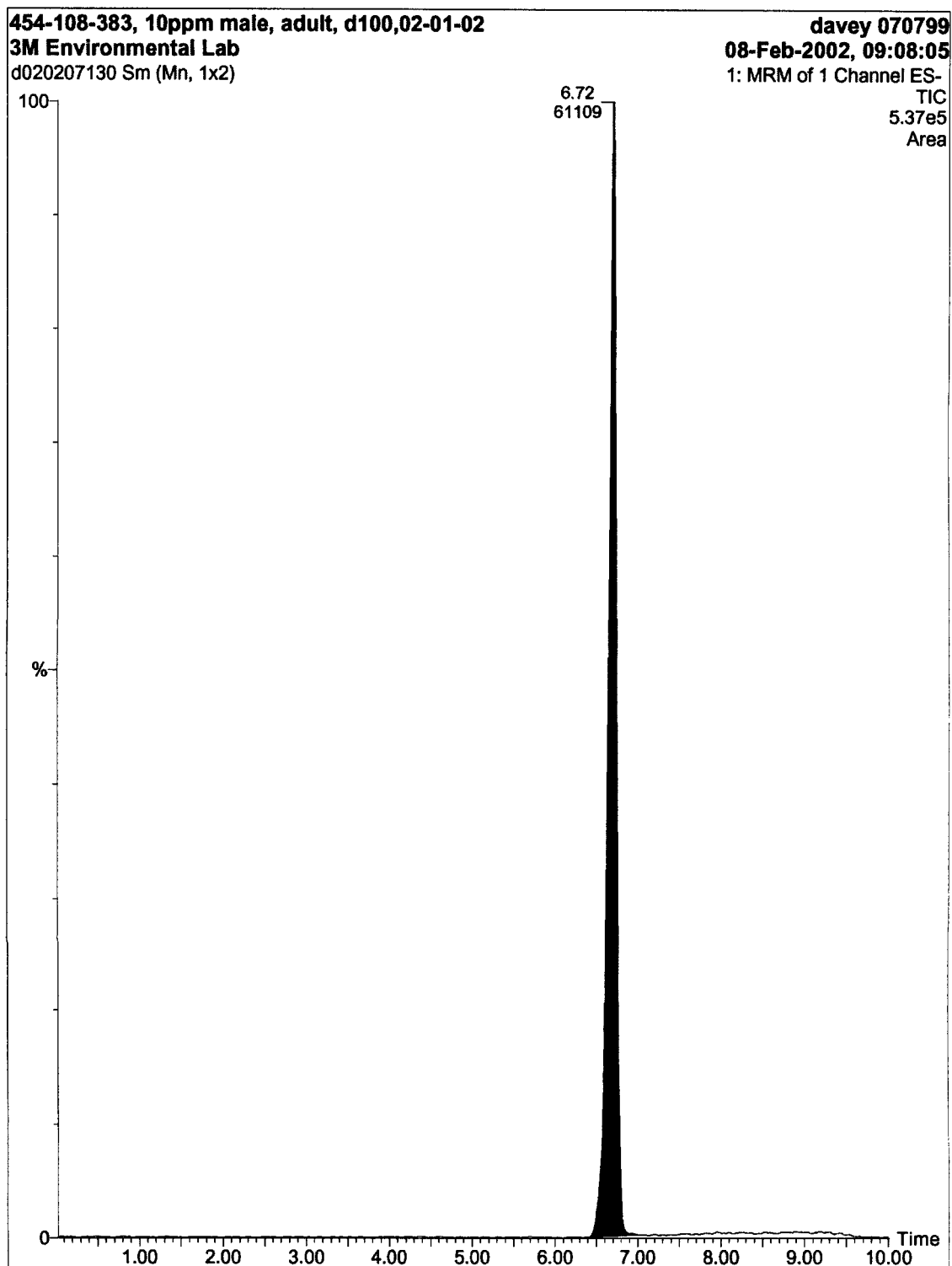


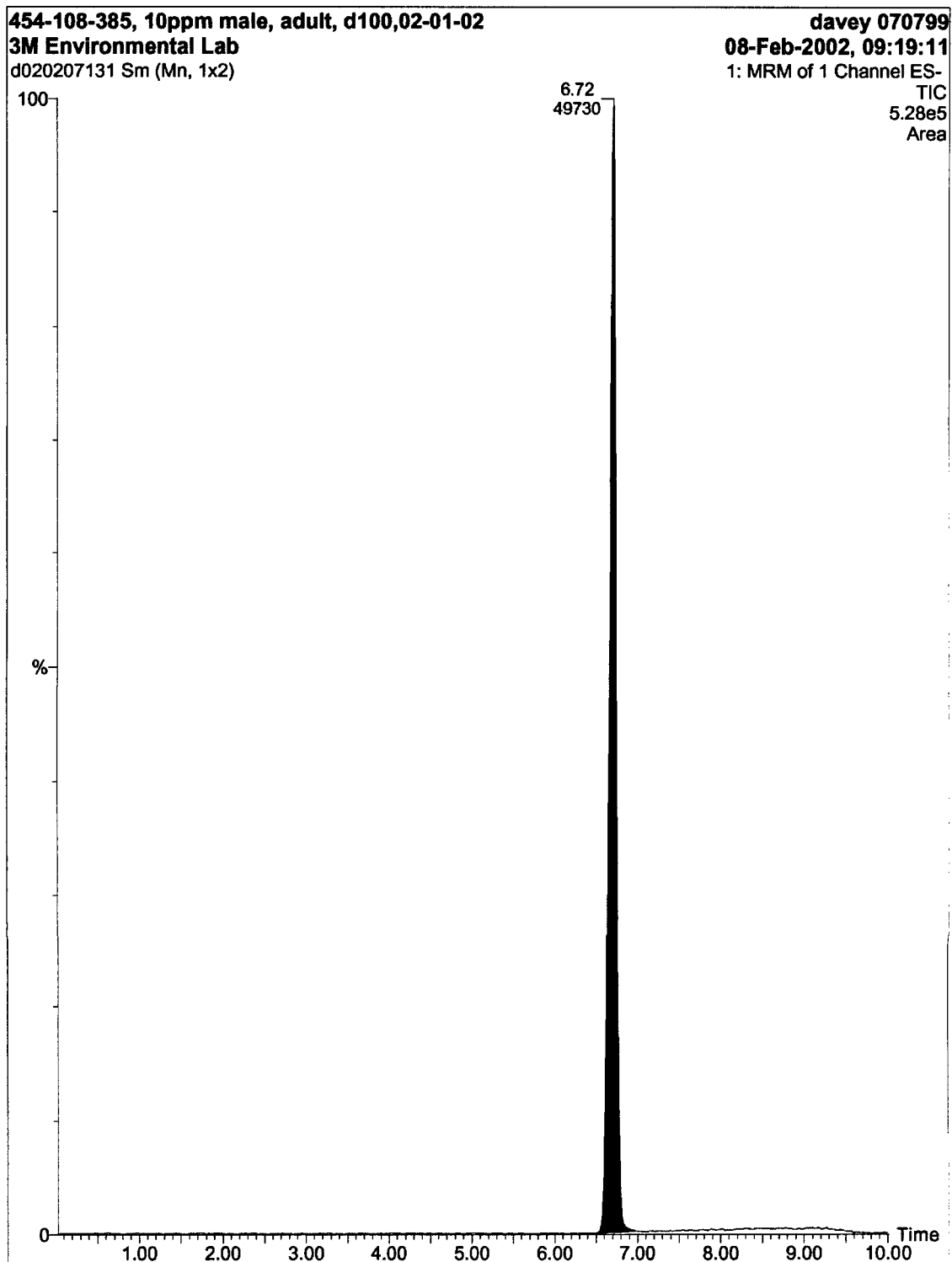


Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite

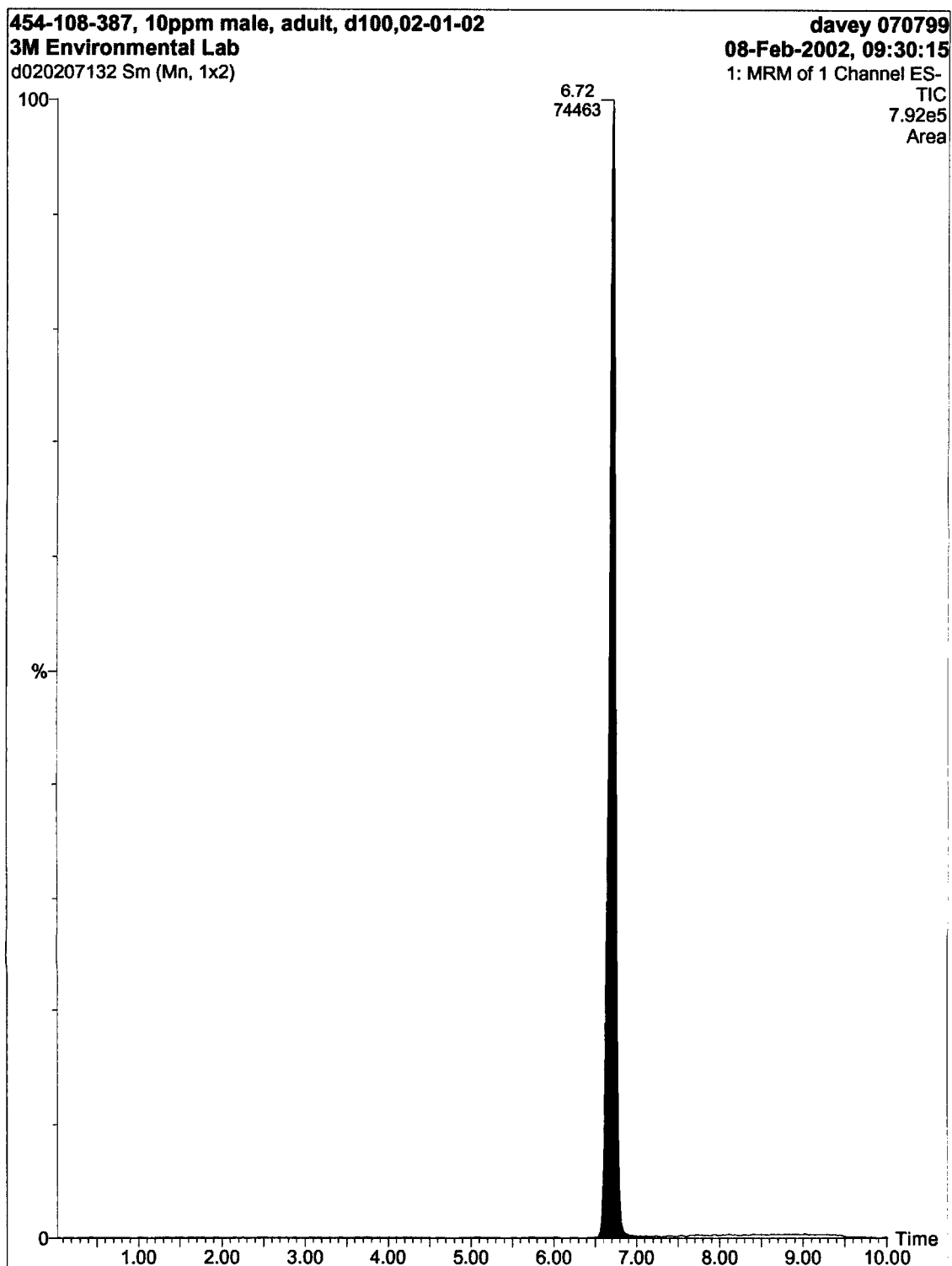






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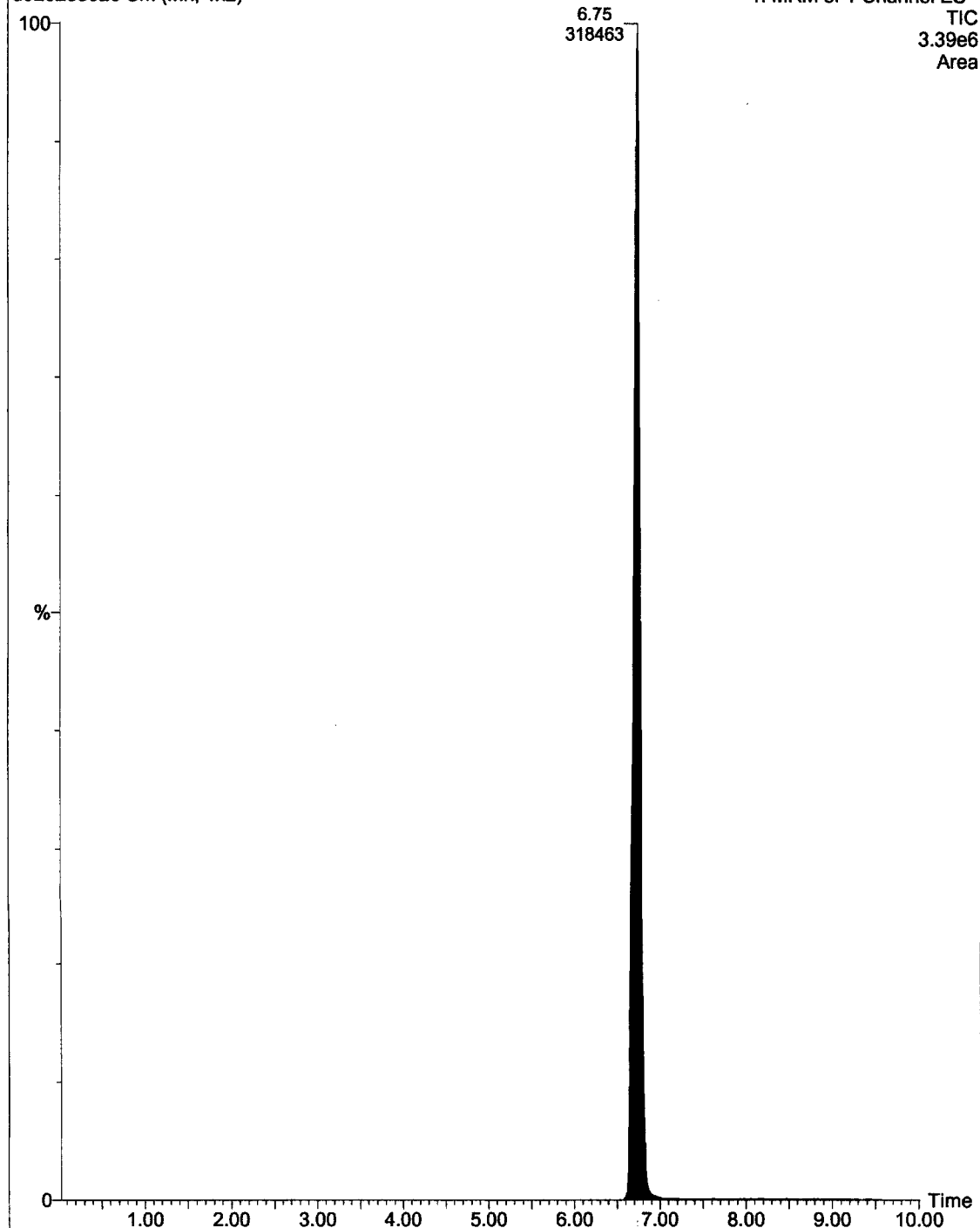
PFOS: A Reproduction Study with the Northern Bobwhite

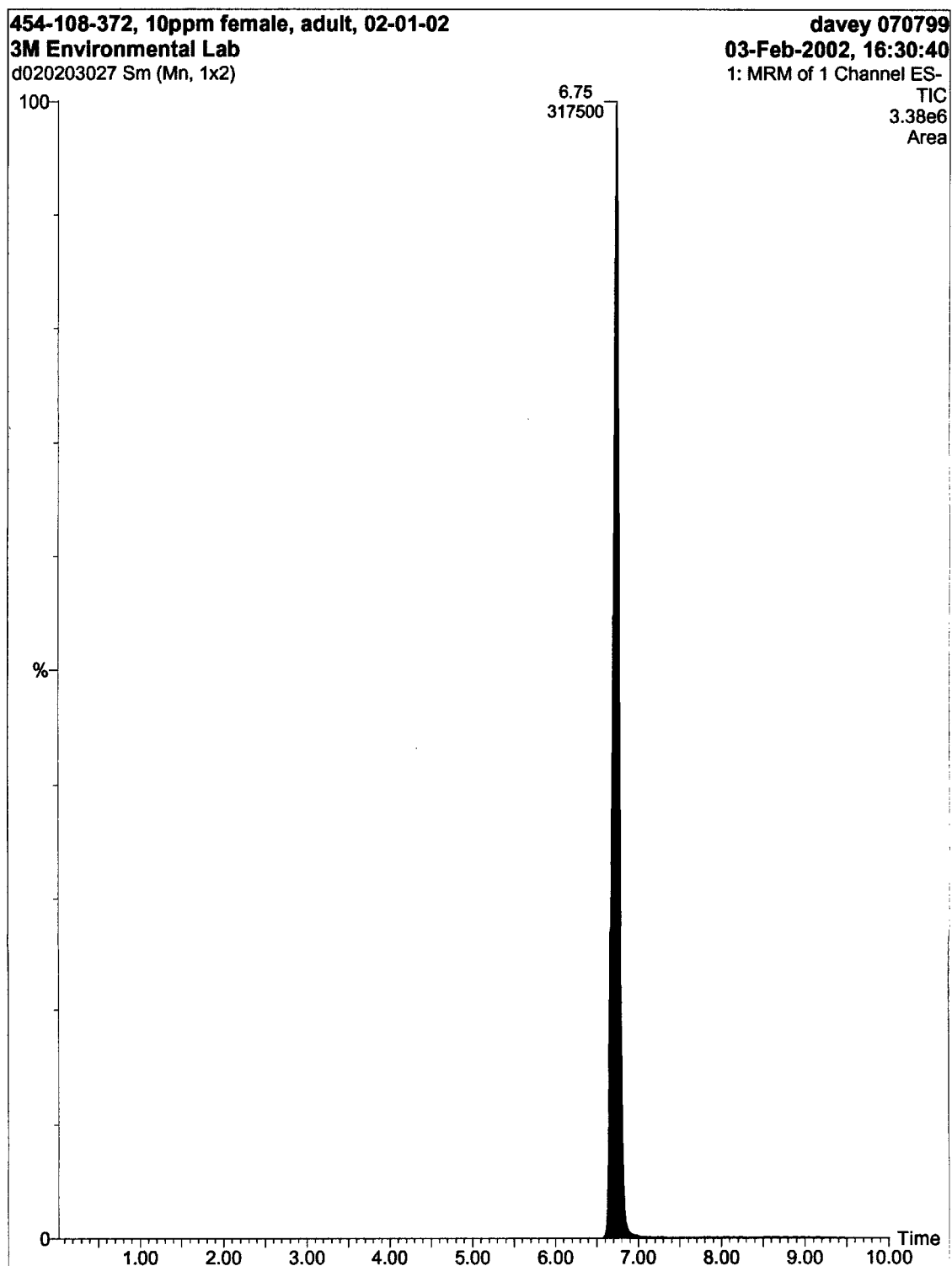


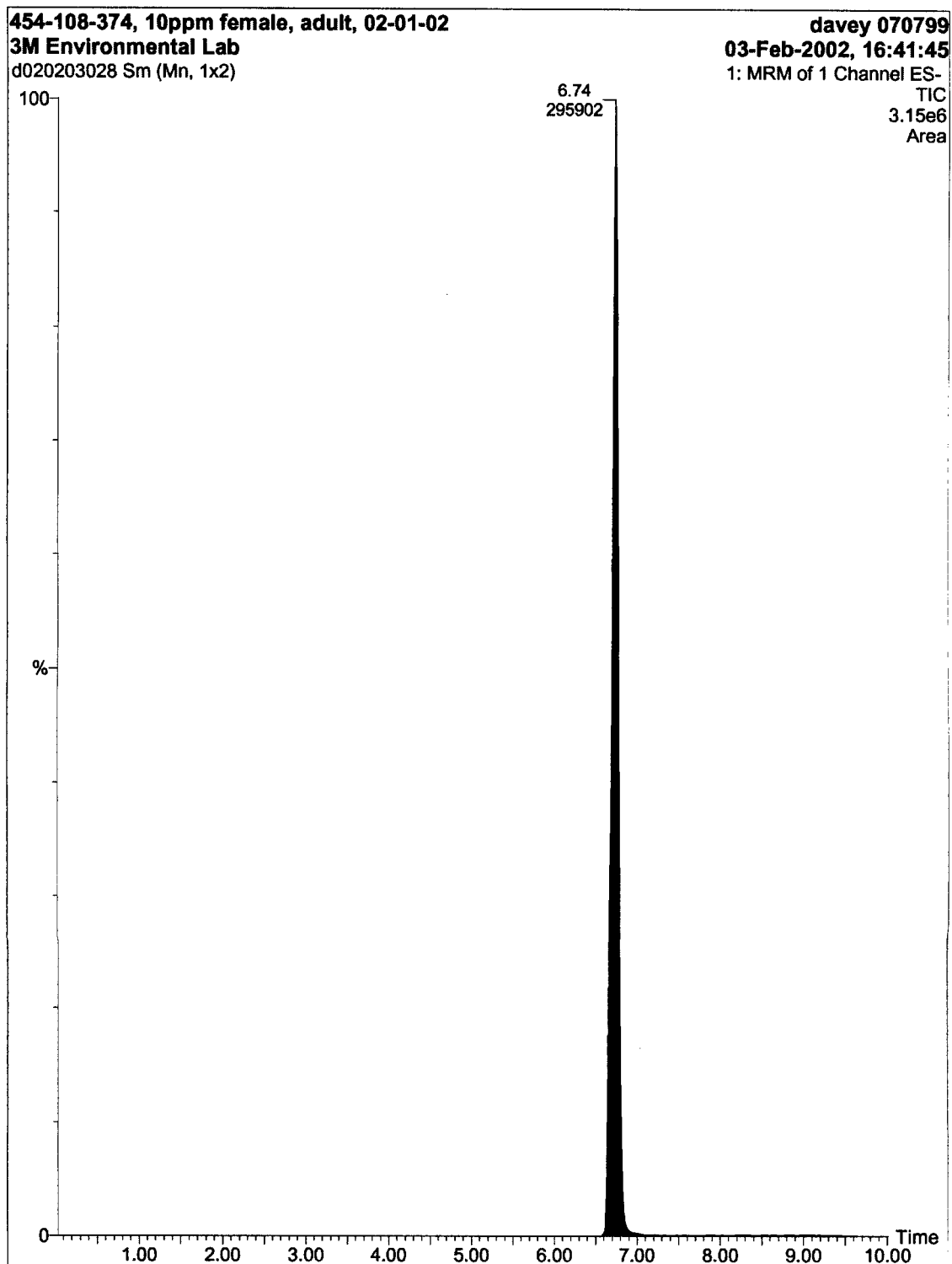
**Dosed Group Adult Female Sera Samples**

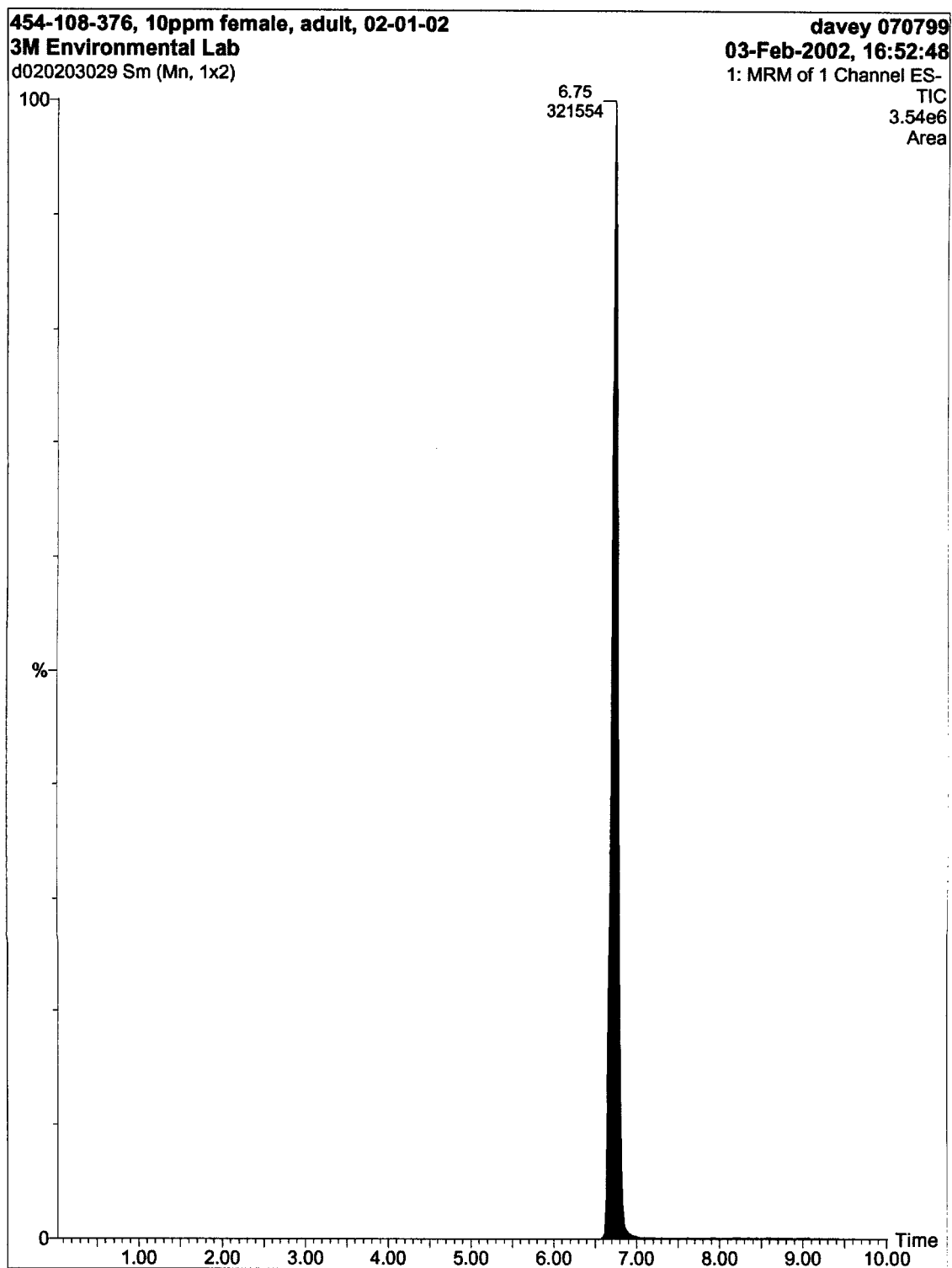
454-108-370, 10ppm female, adult, 02-01-02  
3M Environmental Lab  
d020203026 Sm (Mn, 1x2)

davey 070799  
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1: MRM of 1 Channel ES-  
TIC  
3.39e6  
Area



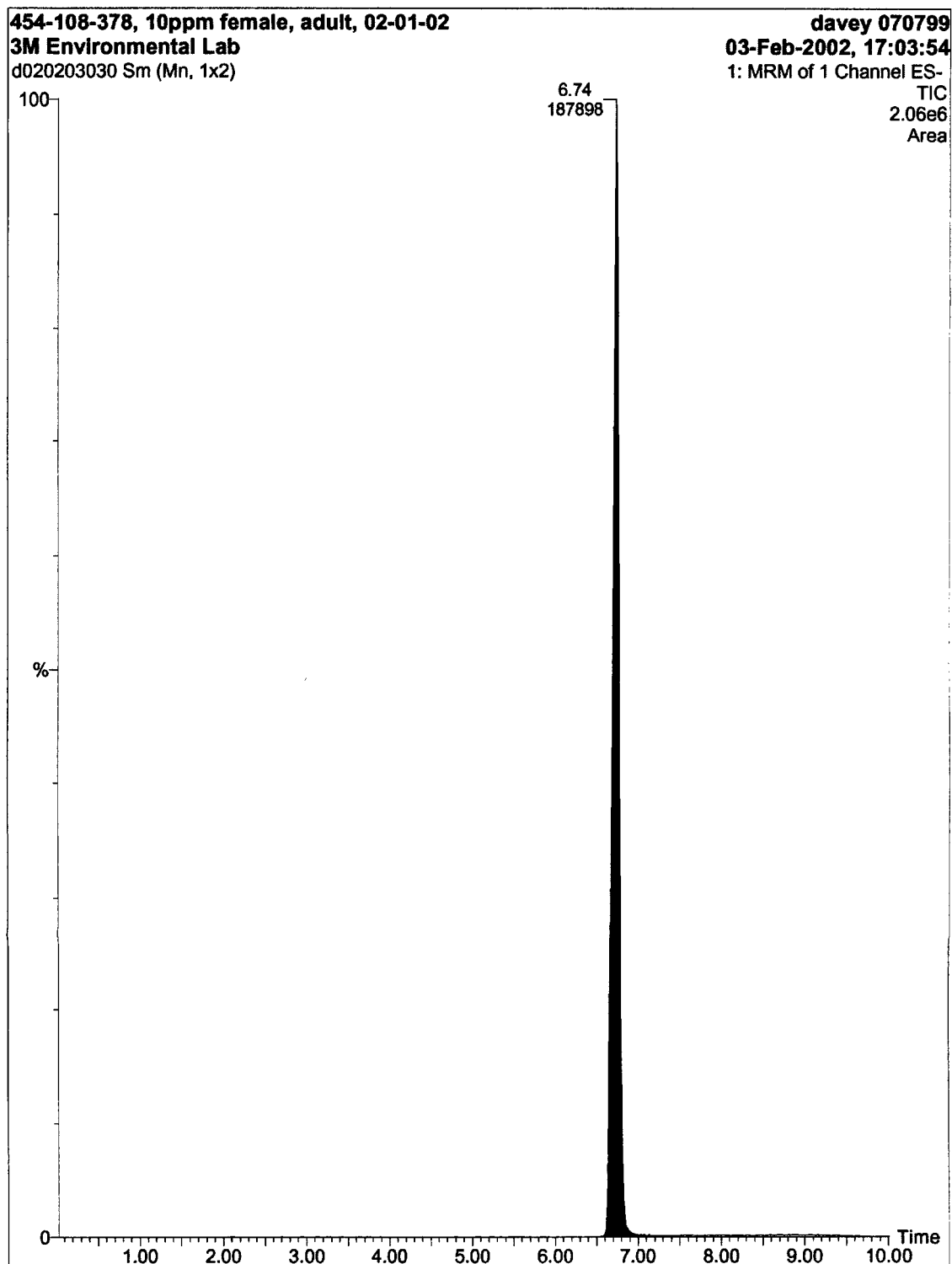






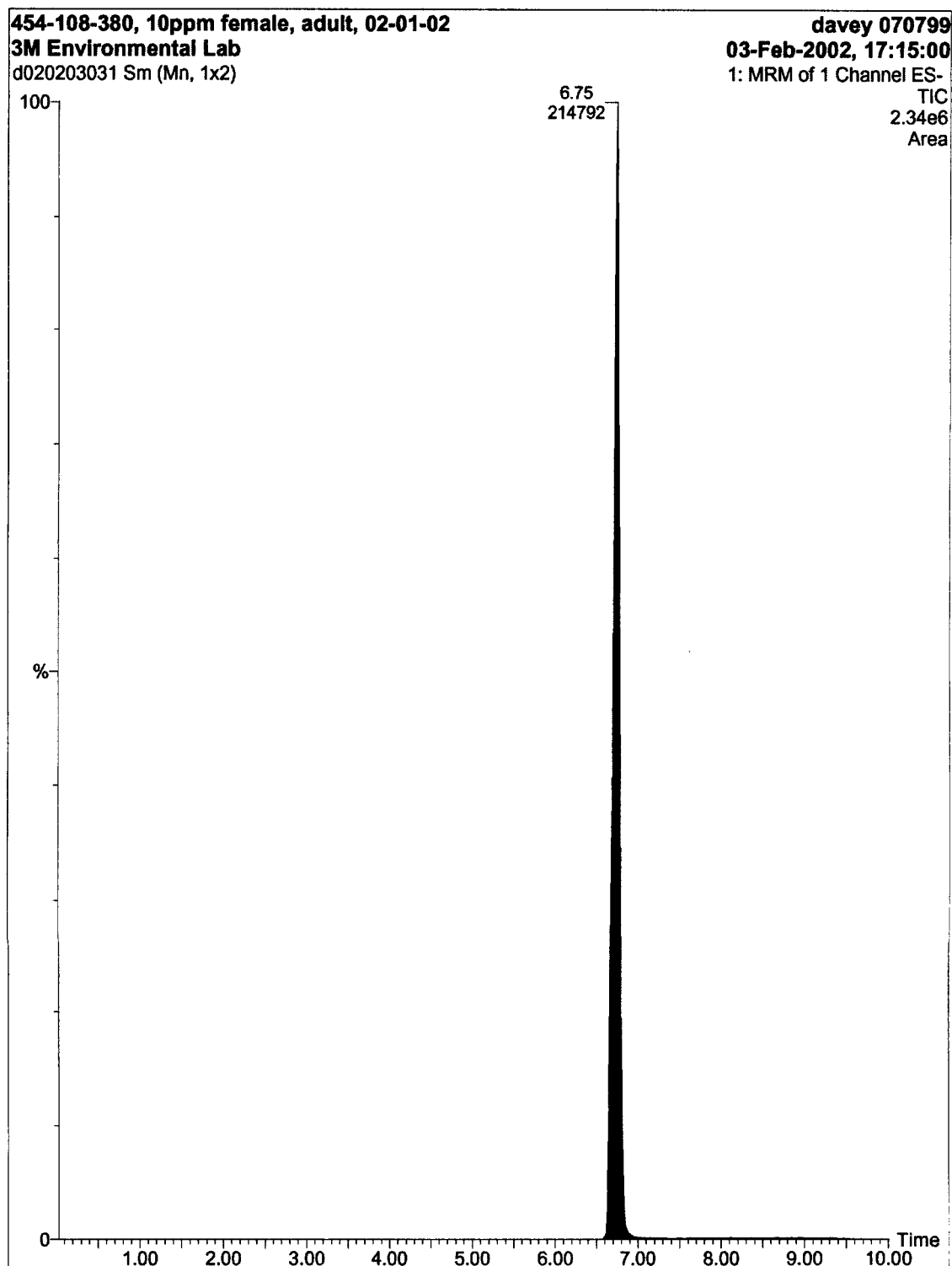
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



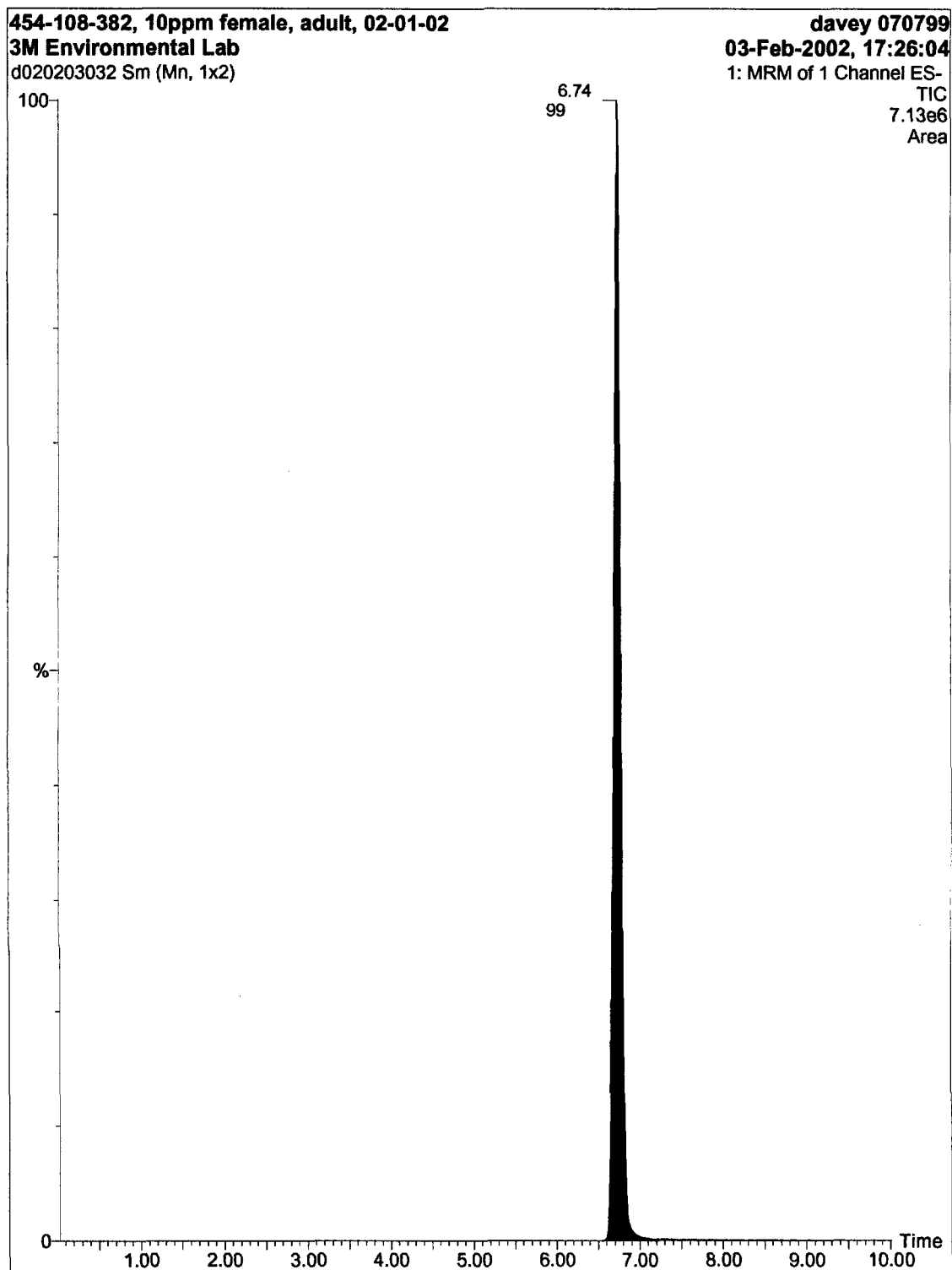
Report E01-1245

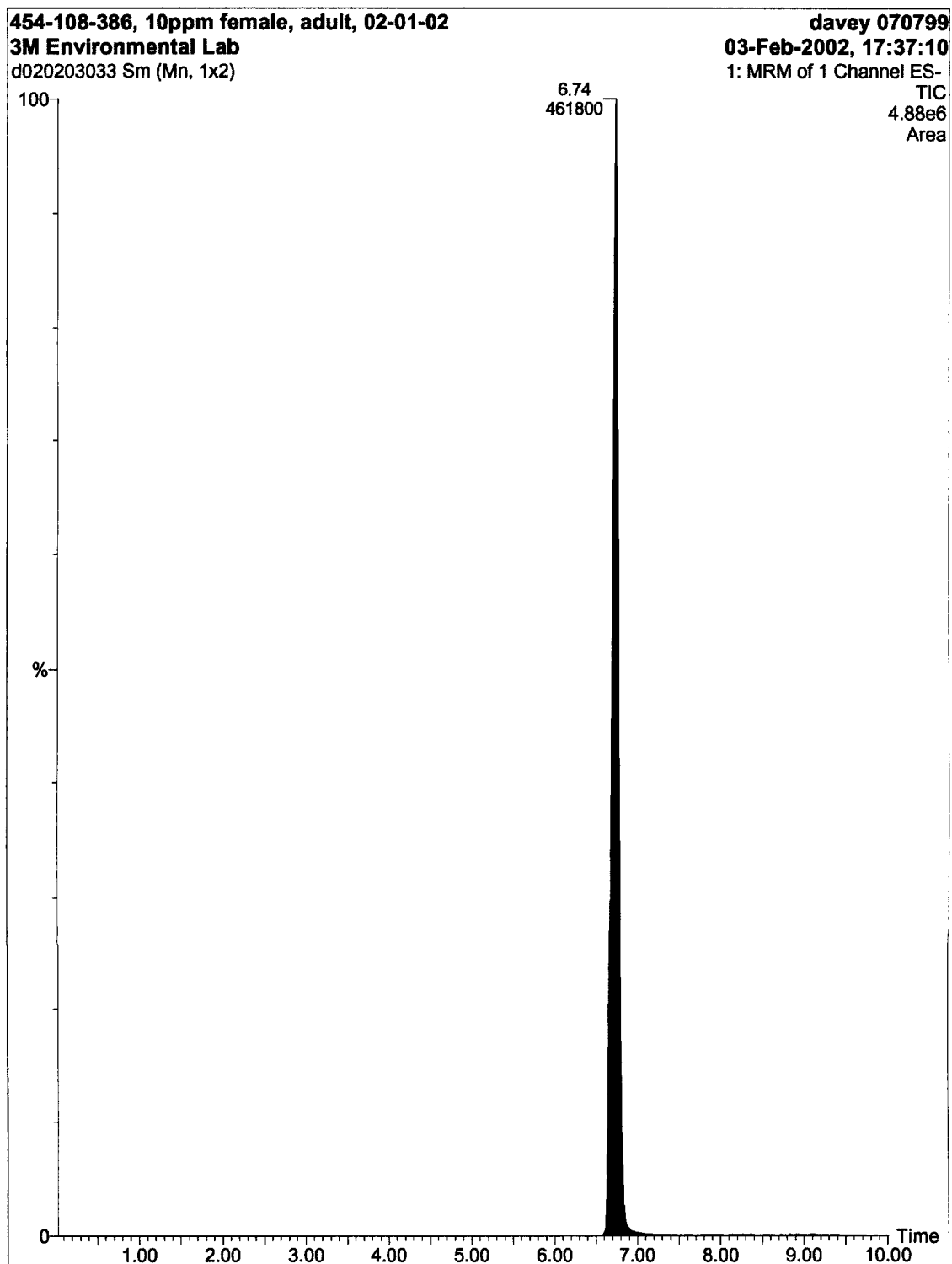
PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245

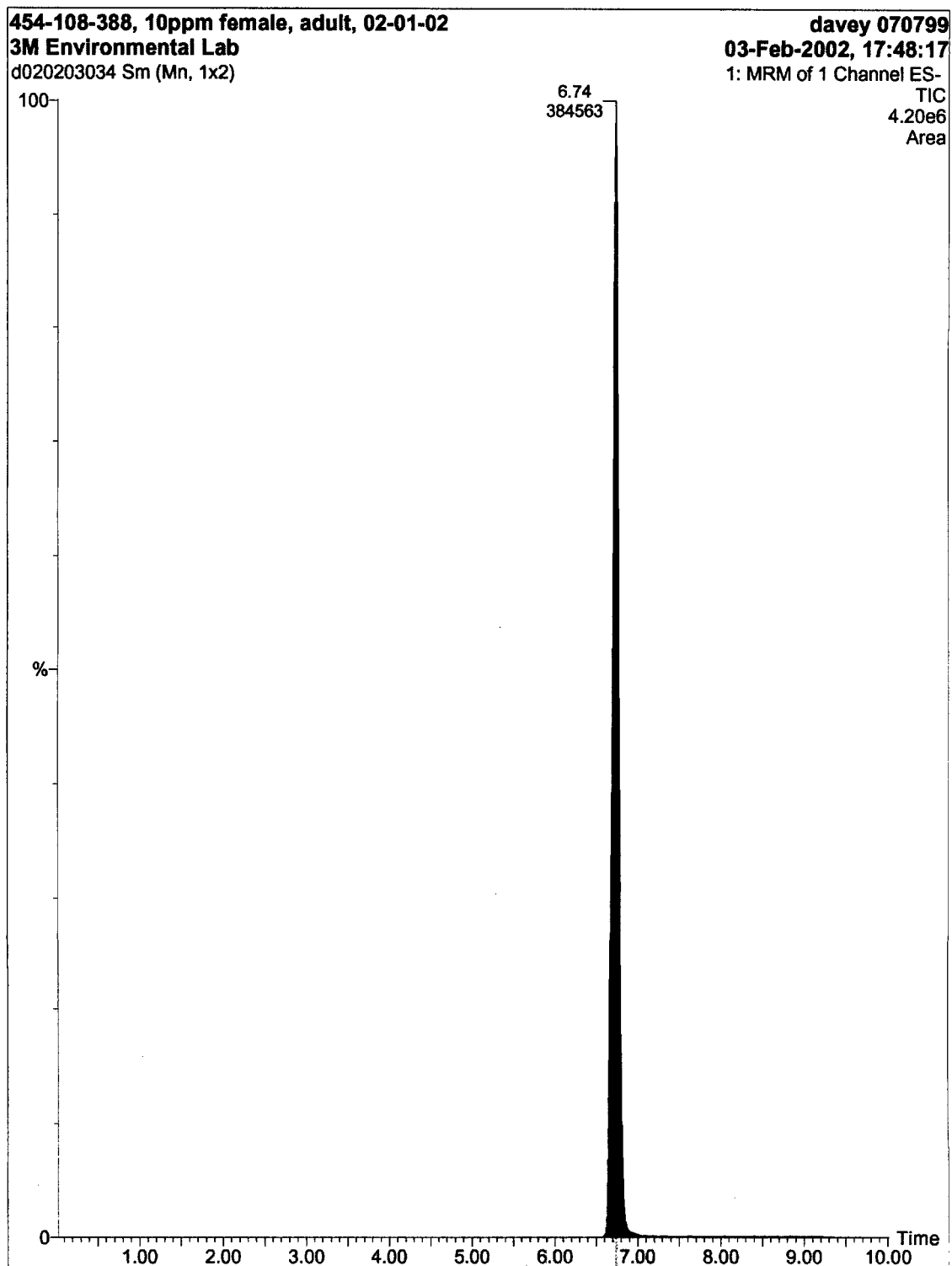
PFOS: A Reproduction Study with the Northern Bobwhite





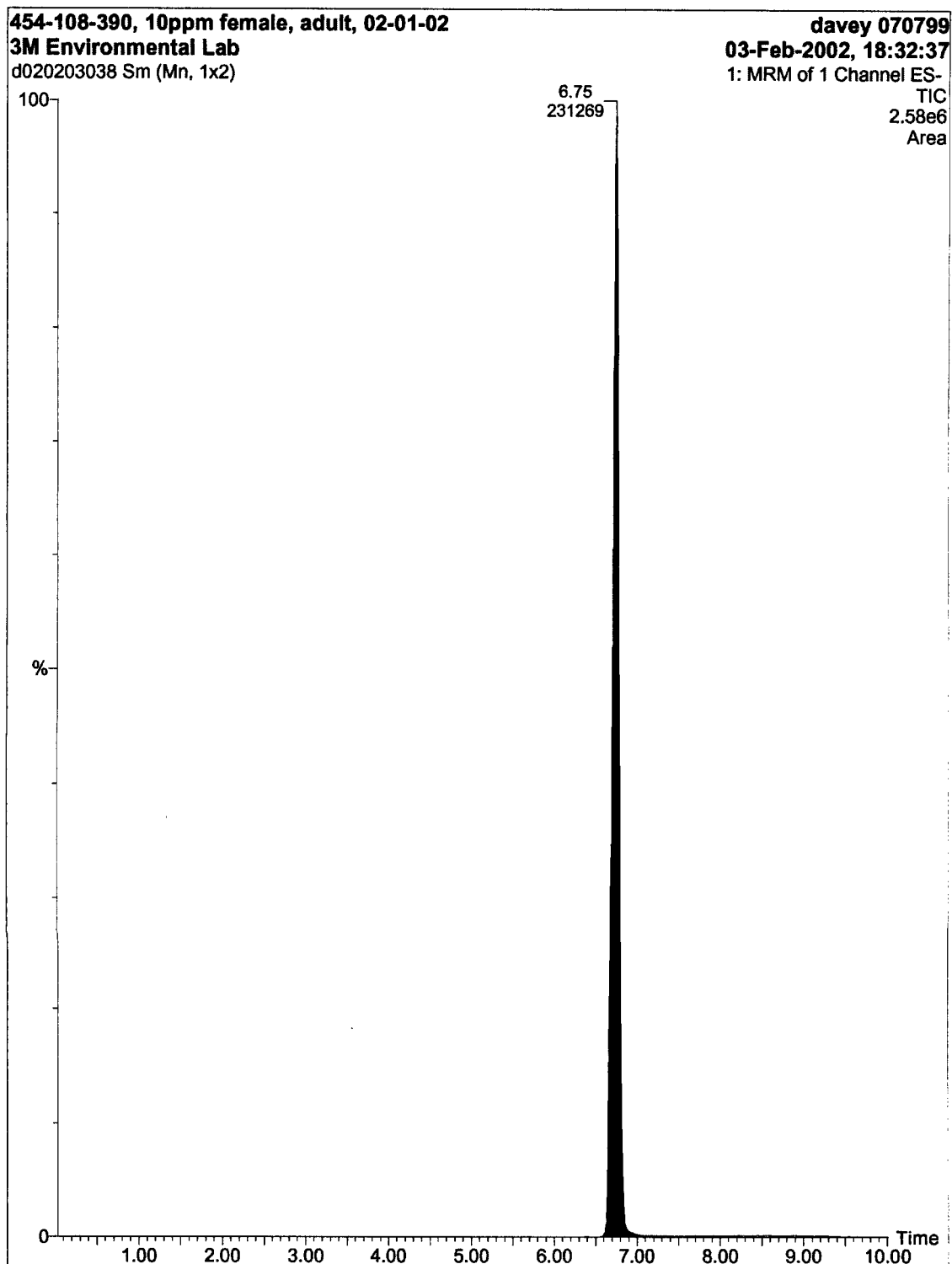
Report E01-1245

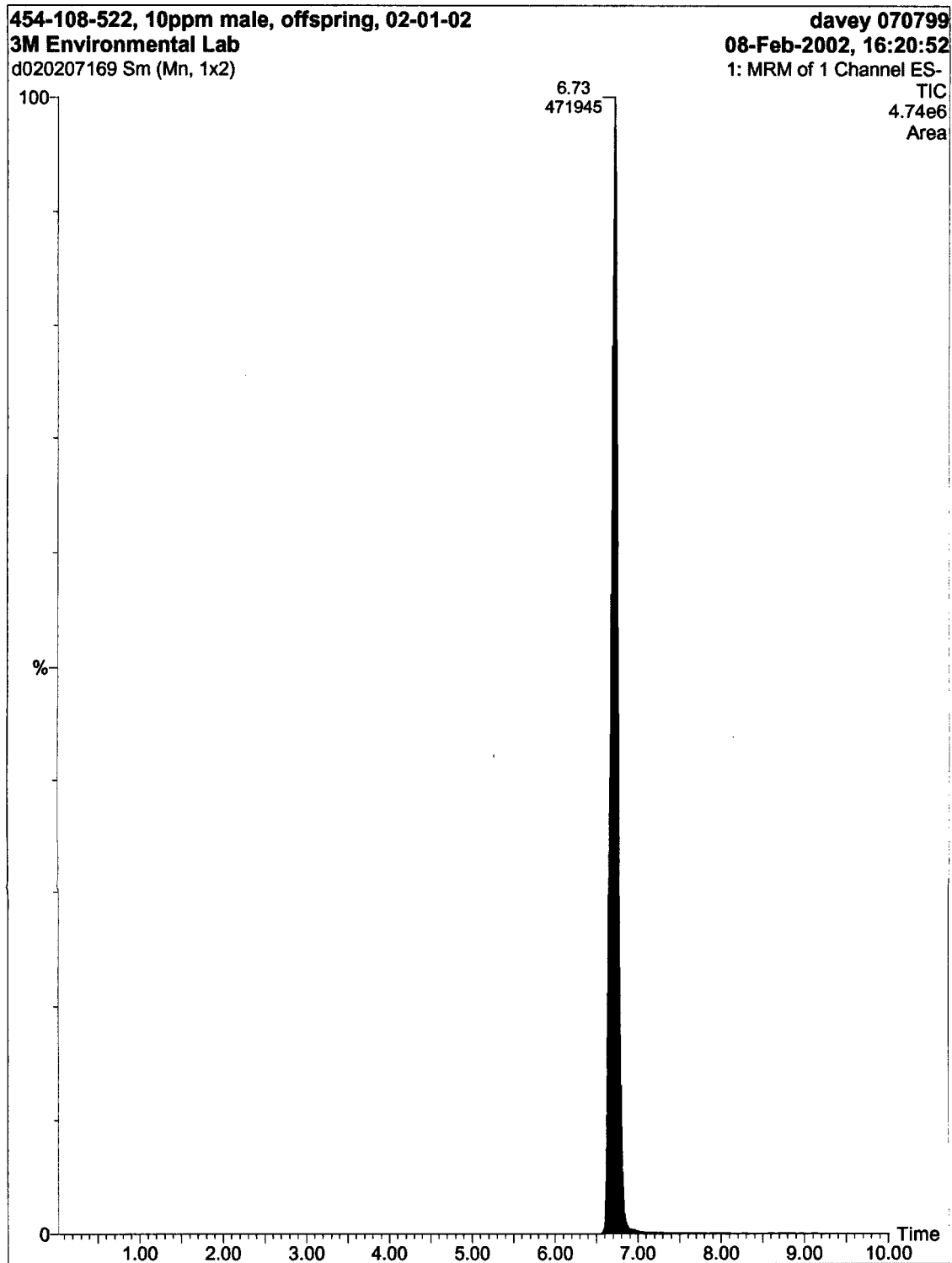
PFOS: A Reproduction Study with the Northern Bobwhite

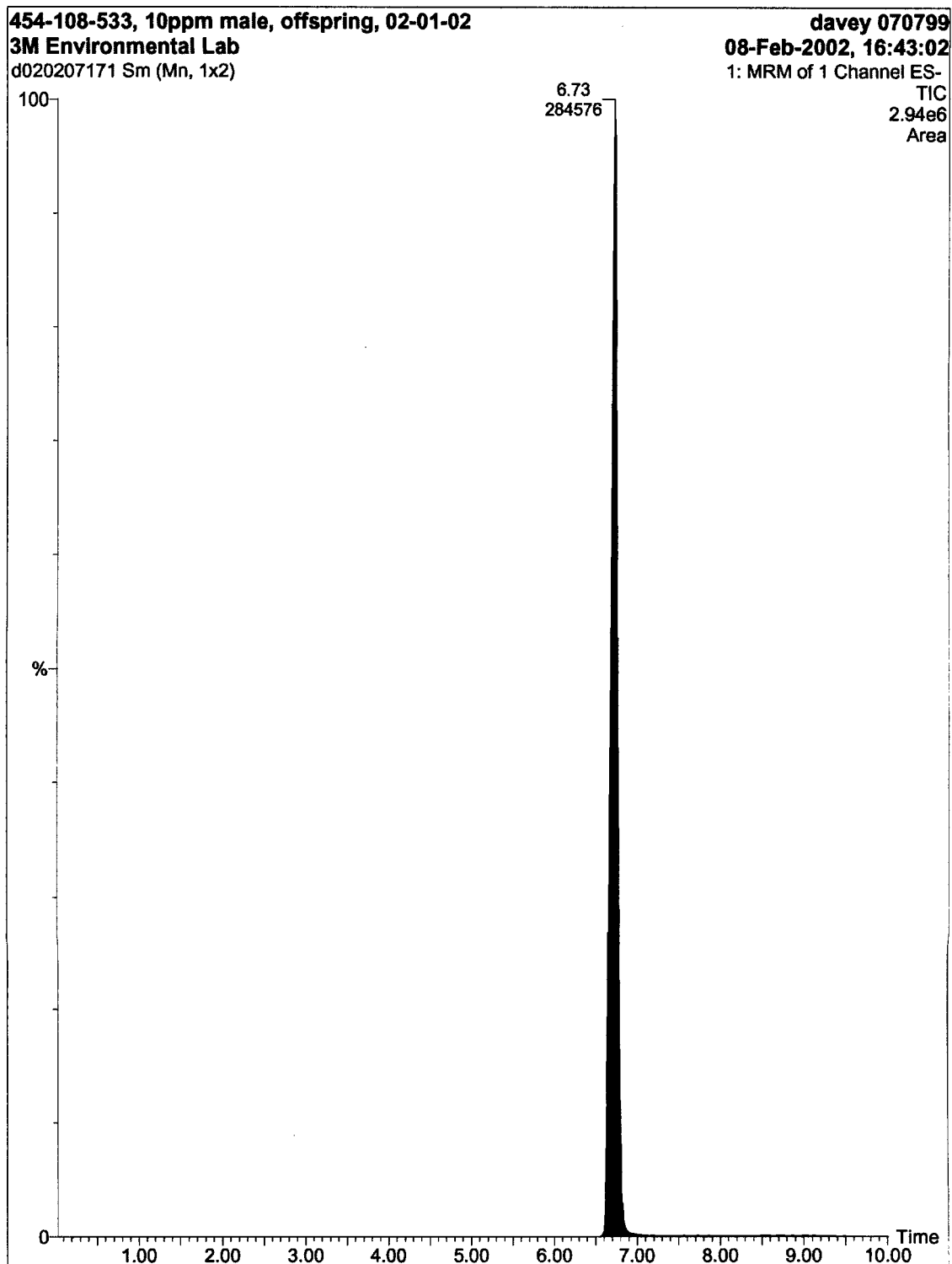


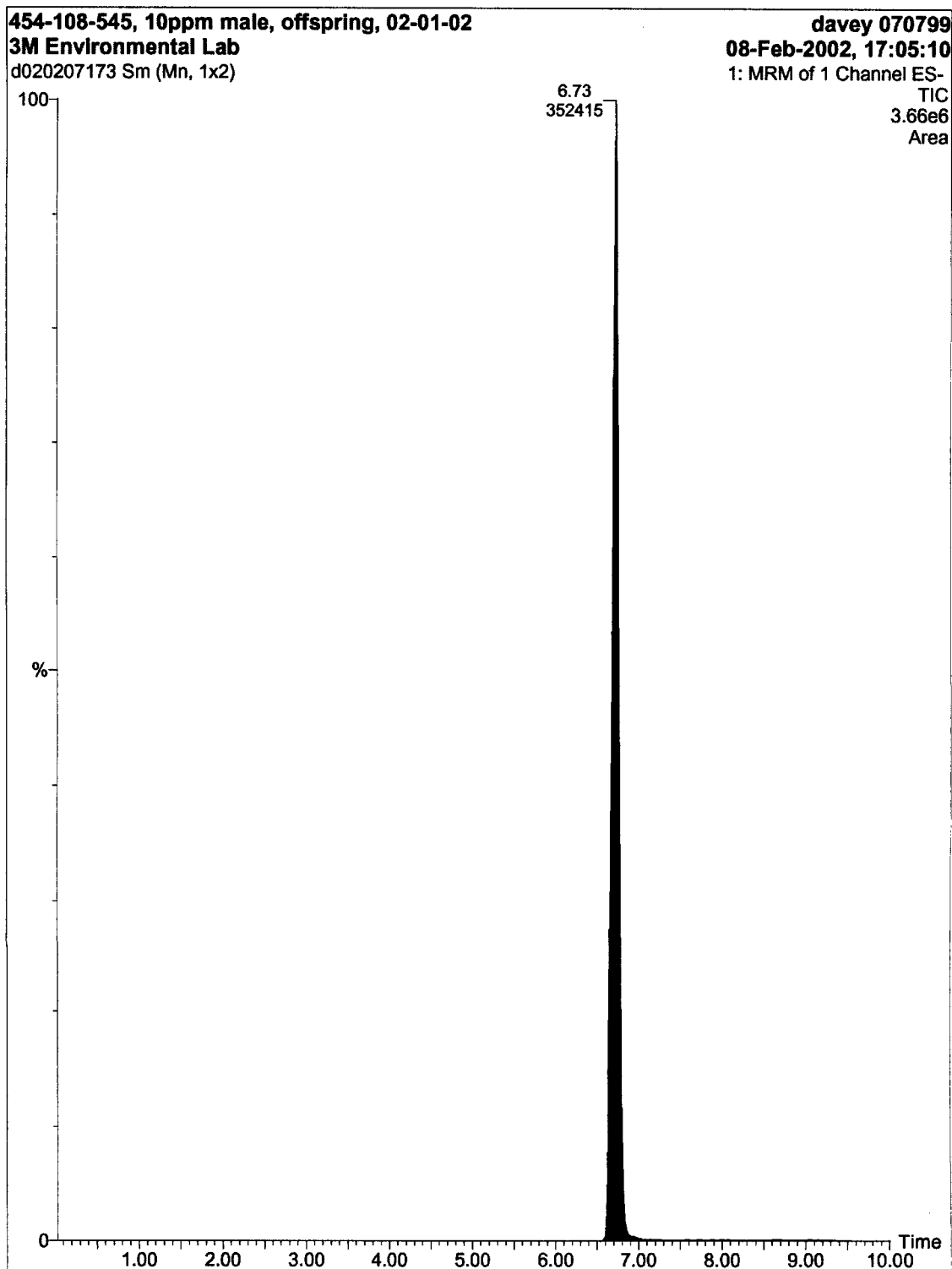
Report E01-1245

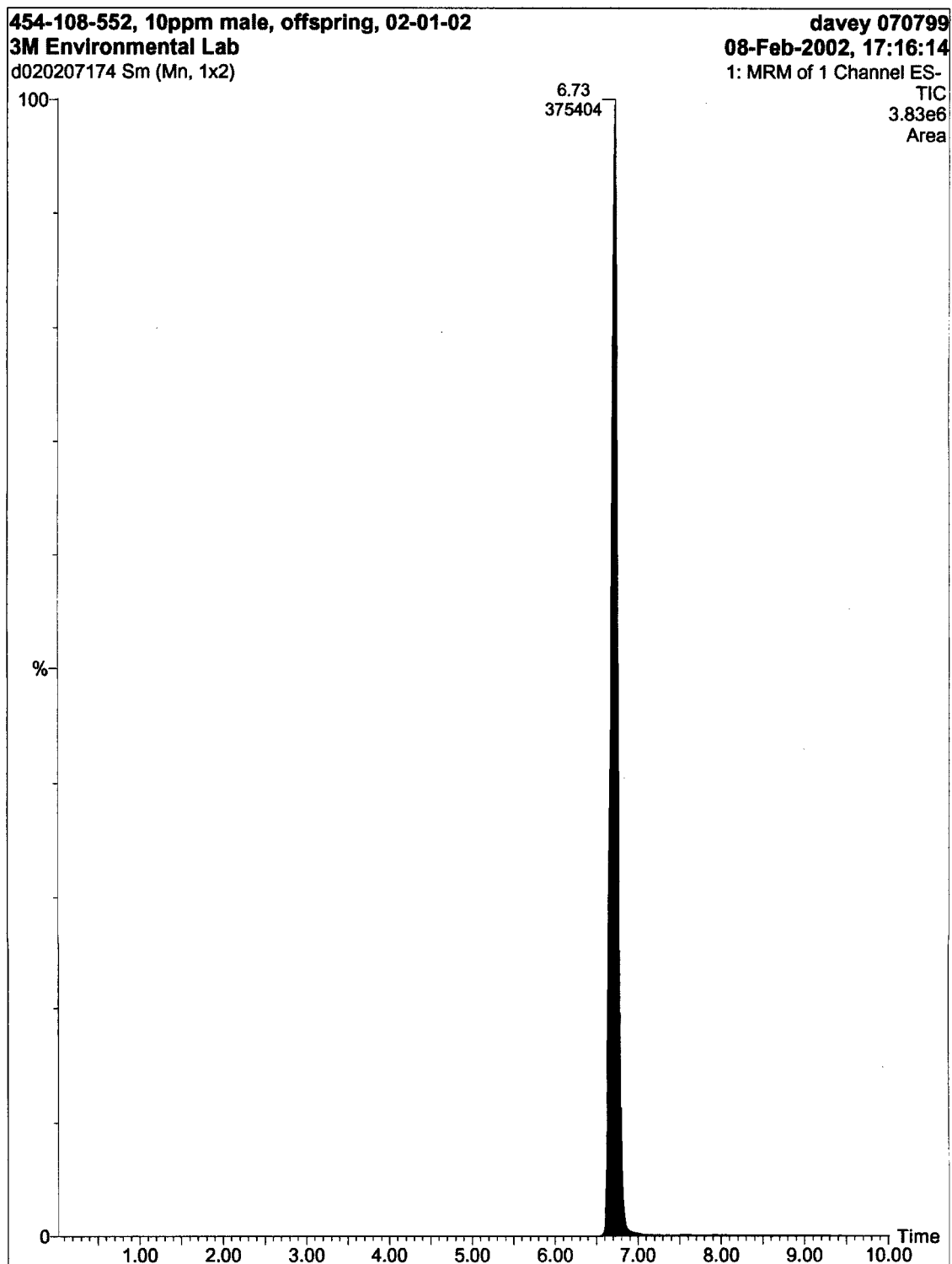
PFOS: A Reproduction Study with the Northern Bobwhite



**Dosed Group Male Offspring Sera Samples**

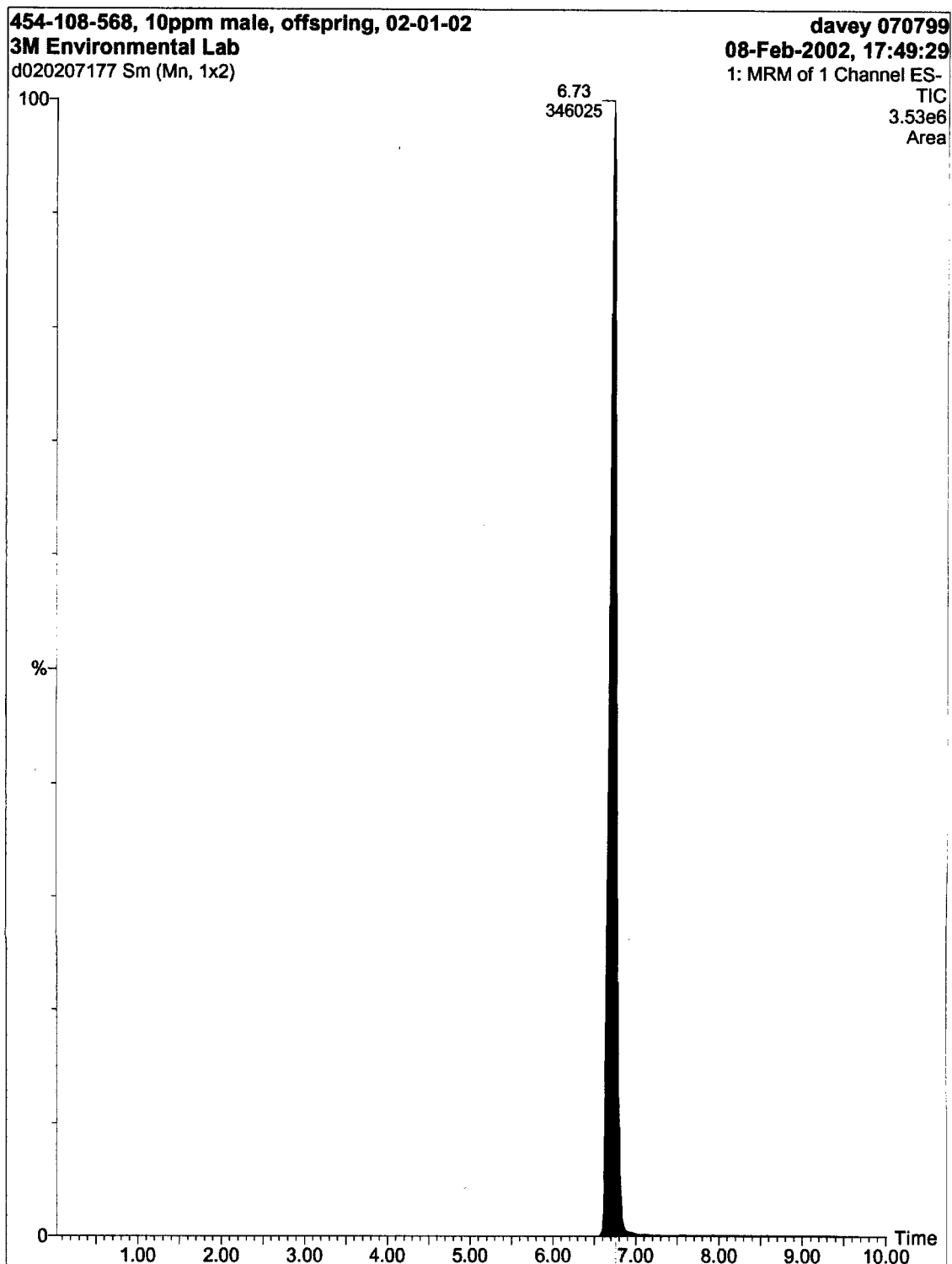






Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



**Dosed Group Female Offspring Sera Samples**

454-108-517, 10ppm female, offspring, 02-01-02

3M Environmental Lab

d020207168 Sm (Mn, 1x2)

davey 070799

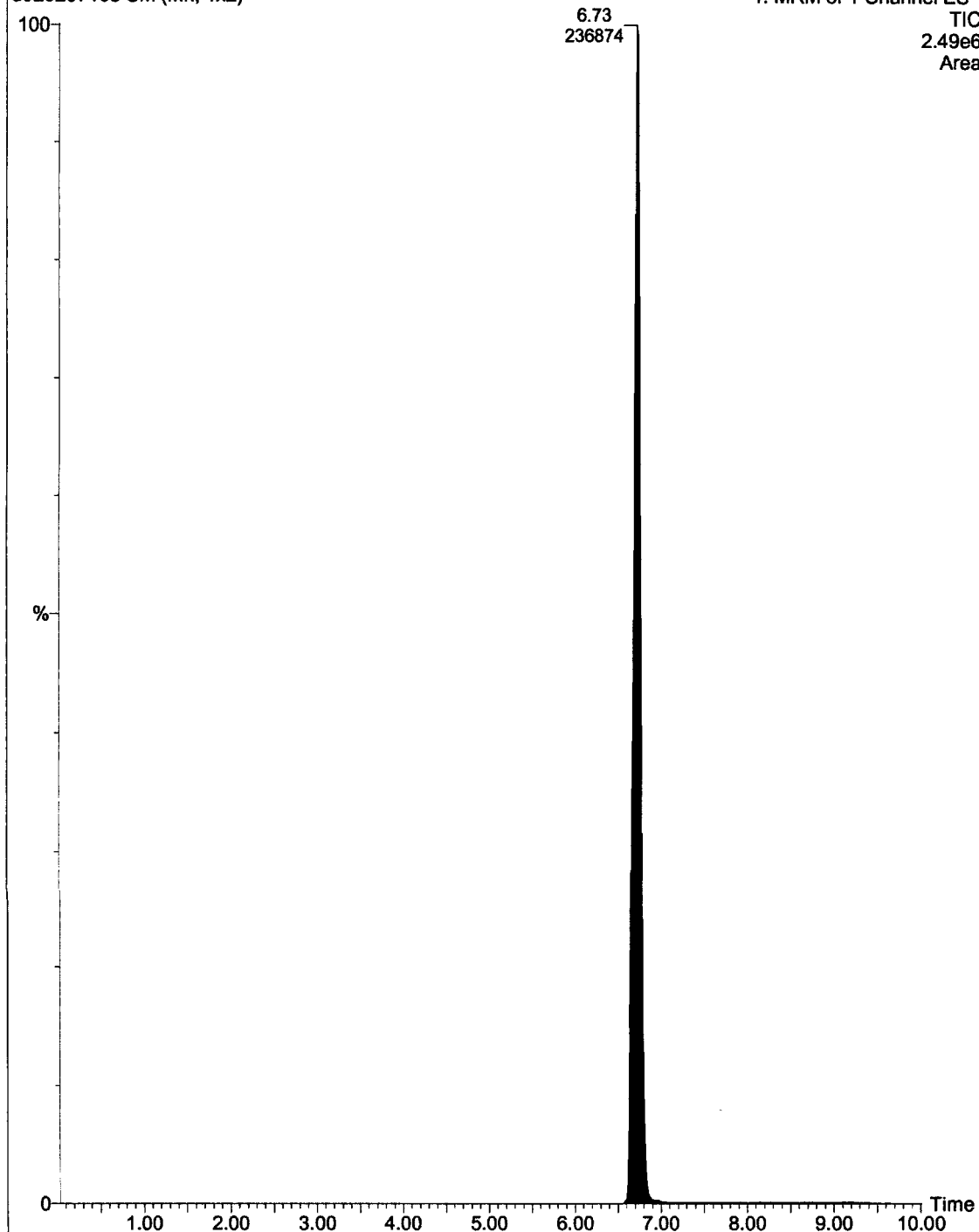
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TIC

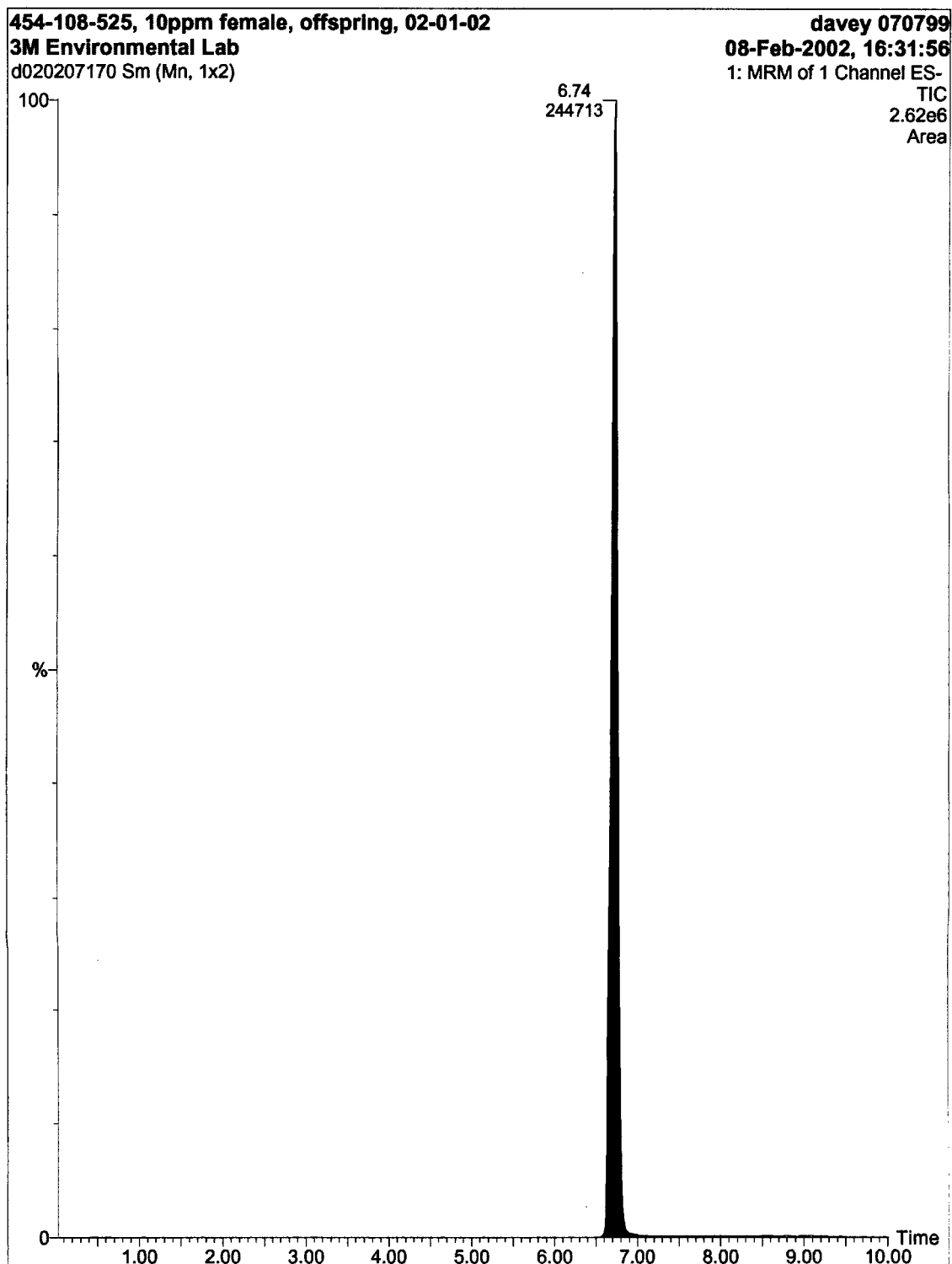
2.49e6

Area



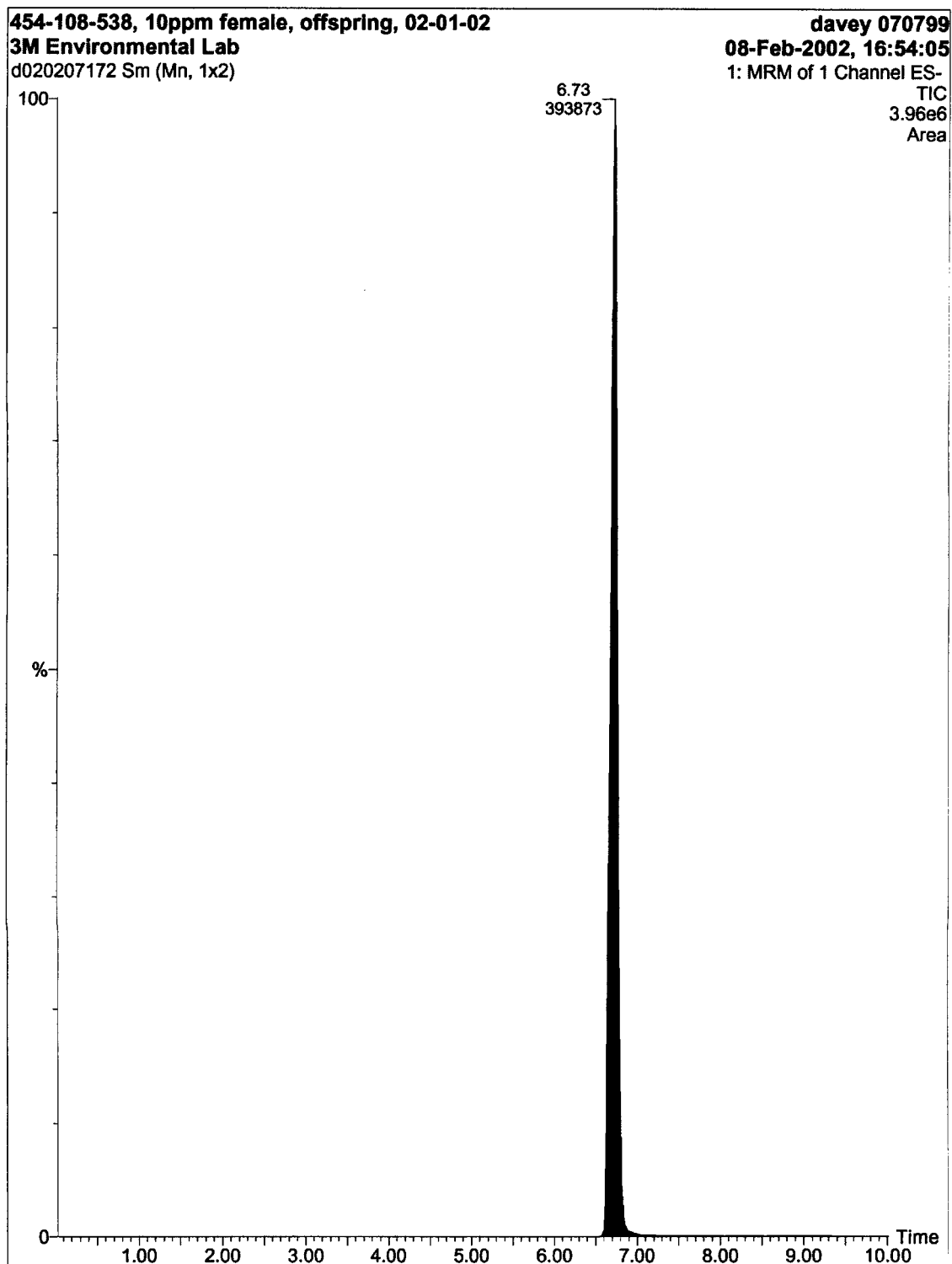
Report E01-1245

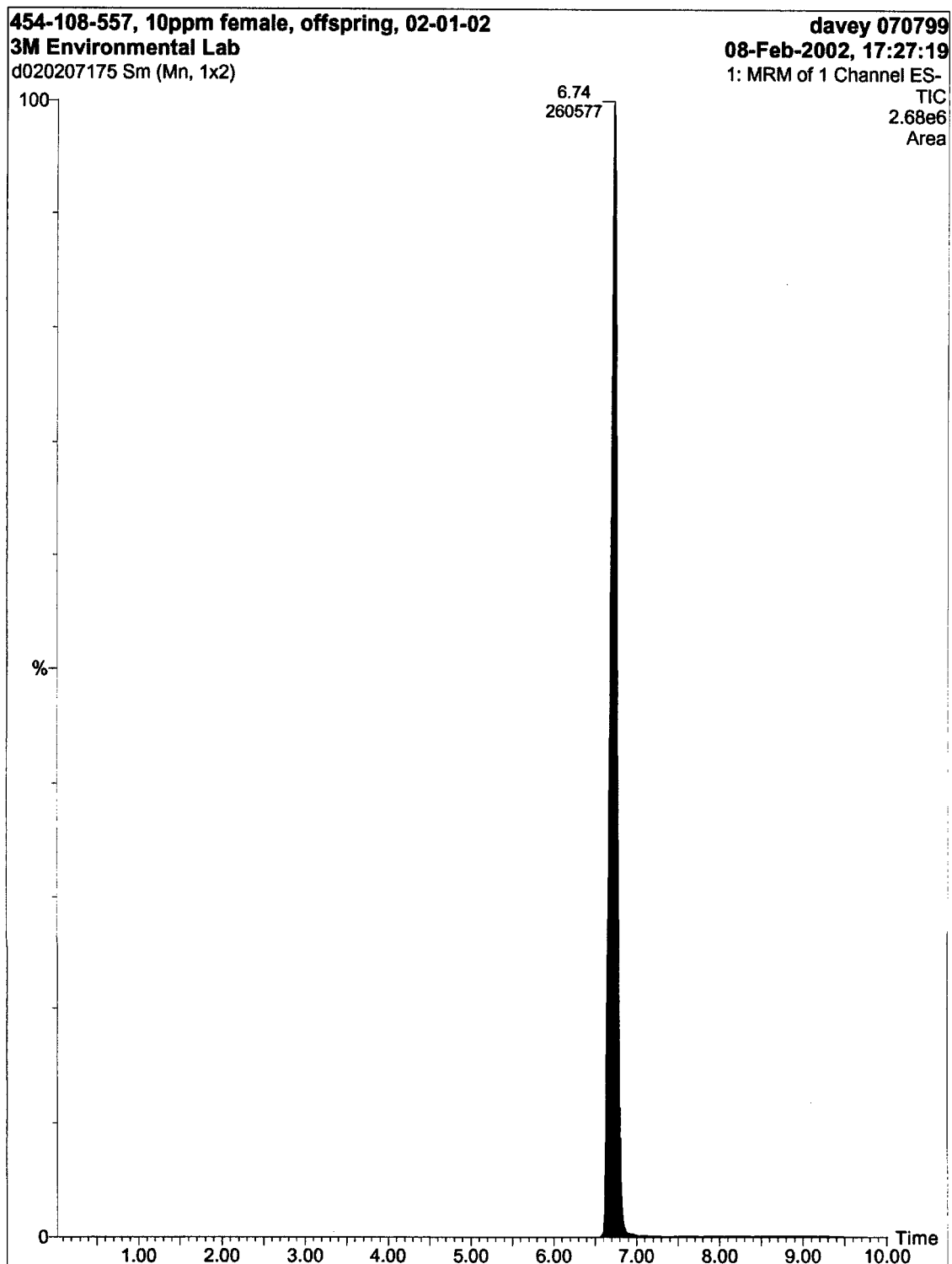
PFOS: A Reproduction Study with the Northern Bobwhite

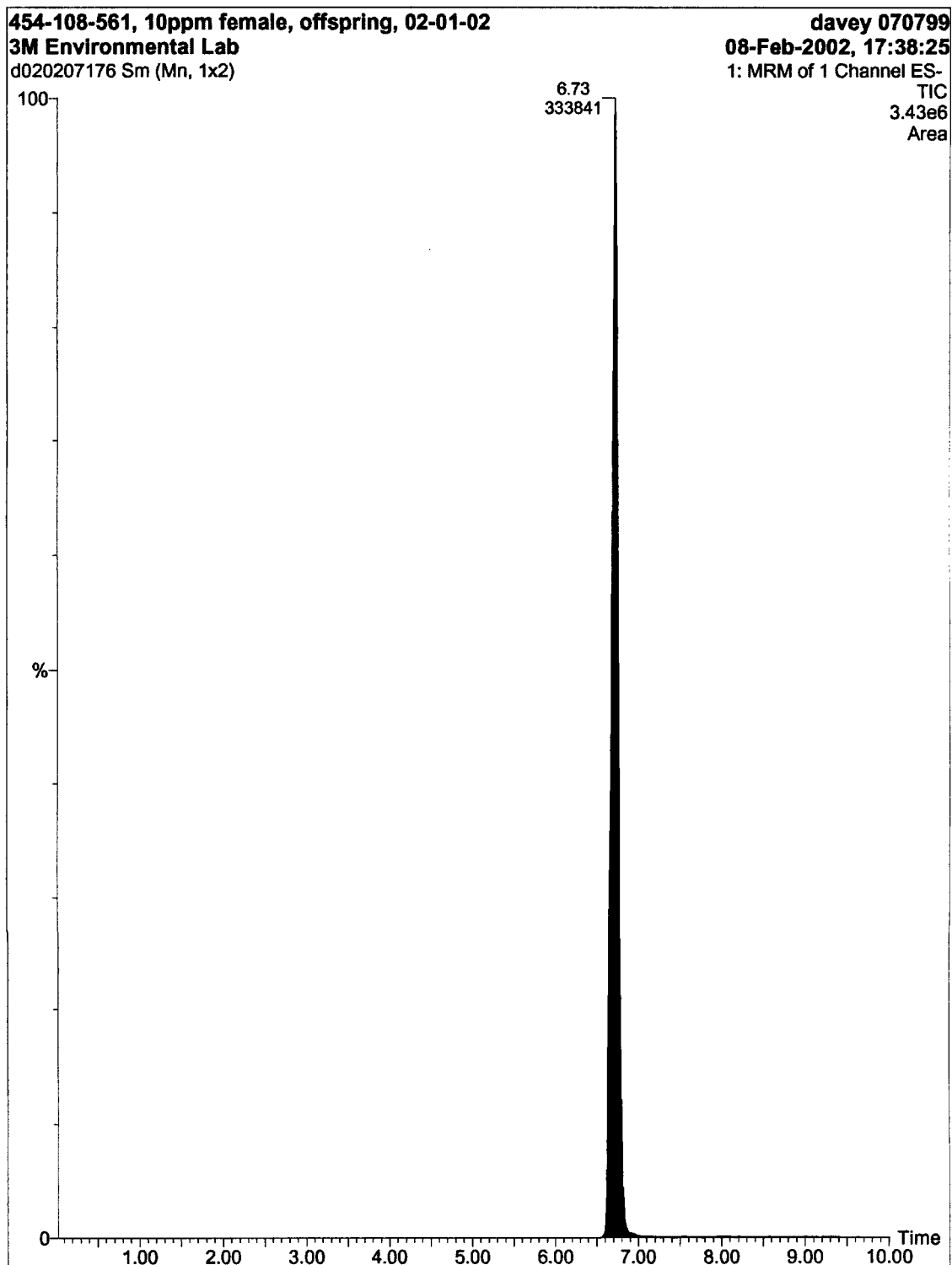


Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite







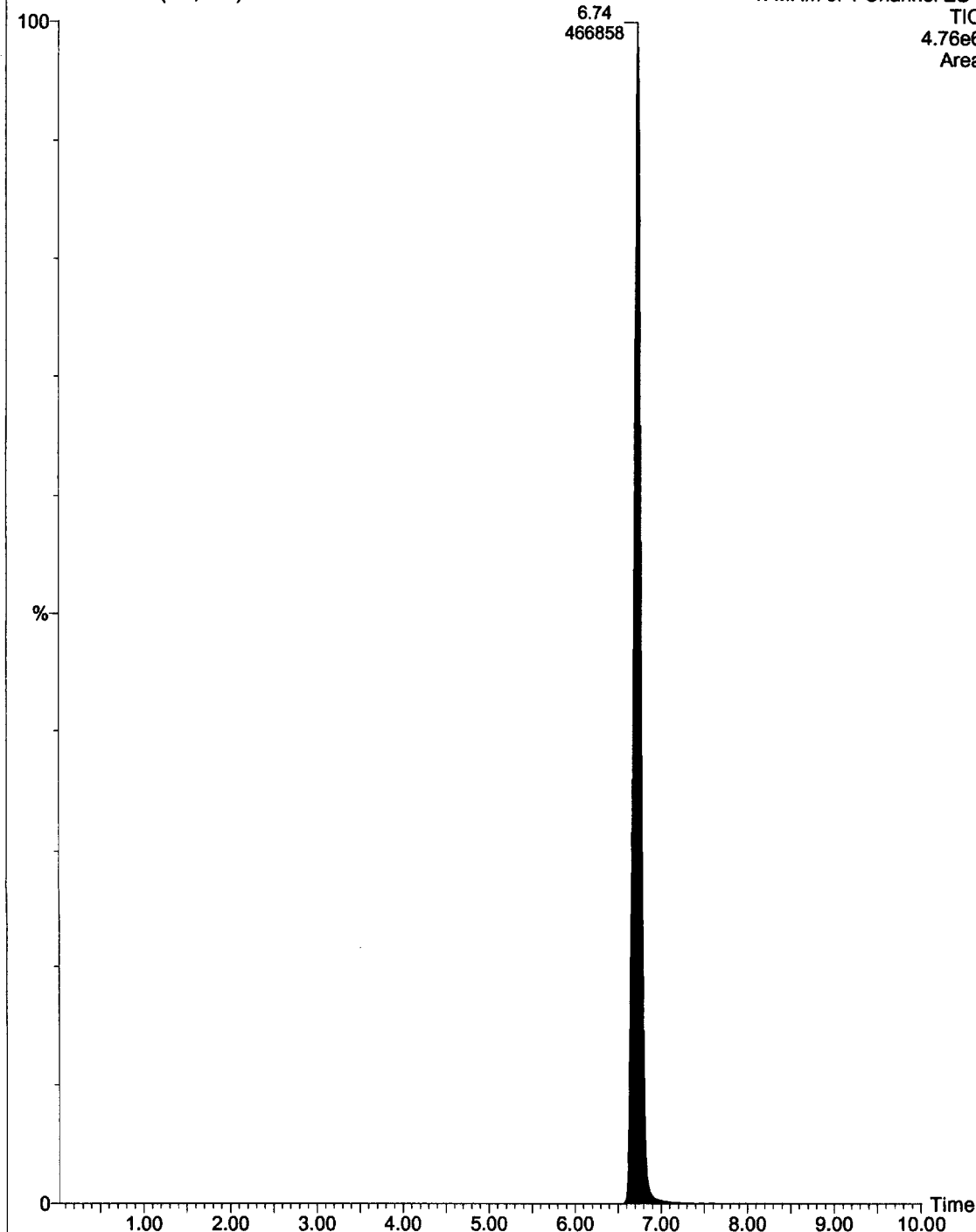
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite

**Dosed Group Male Liver Samples**

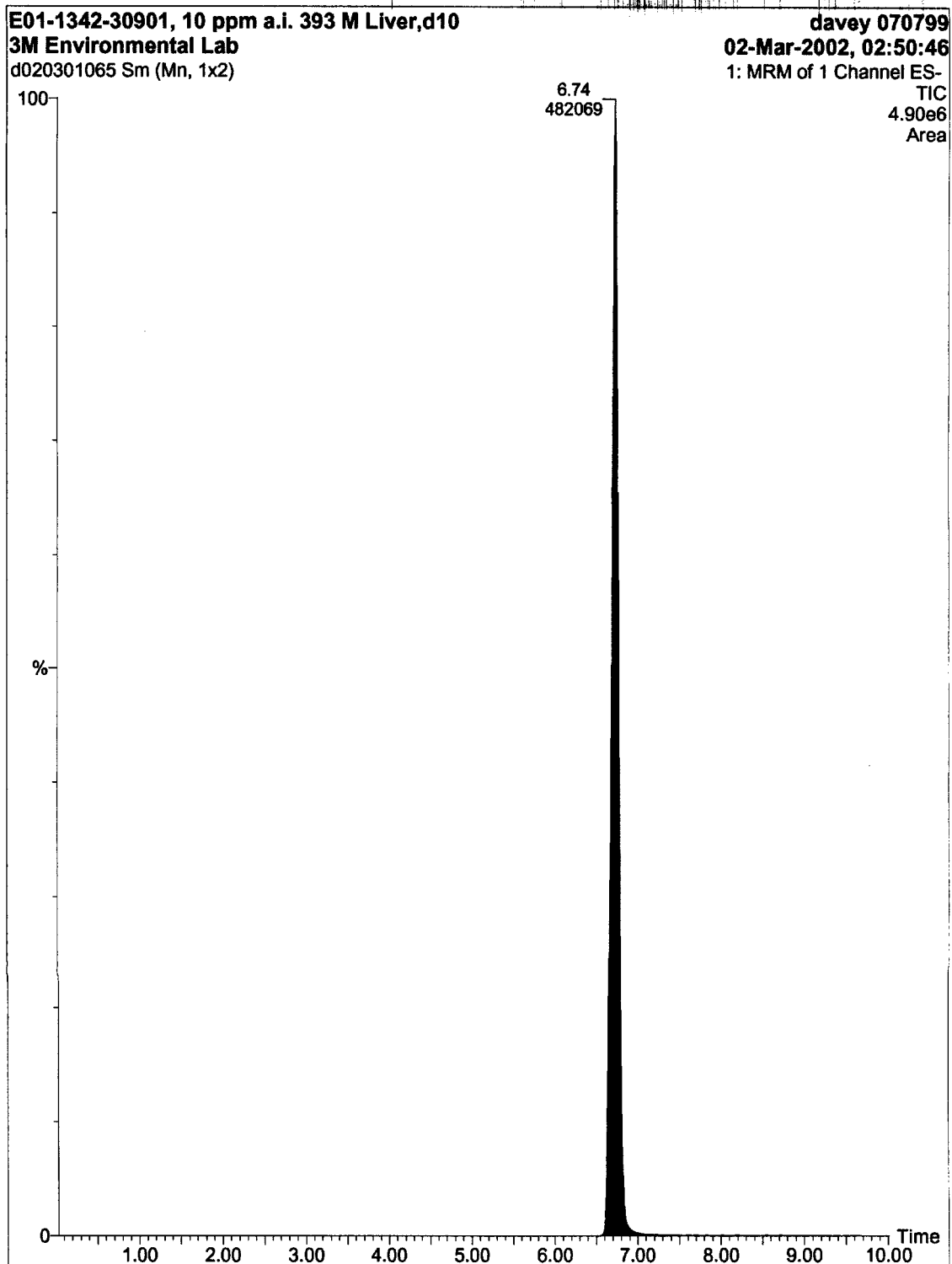
E01-1342-30762, 10 ppm a.i. 379 M Liver,d10  
3M Environmental Lab  
d020301058 Sm (Mn, 1x2)

davey 070799  
02-Mar-2002, 01:32:50  
1: MRM of 1 Channel ES-  
TIC  
4.76e6  
Area



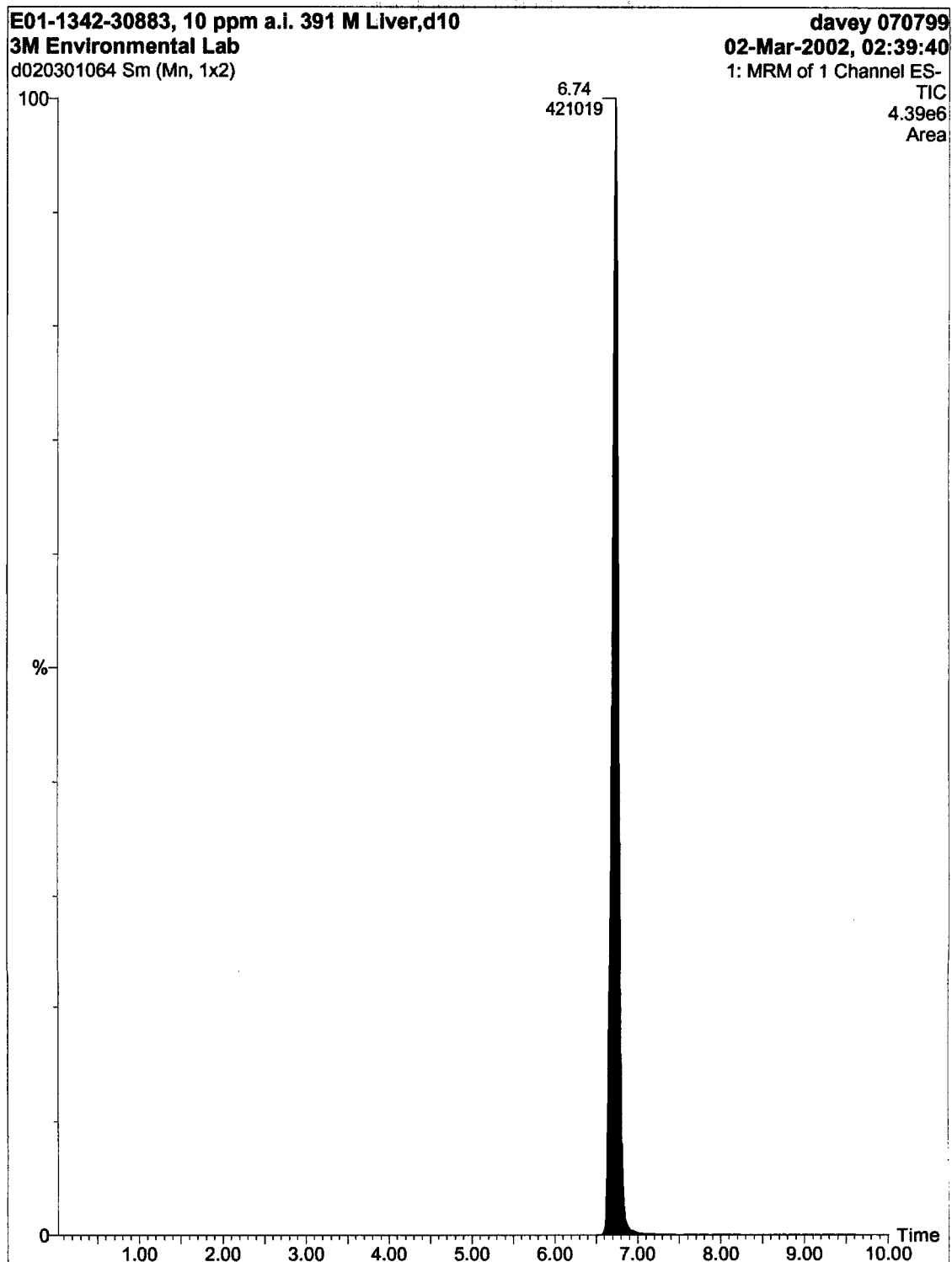
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



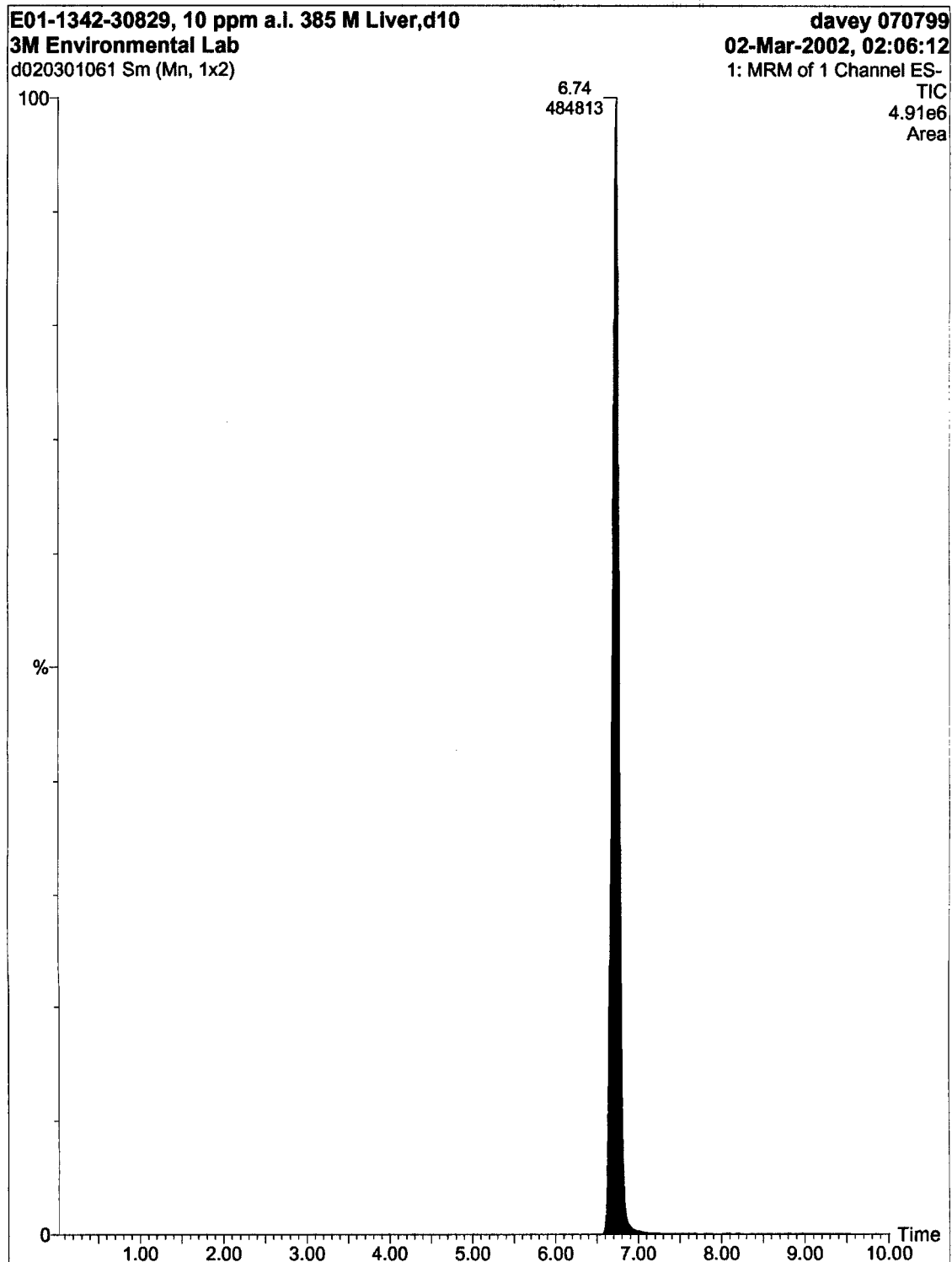
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



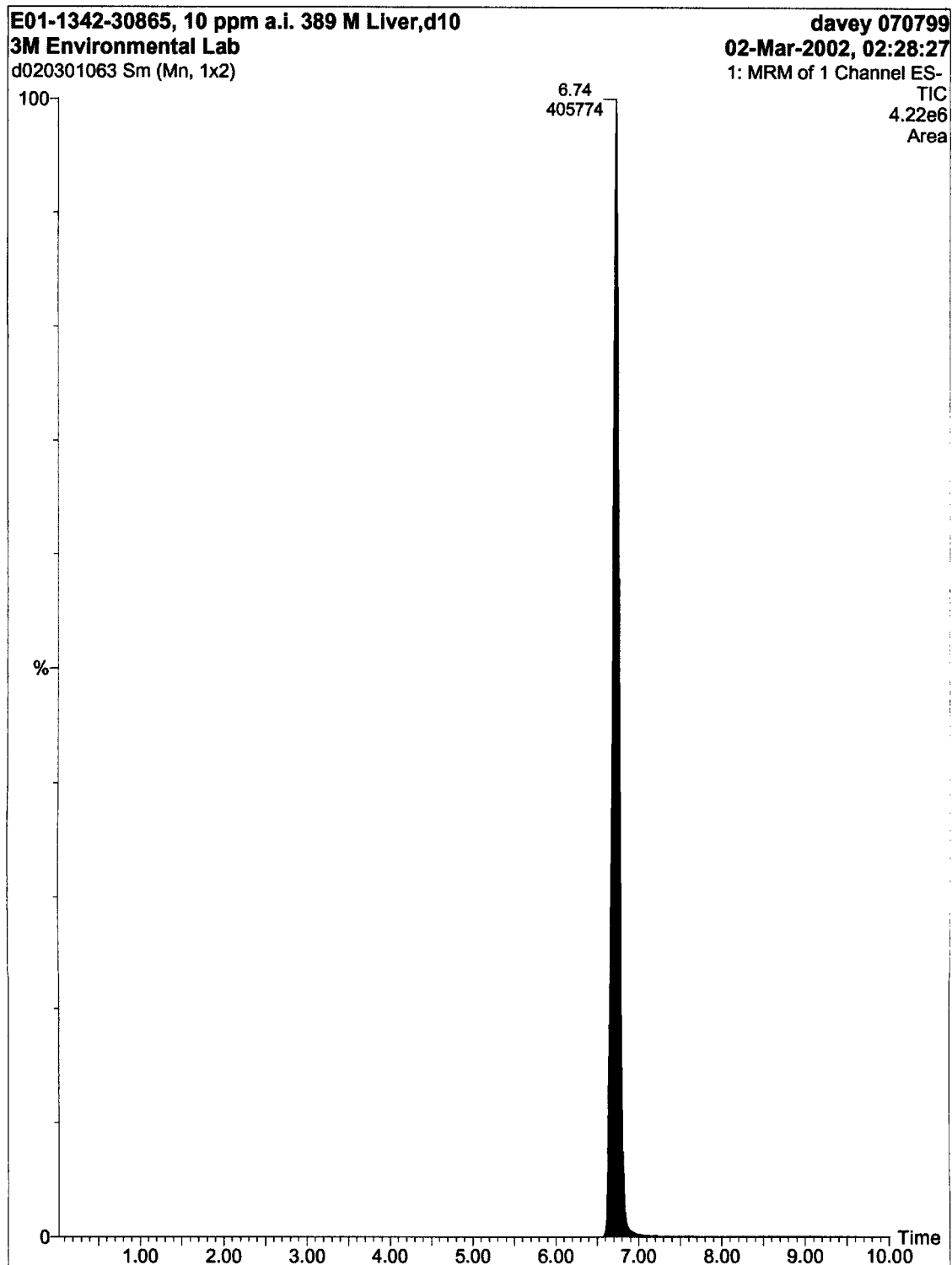
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



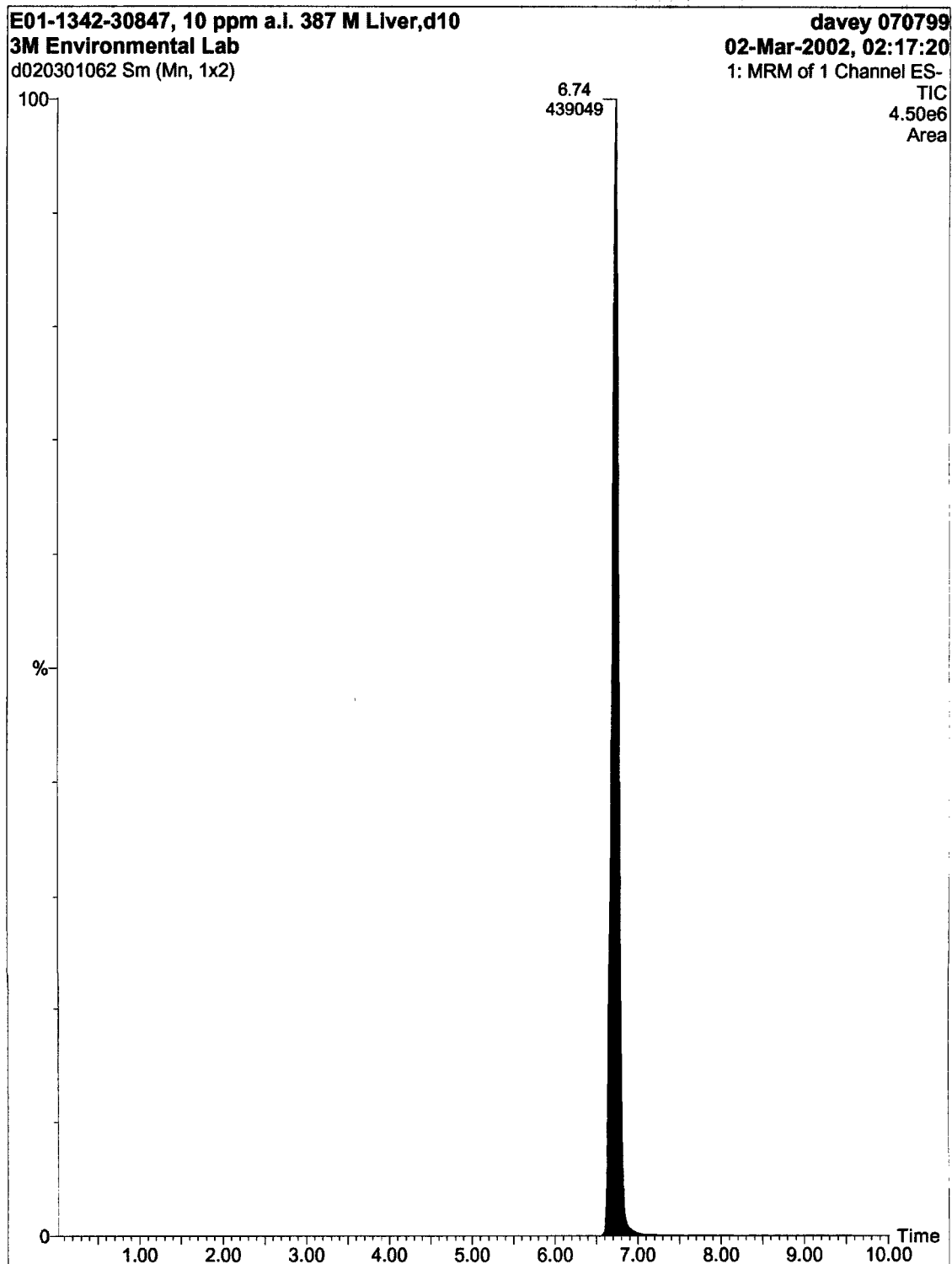
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



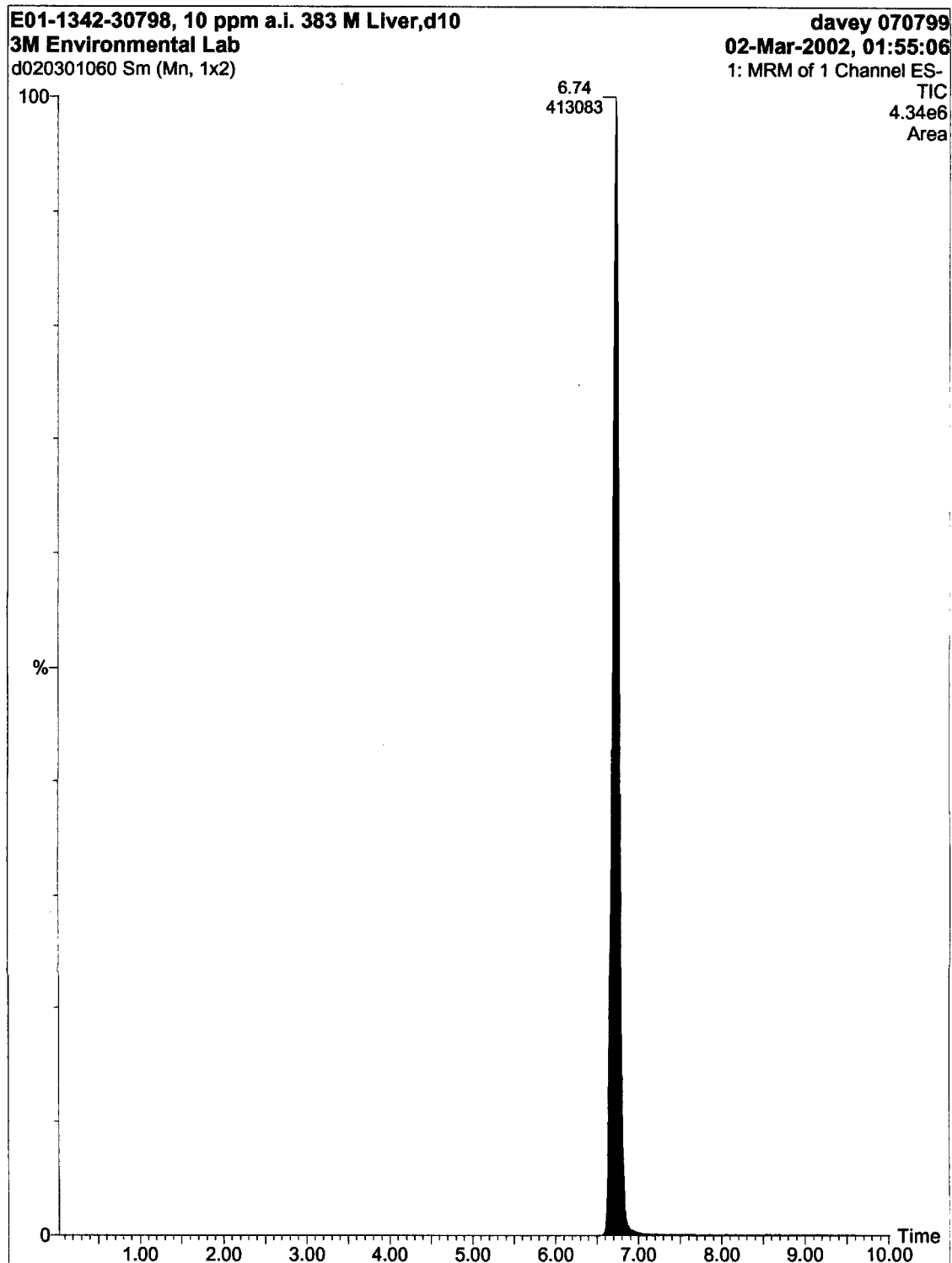
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



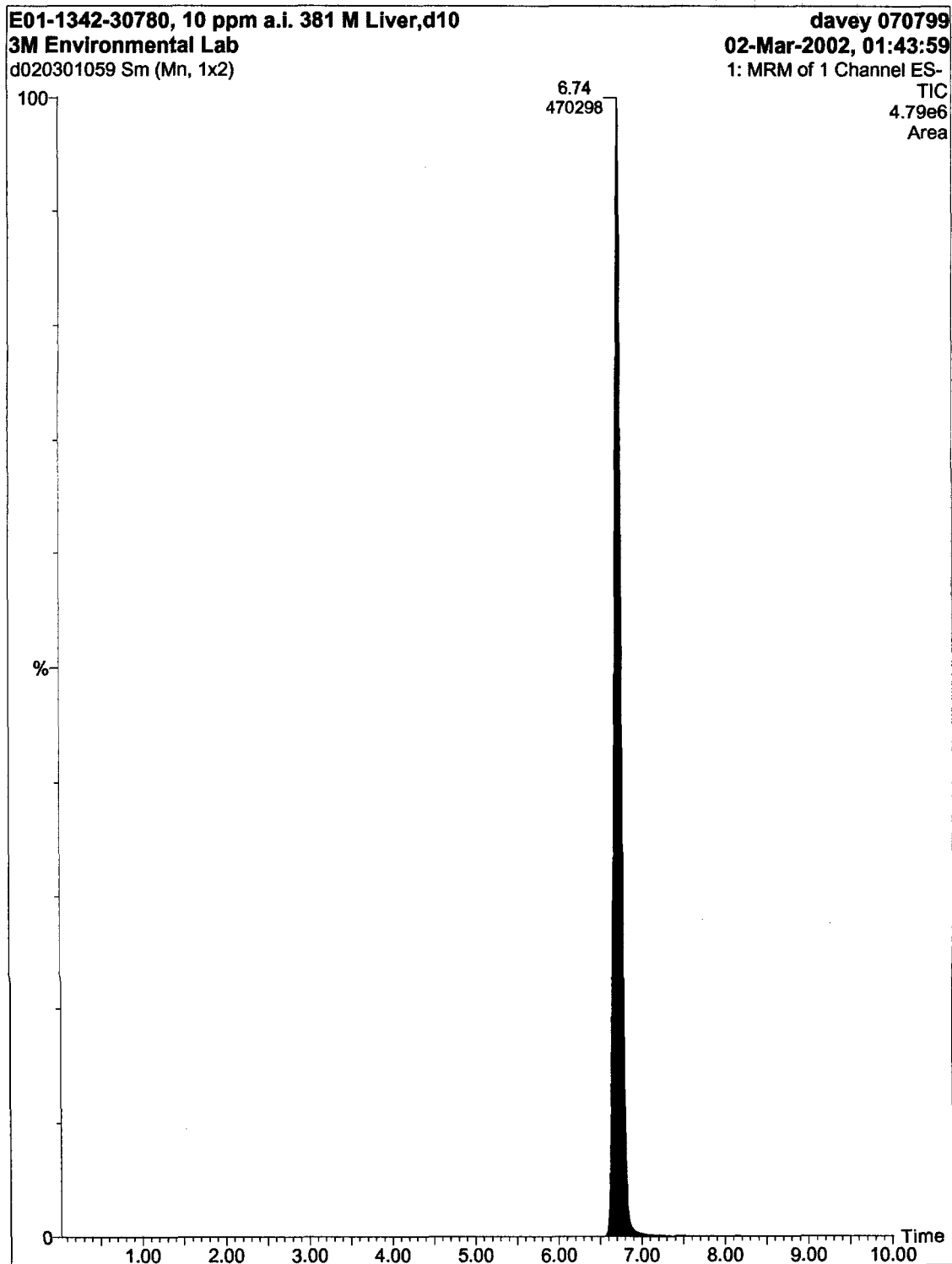
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



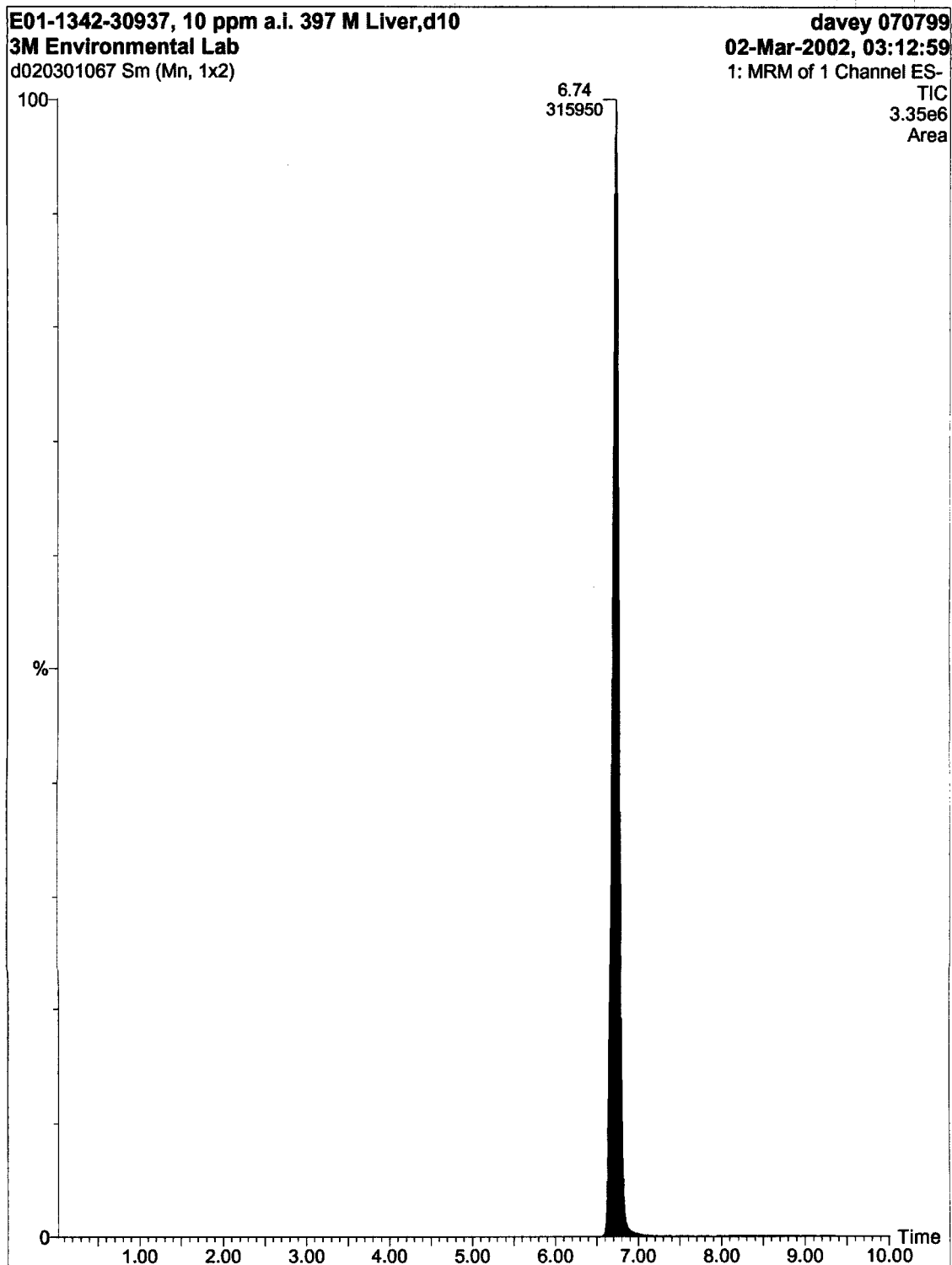
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



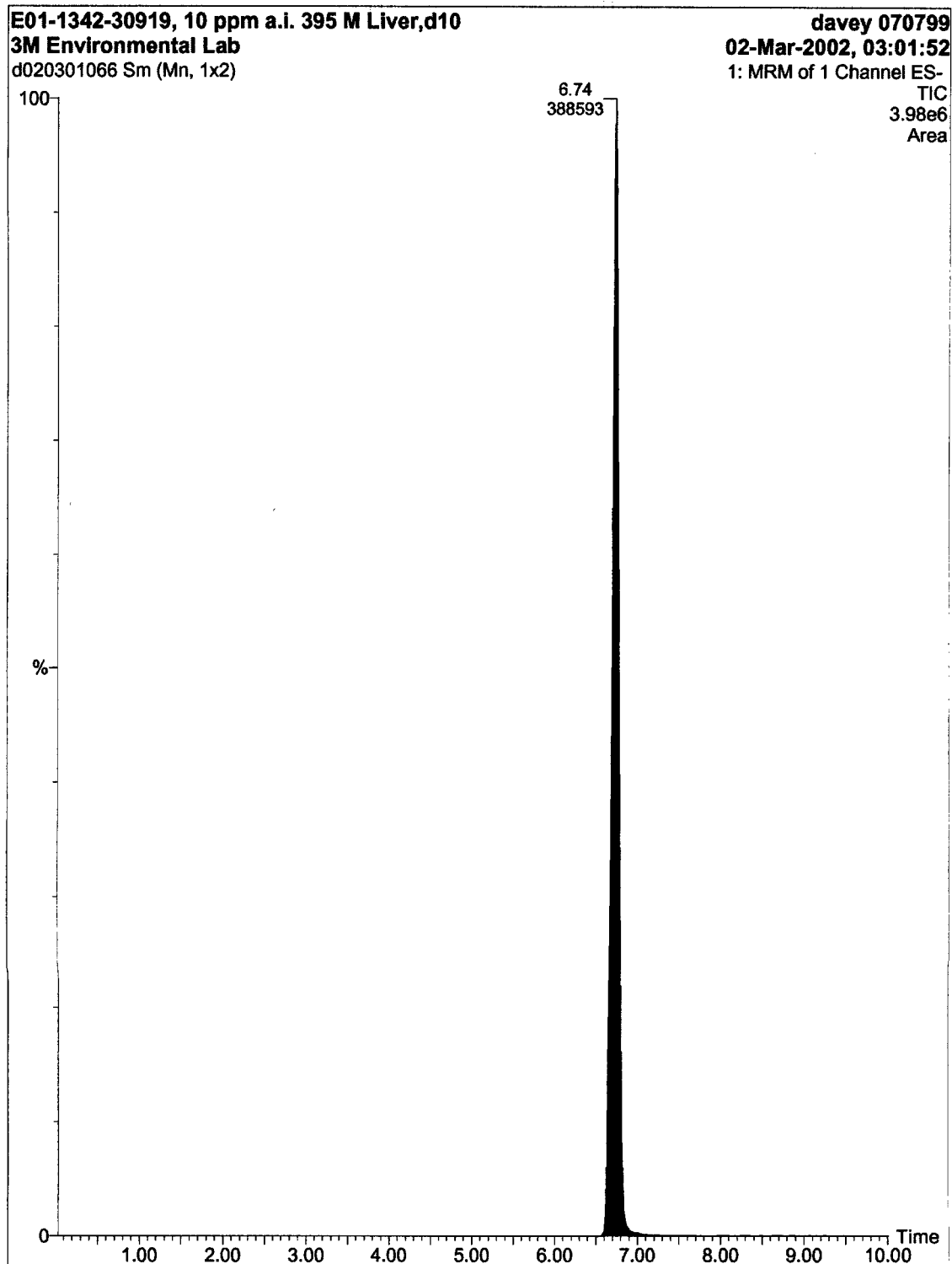
Report E01-1245

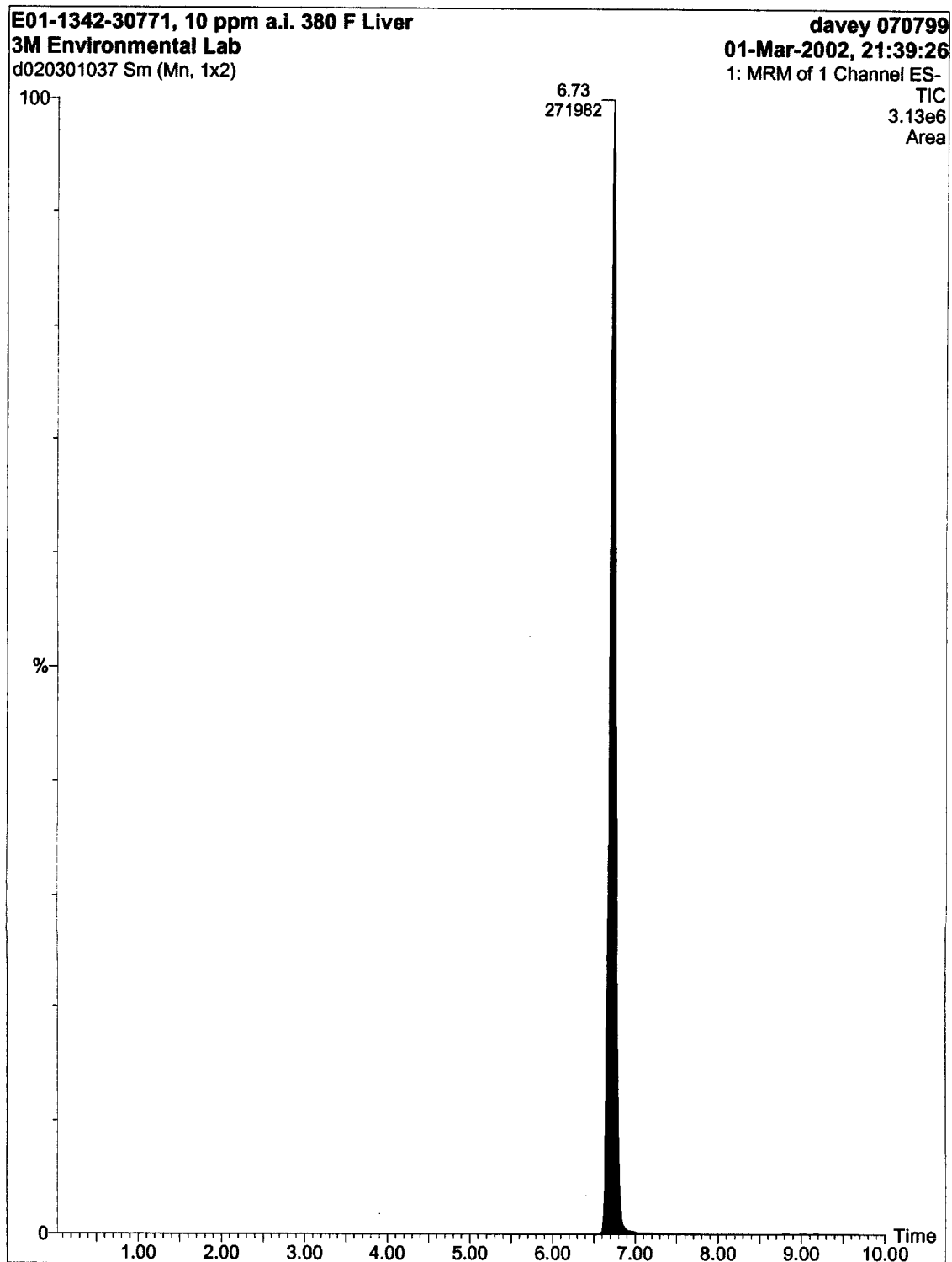
PFOS: A Reproduction Study with the Northern Bobwhite

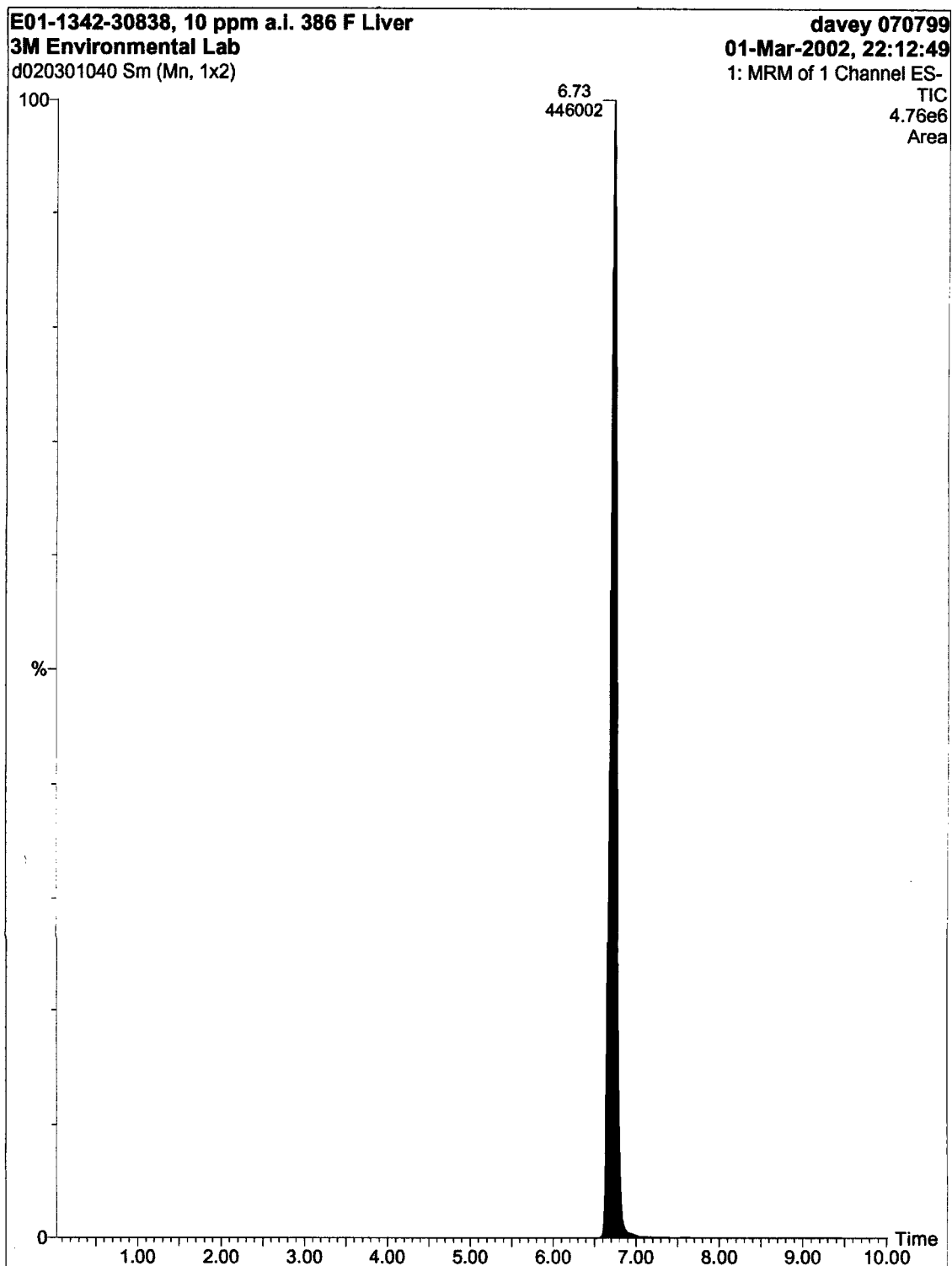


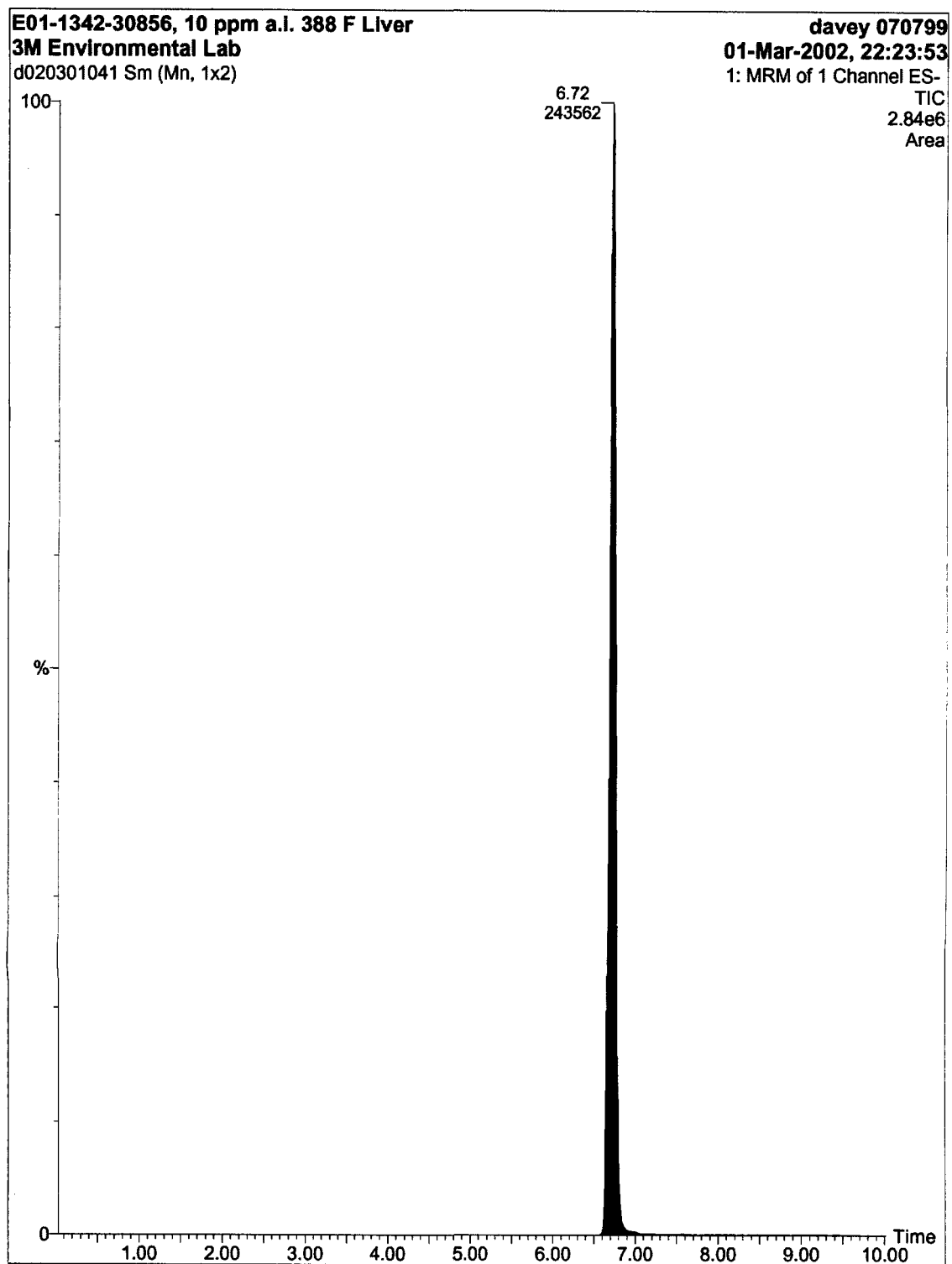
Report E01-1245

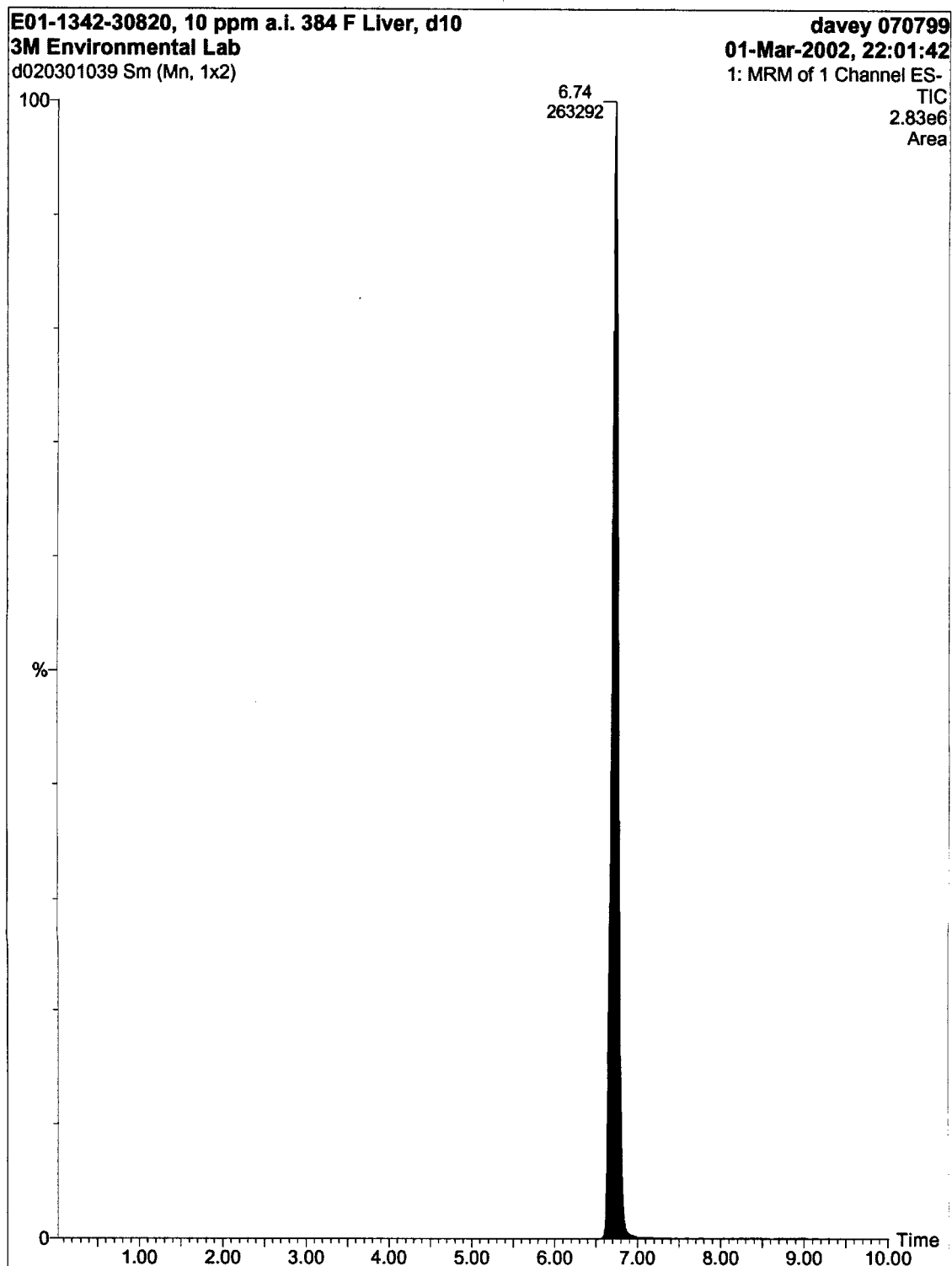
PFOS: A Reproduction Study with the Northern Bobwhite

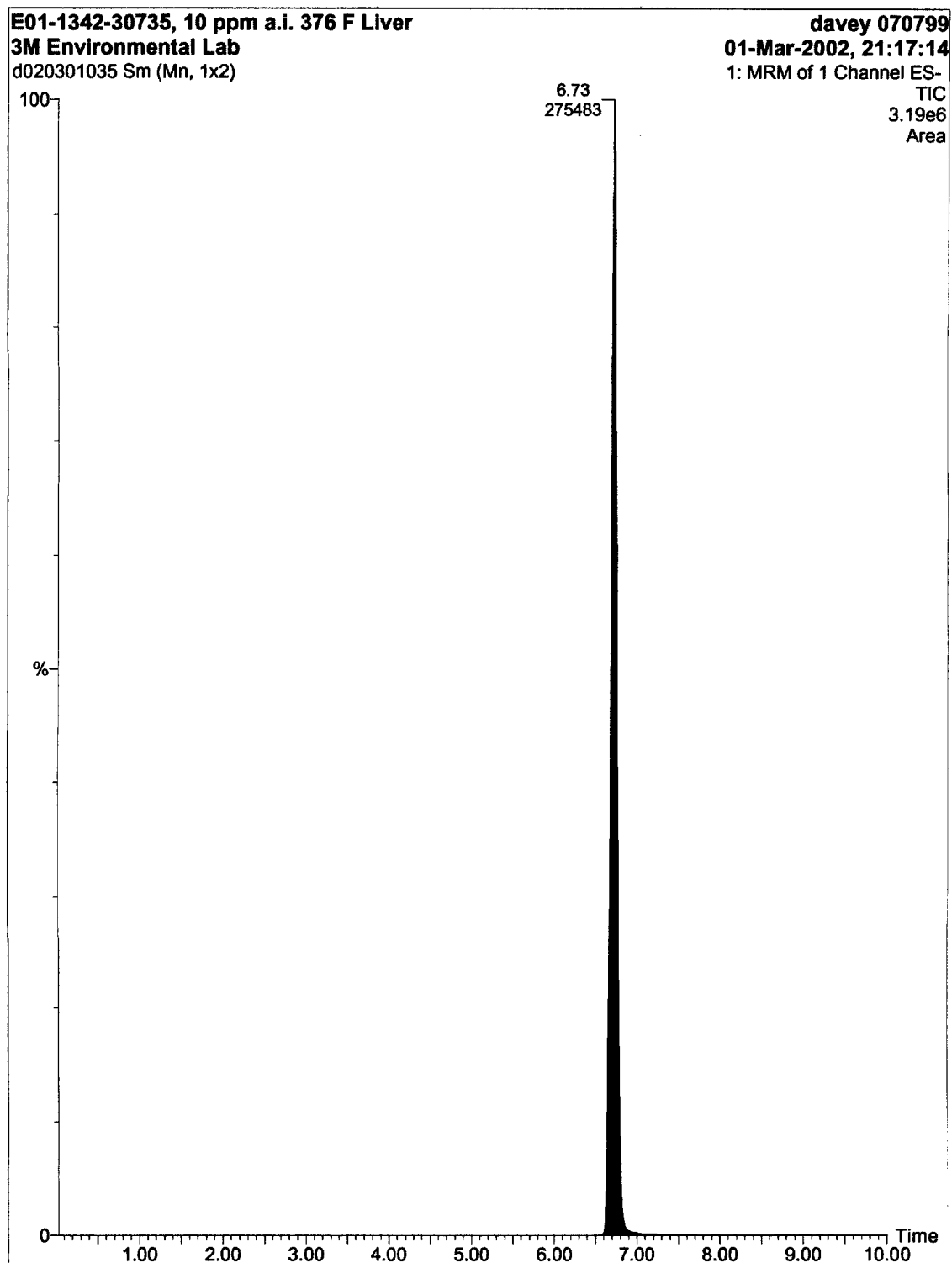


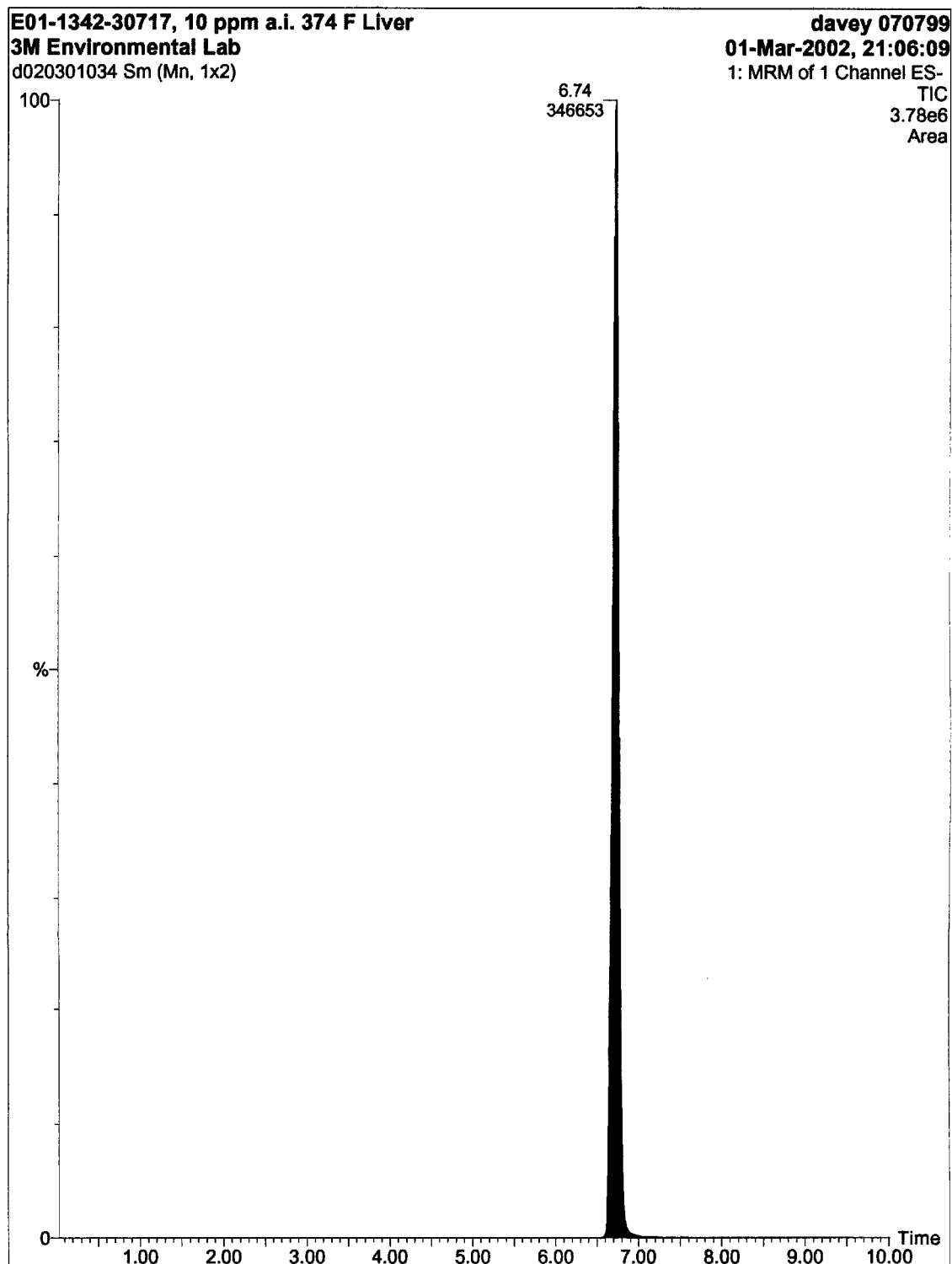
**Dosed Group Female Liver Samples**

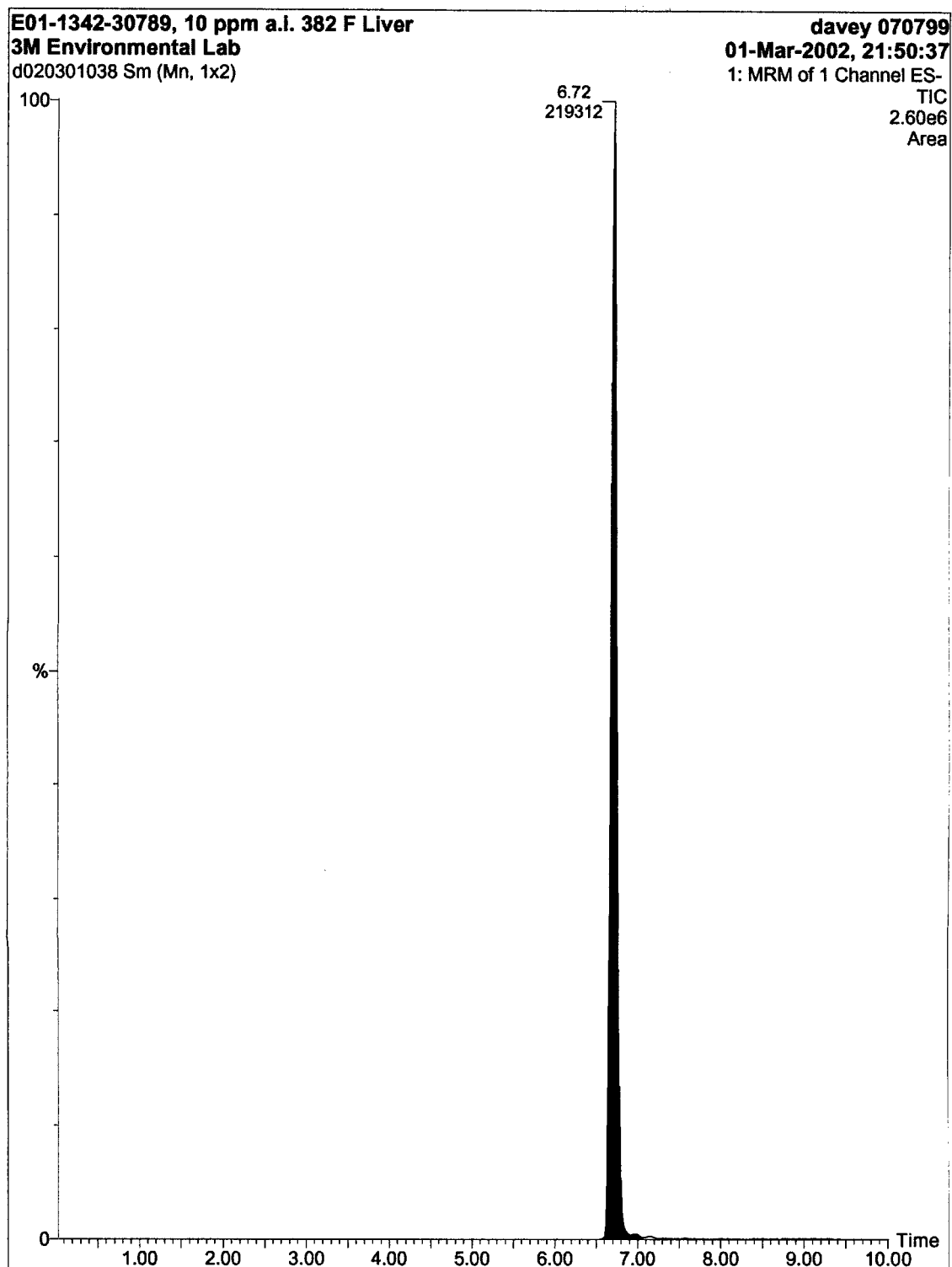


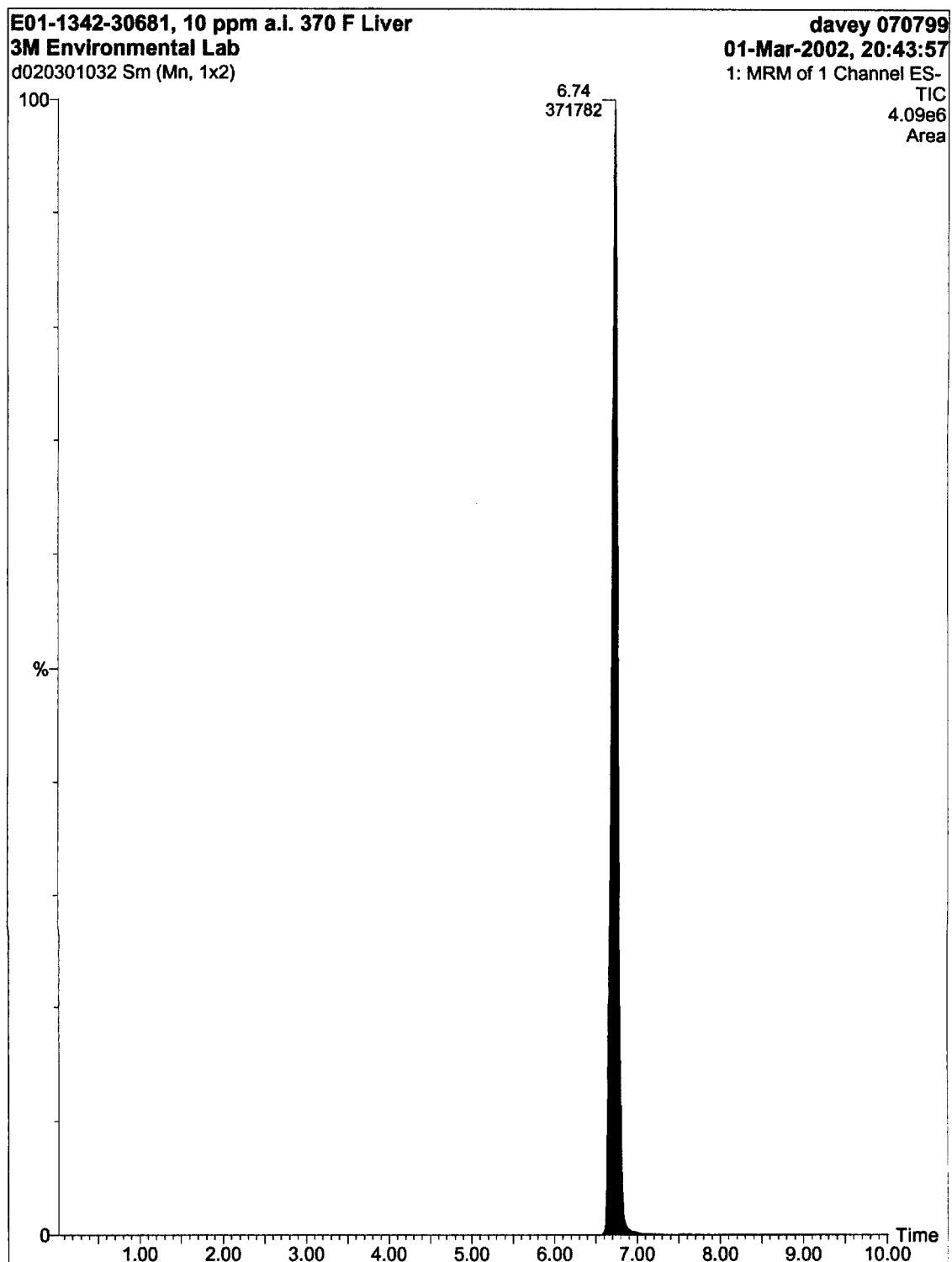


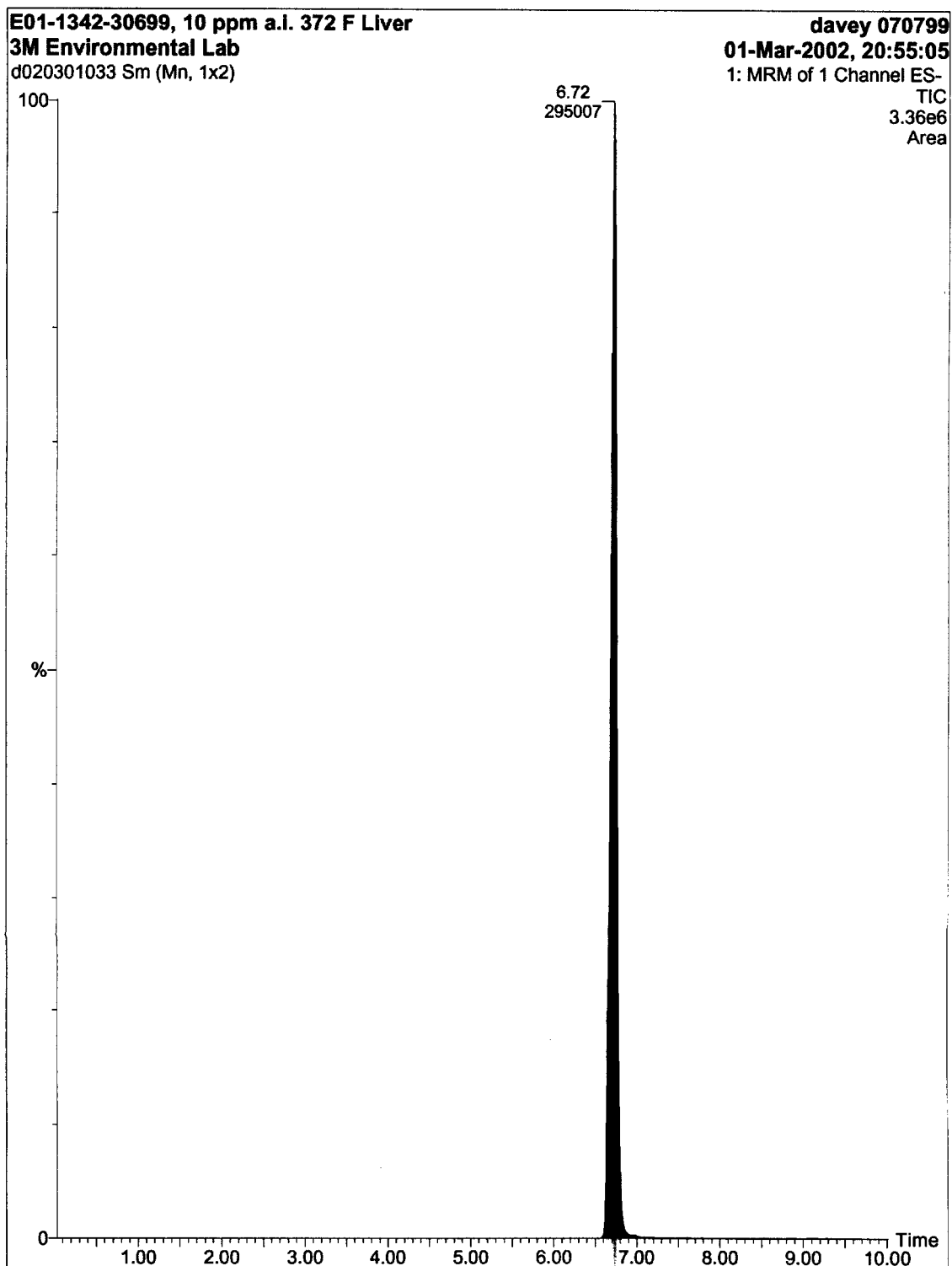






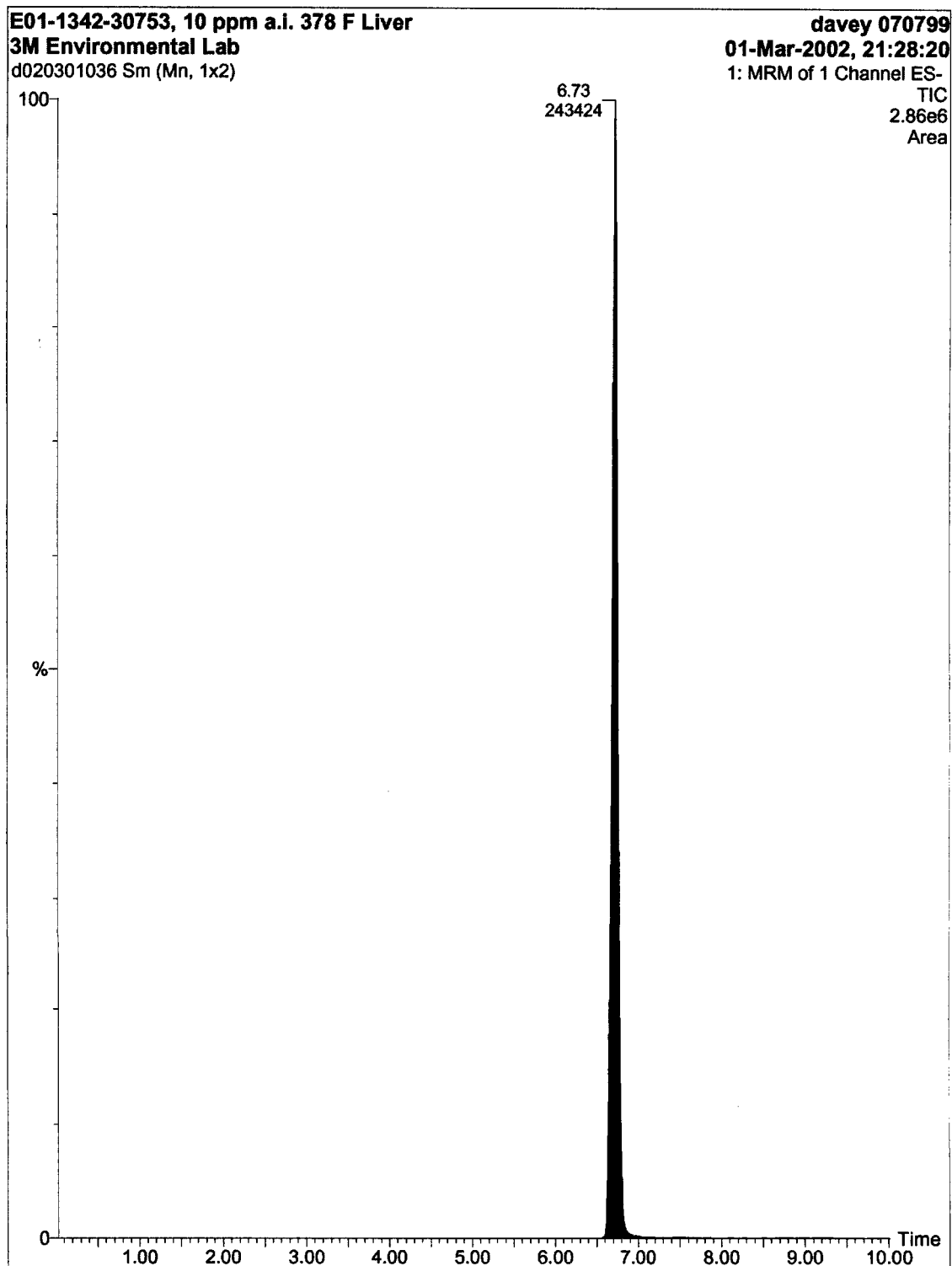






Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



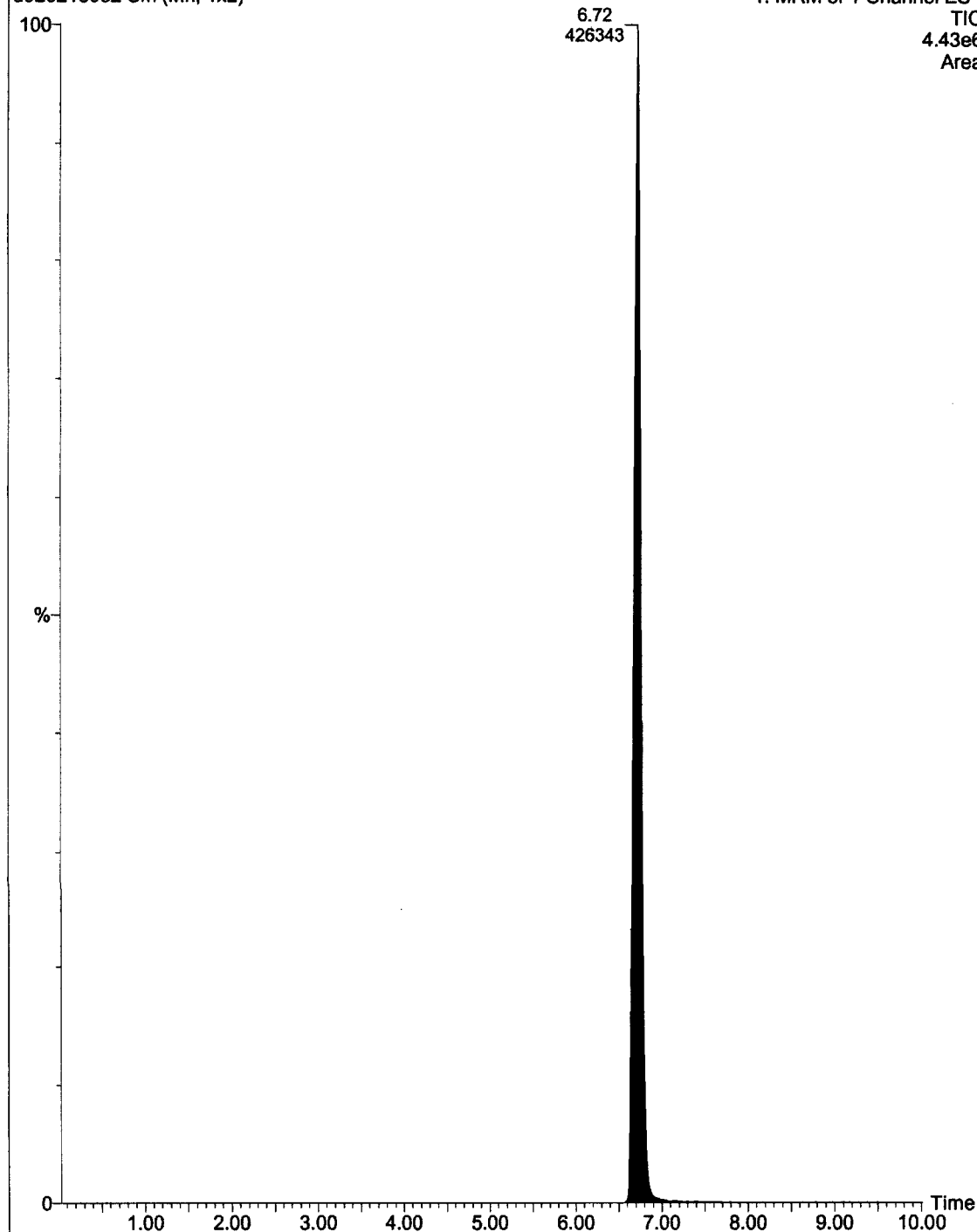
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite

**Dosed Group Male Offspring Liver Samples**

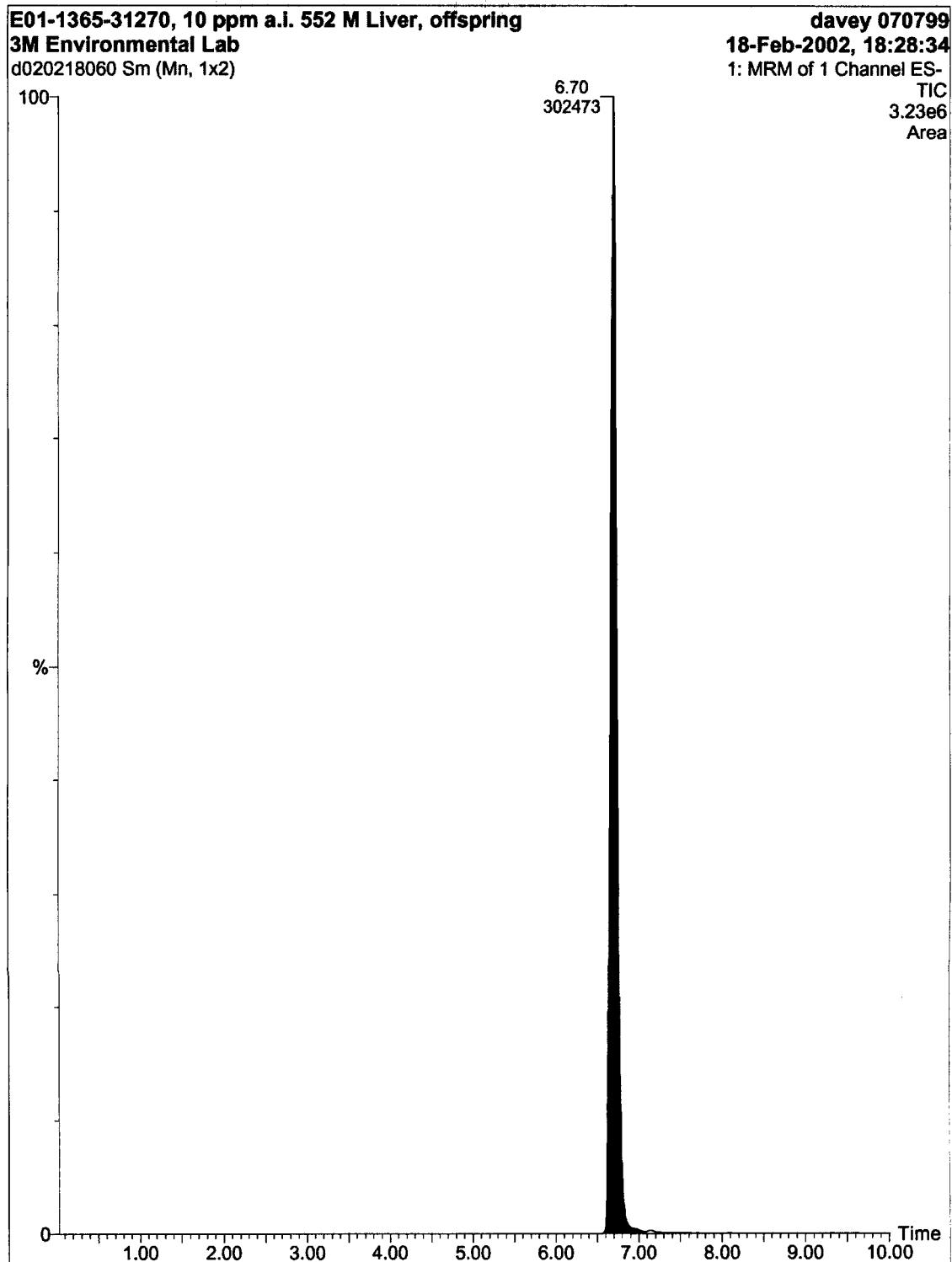
E01-1365-31245, 10 ppm a.i. 522 M Liver, offspring  
3M Environmental Lab  
d020218052 Sm (Mn, 1x2)

davey 070799  
18-Feb-2002, 16:59:46  
1: MRM of 1 Channel ES-  
TIC  
4.43e6  
Area



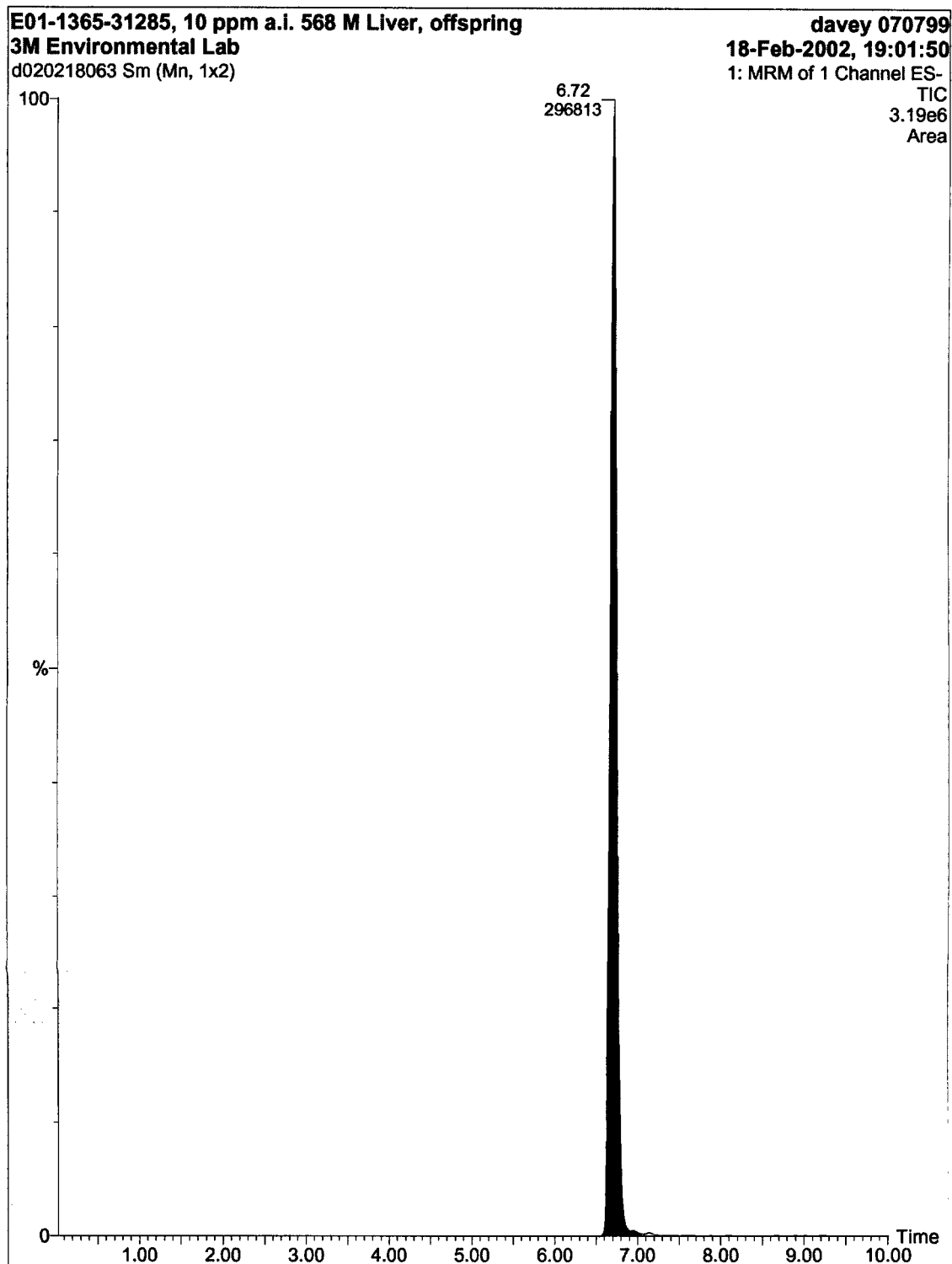
Report E01-1245

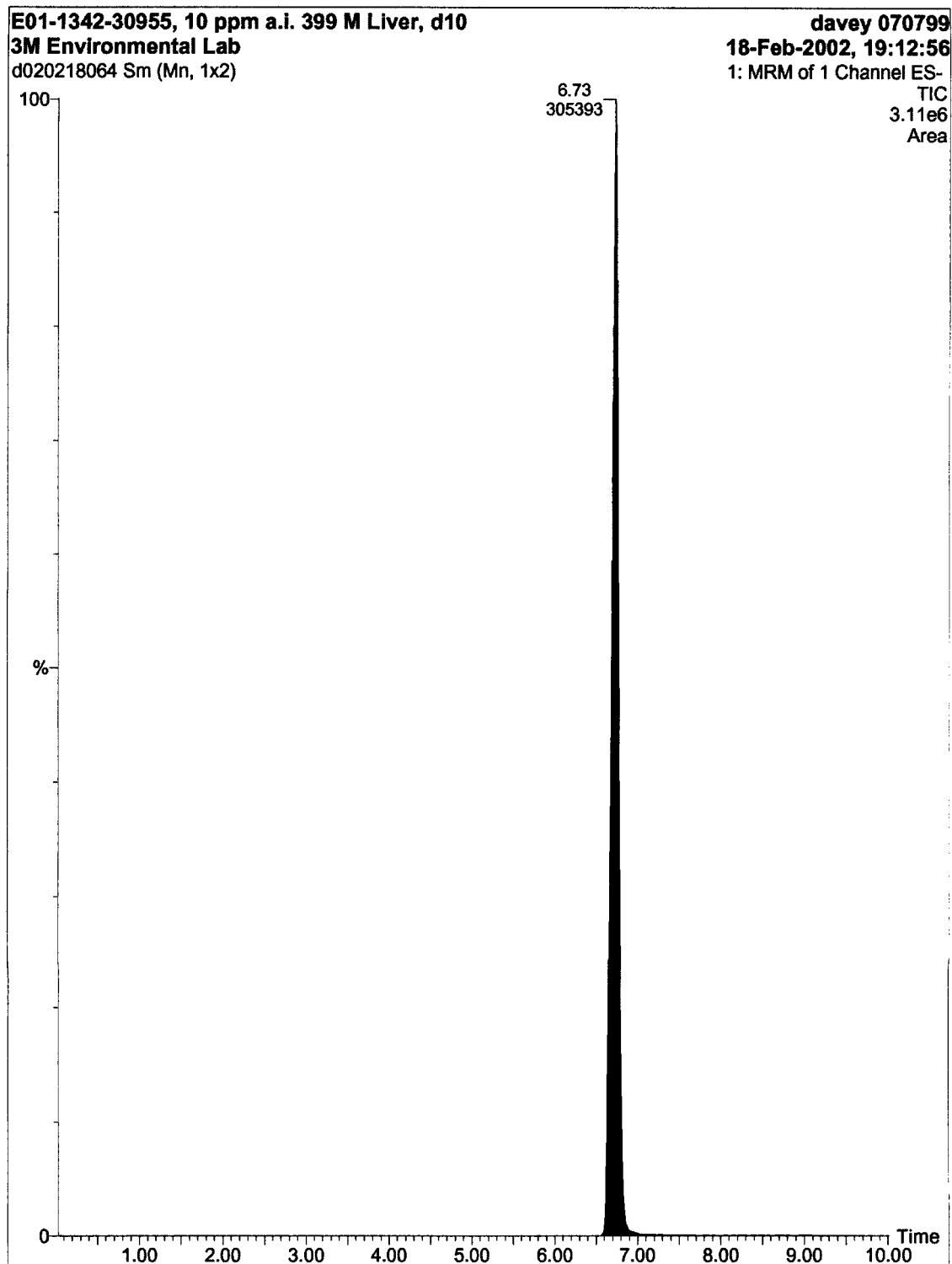
PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245

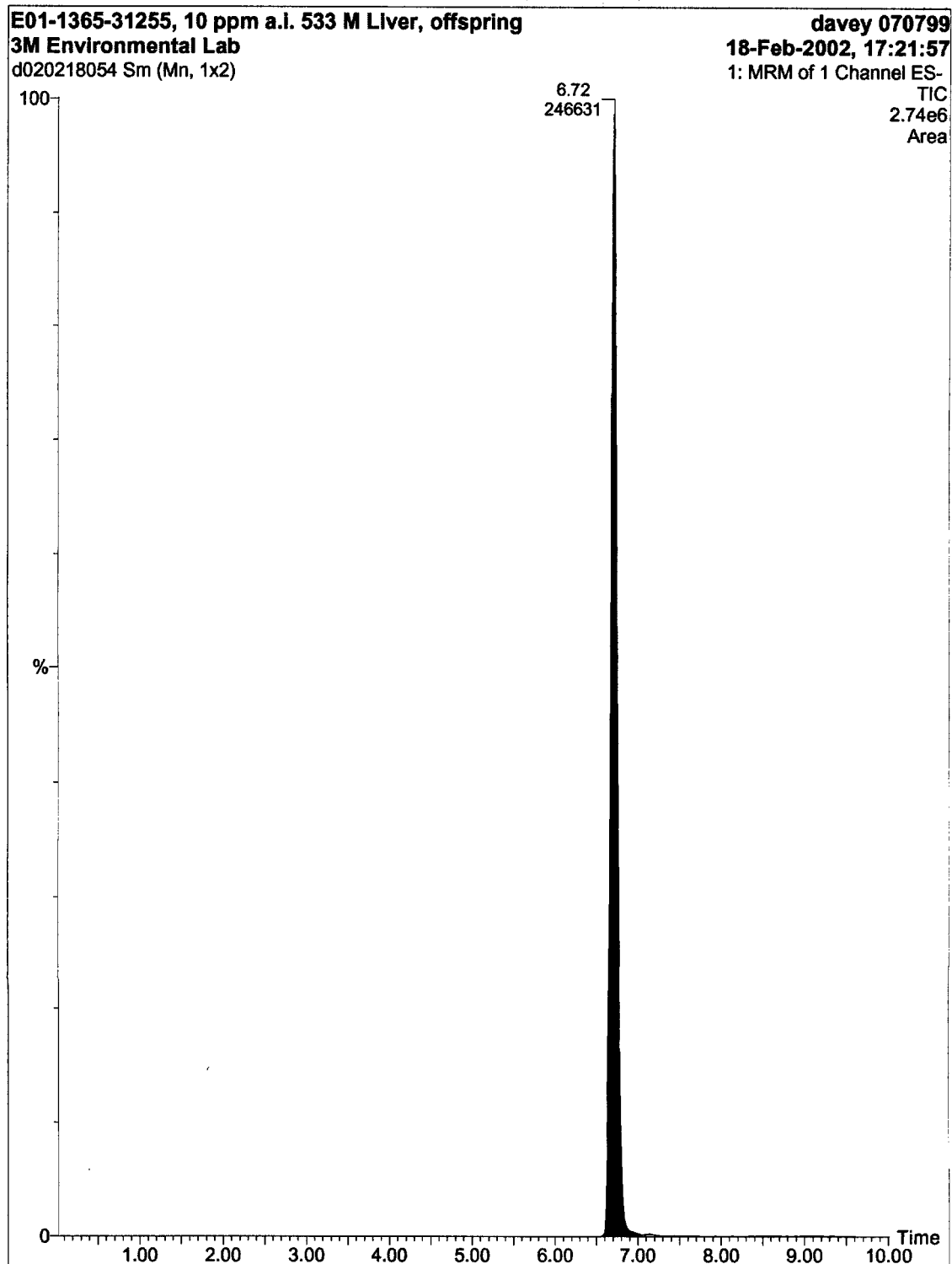
PFOS: A Reproduction Study with the Northern Bobwhite





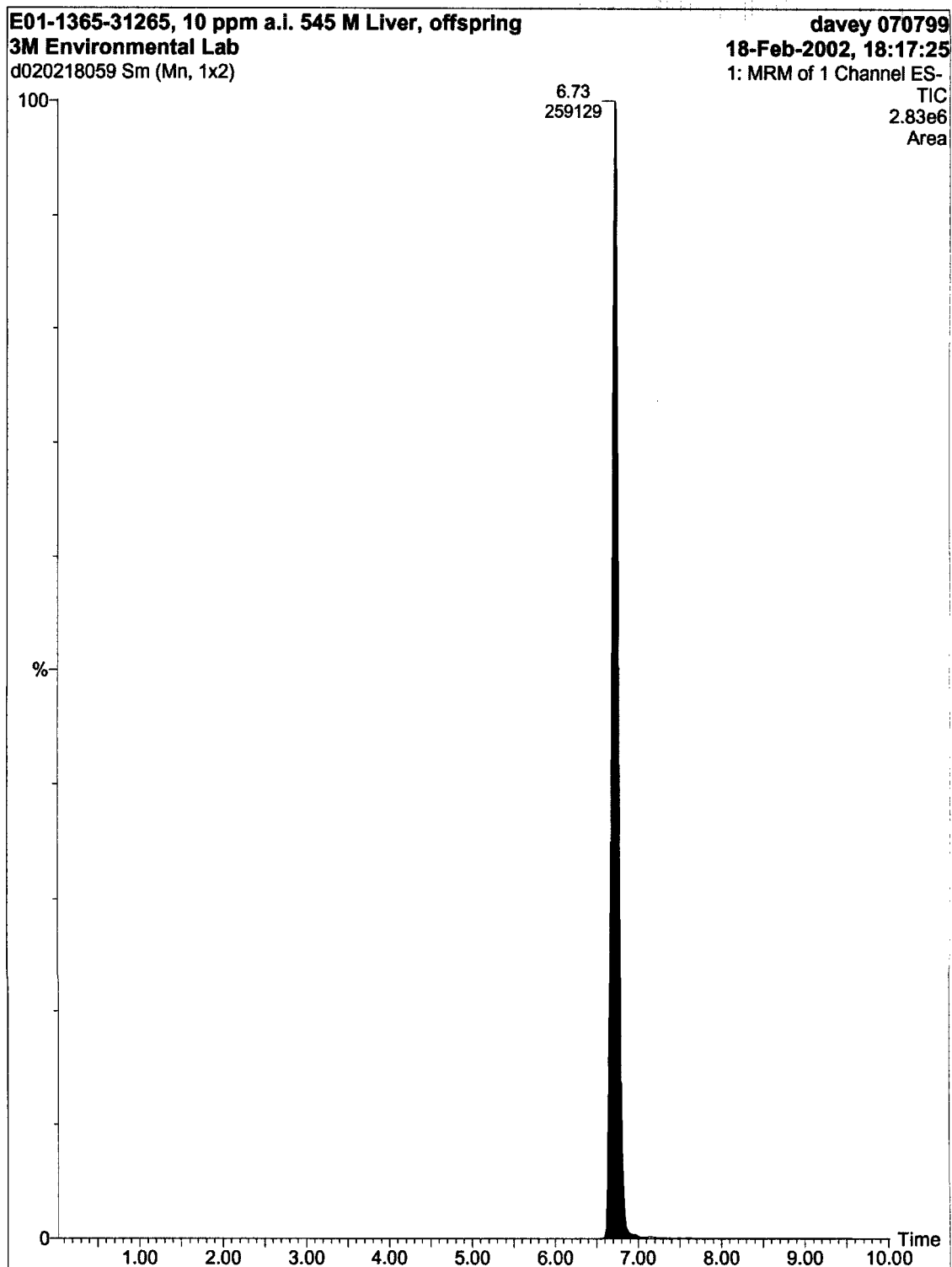
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite



Report E01-1245

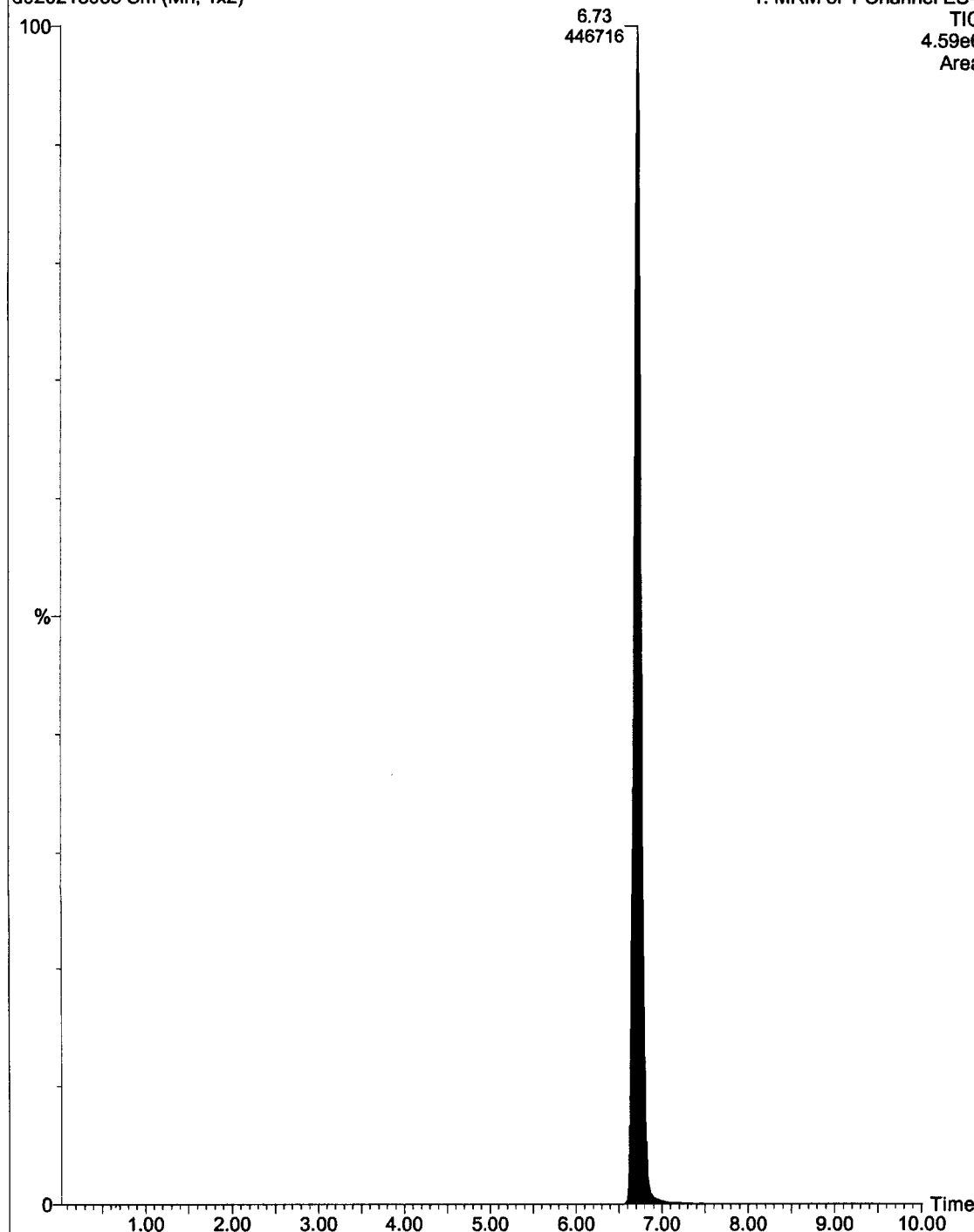
PFOS: A Reproduction Study with the Northern Bobwhite

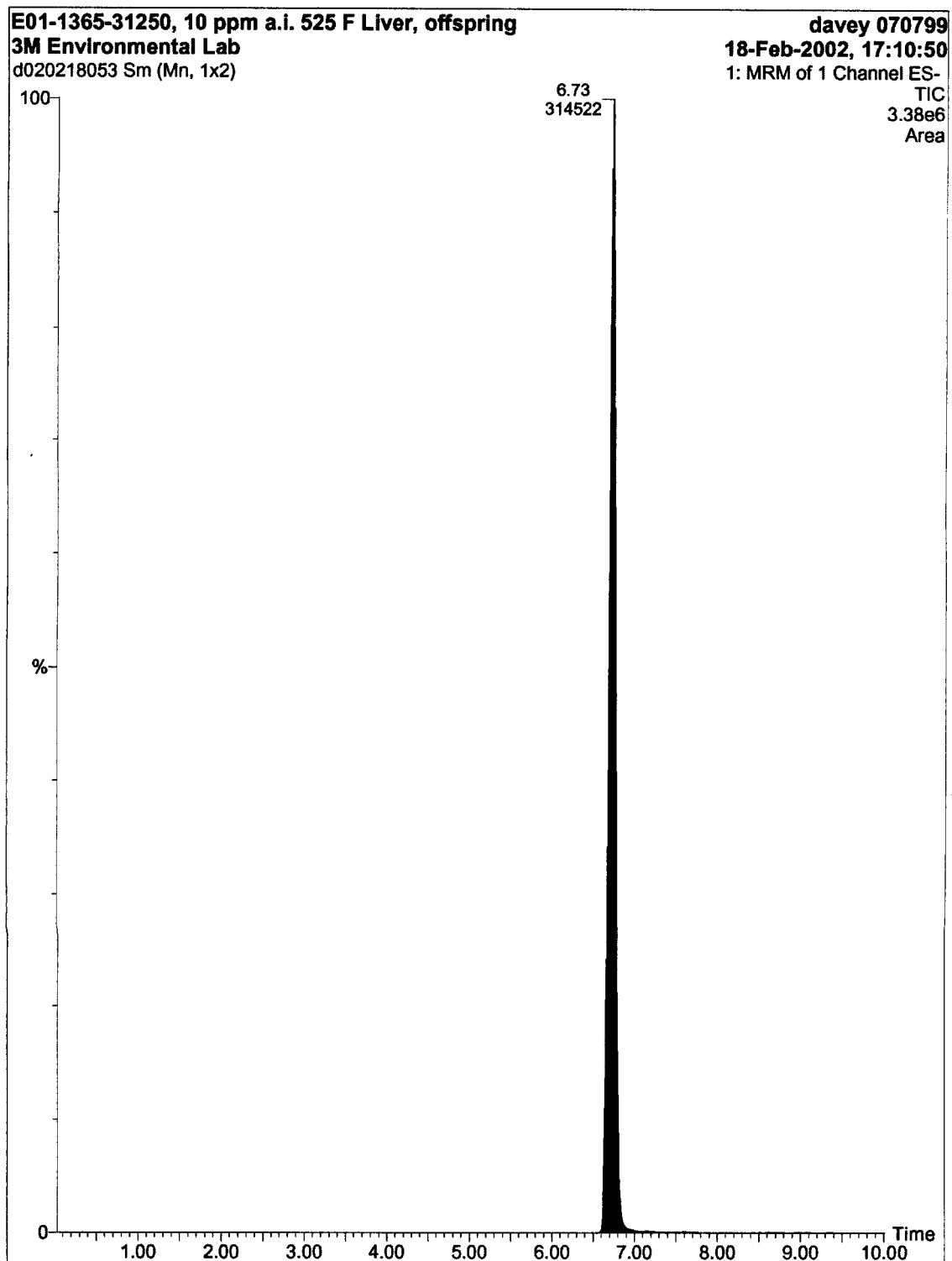


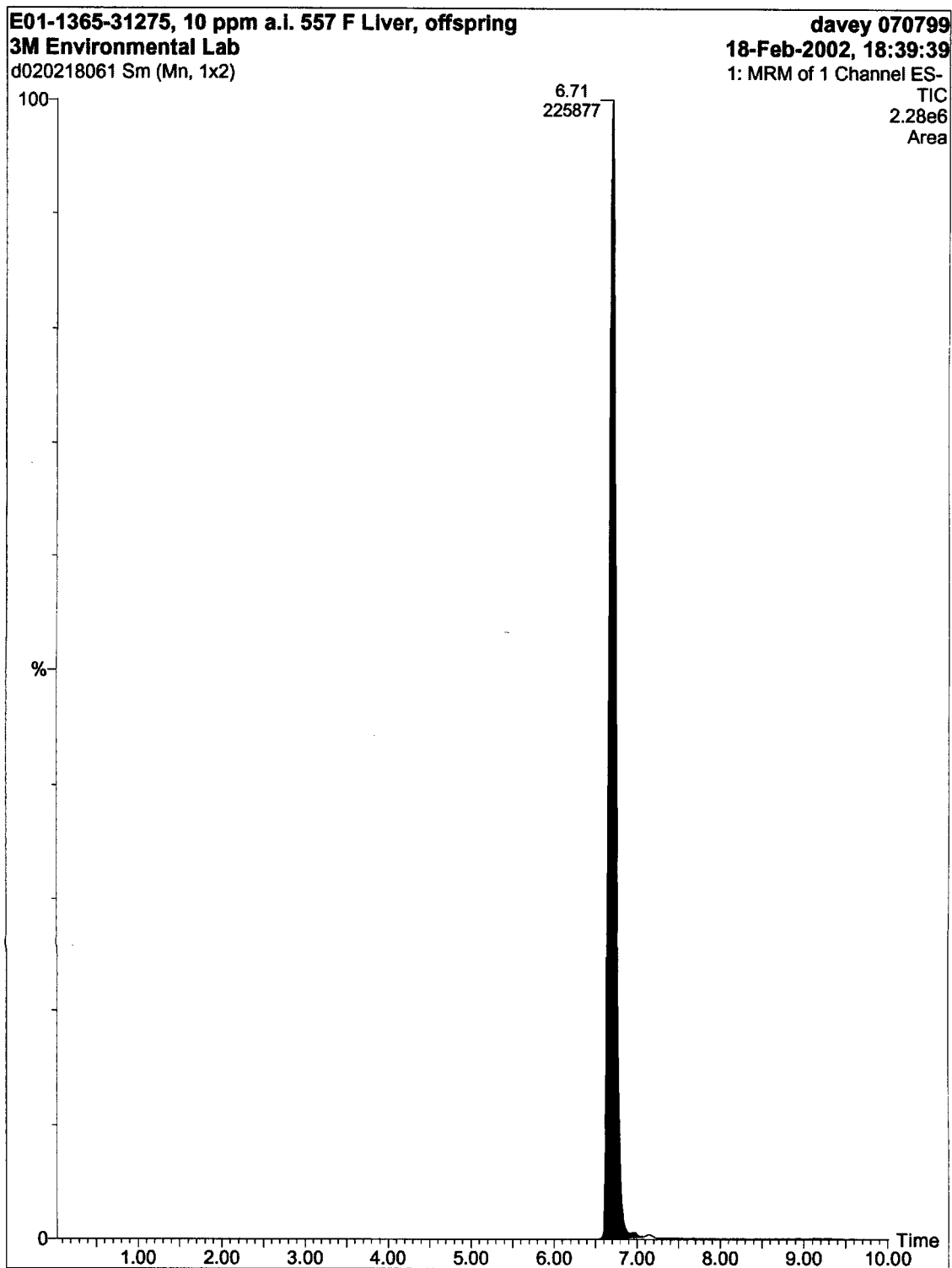
**Dosed Group Female Offspring Liver Samples**

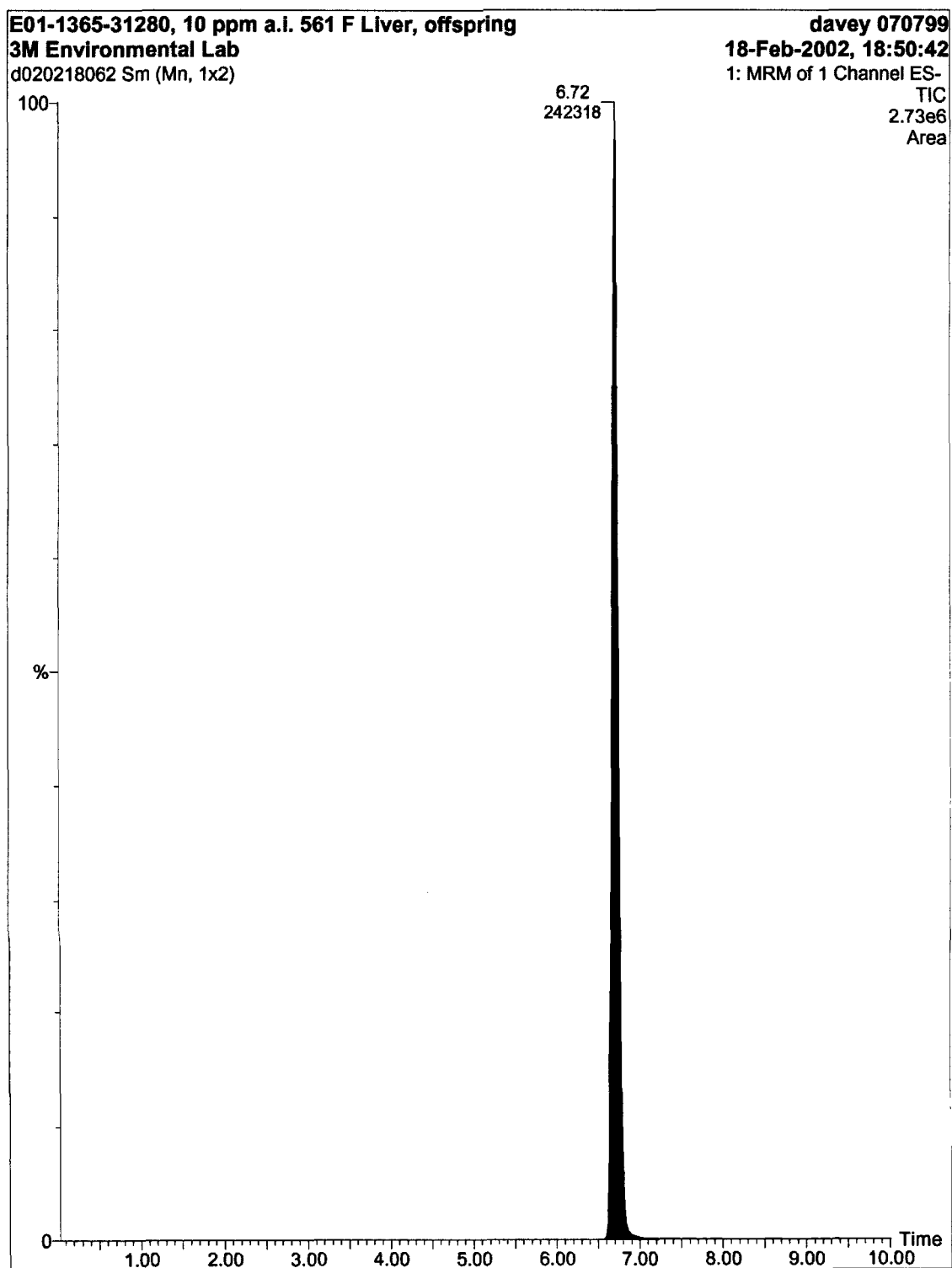
E01-1365-31260, 10 ppm a.i. 538 F Liver, offspring  
3M Environmental Lab  
d020218055 Sm (Mn, 1x2)

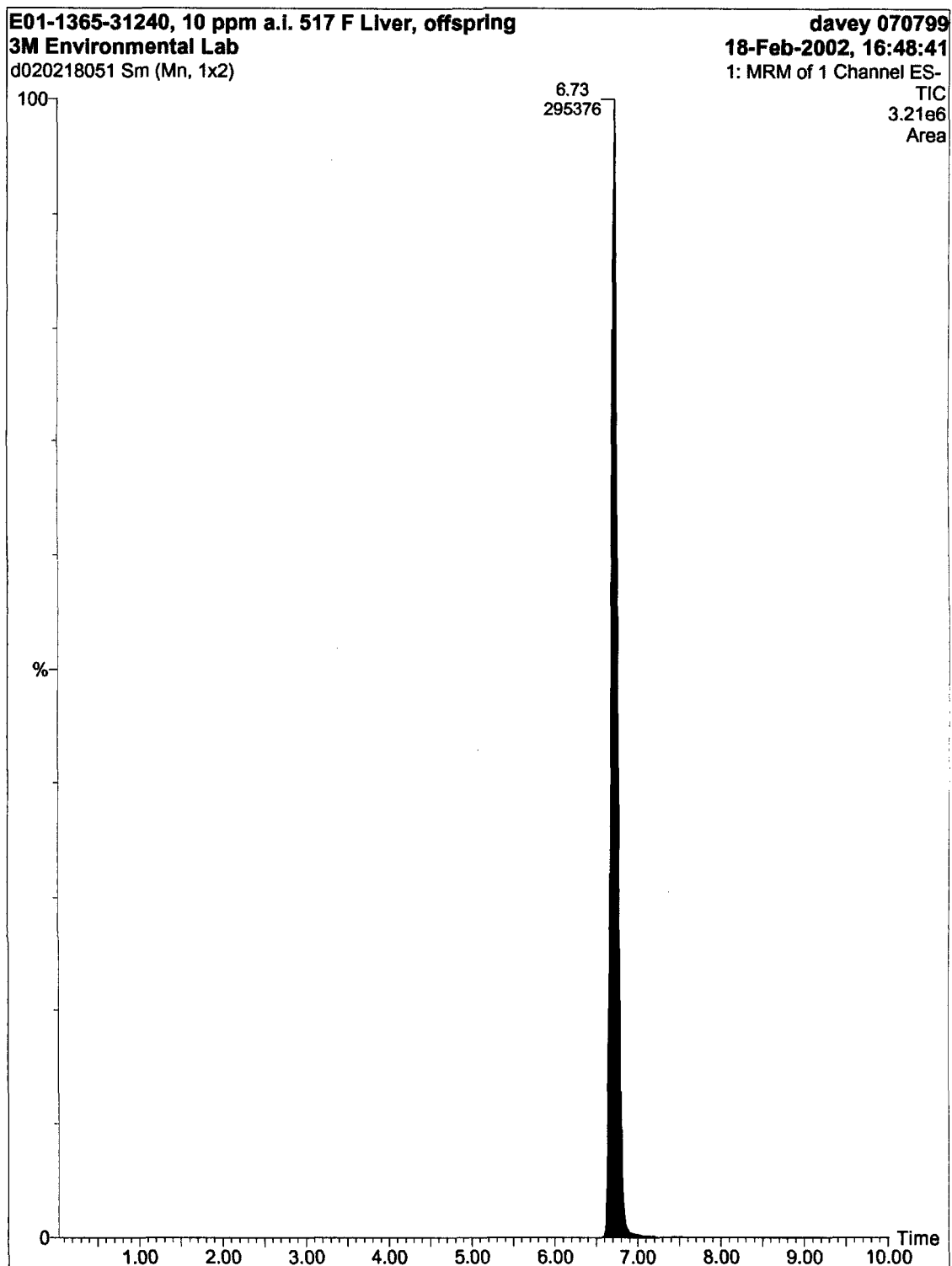
davey 070799  
18-Feb-2002, 17:33:05  
1: MRM of 1 Channel ES-  
TIC  
4.59e6  
Area

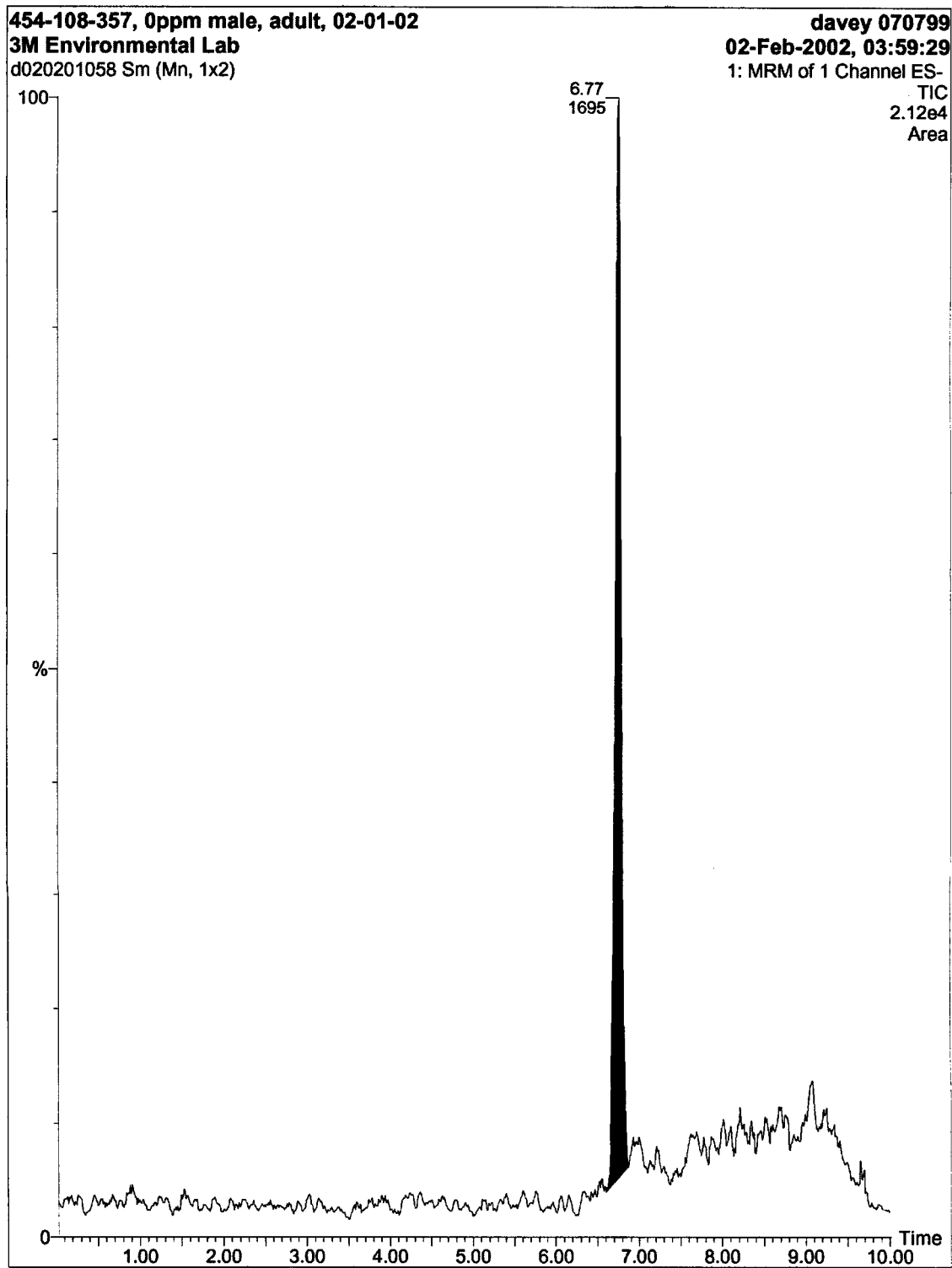






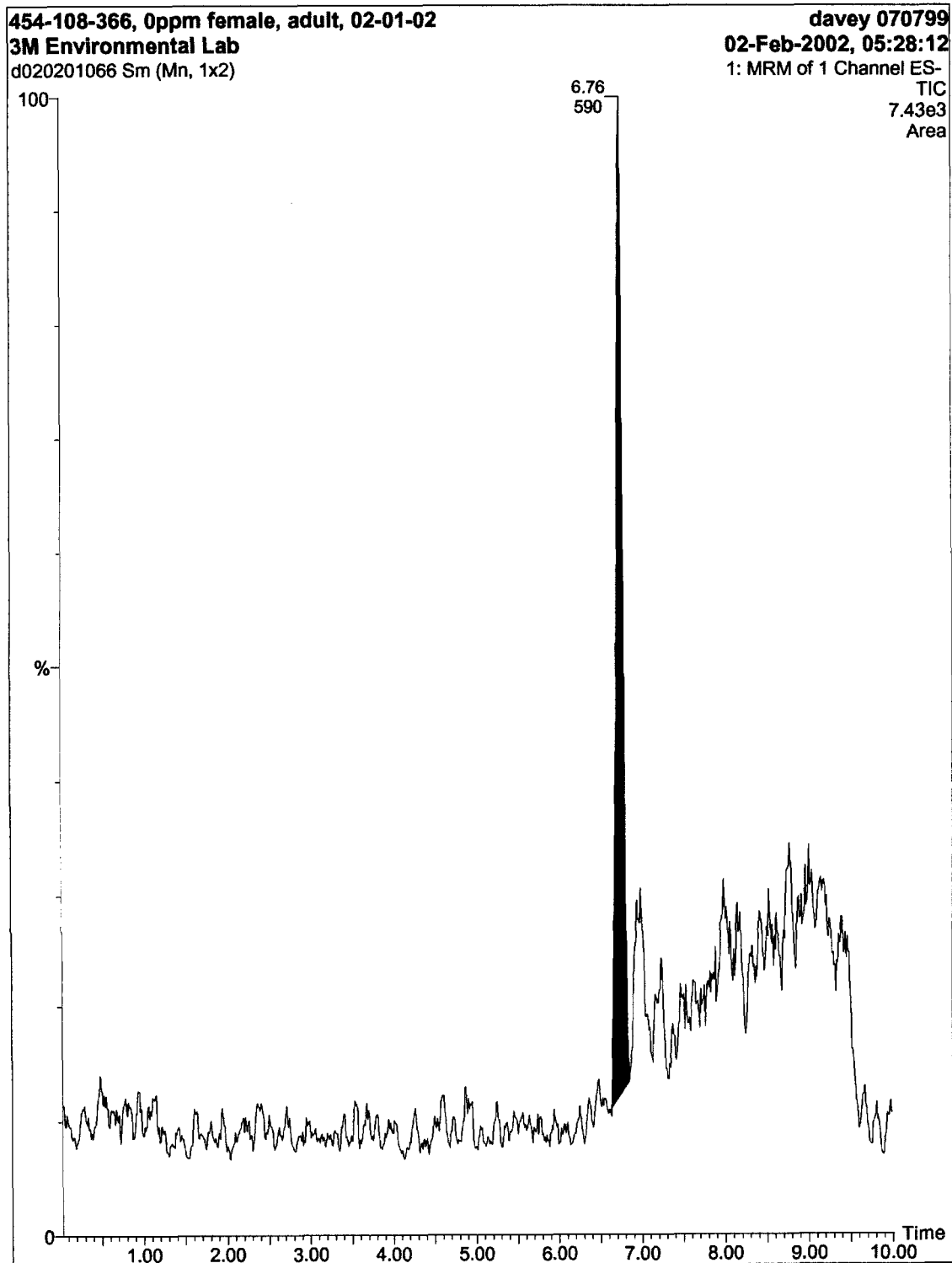




**Control Group Male Sera Sample**

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**

454-108-253, 0ppm male, offspring, 02-01-02

3M Environmental Lab

D 0.0 %

davey 070799

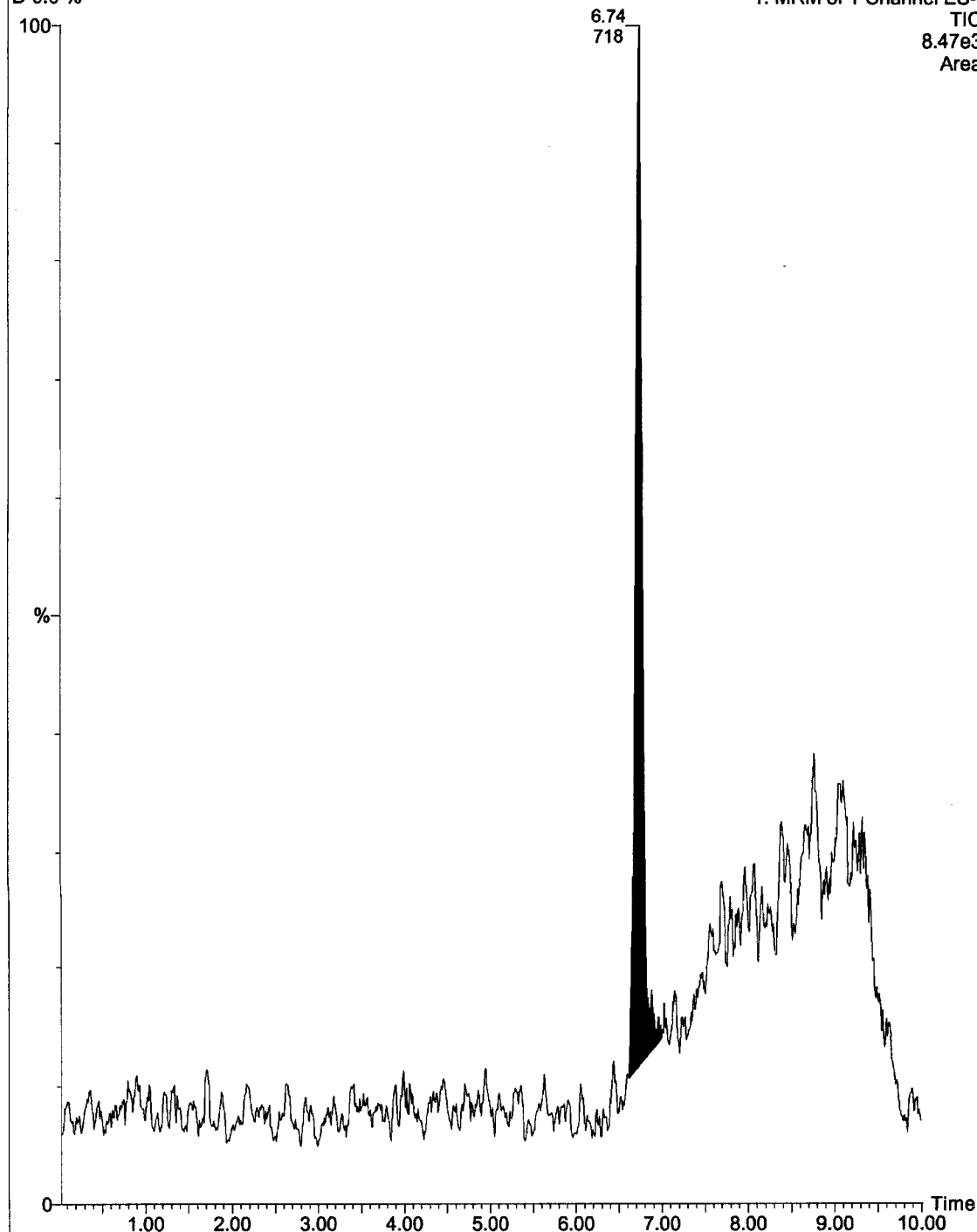
08-Feb-2002, 13:34:19

1: MRM of 1 Channel ES-

TIC

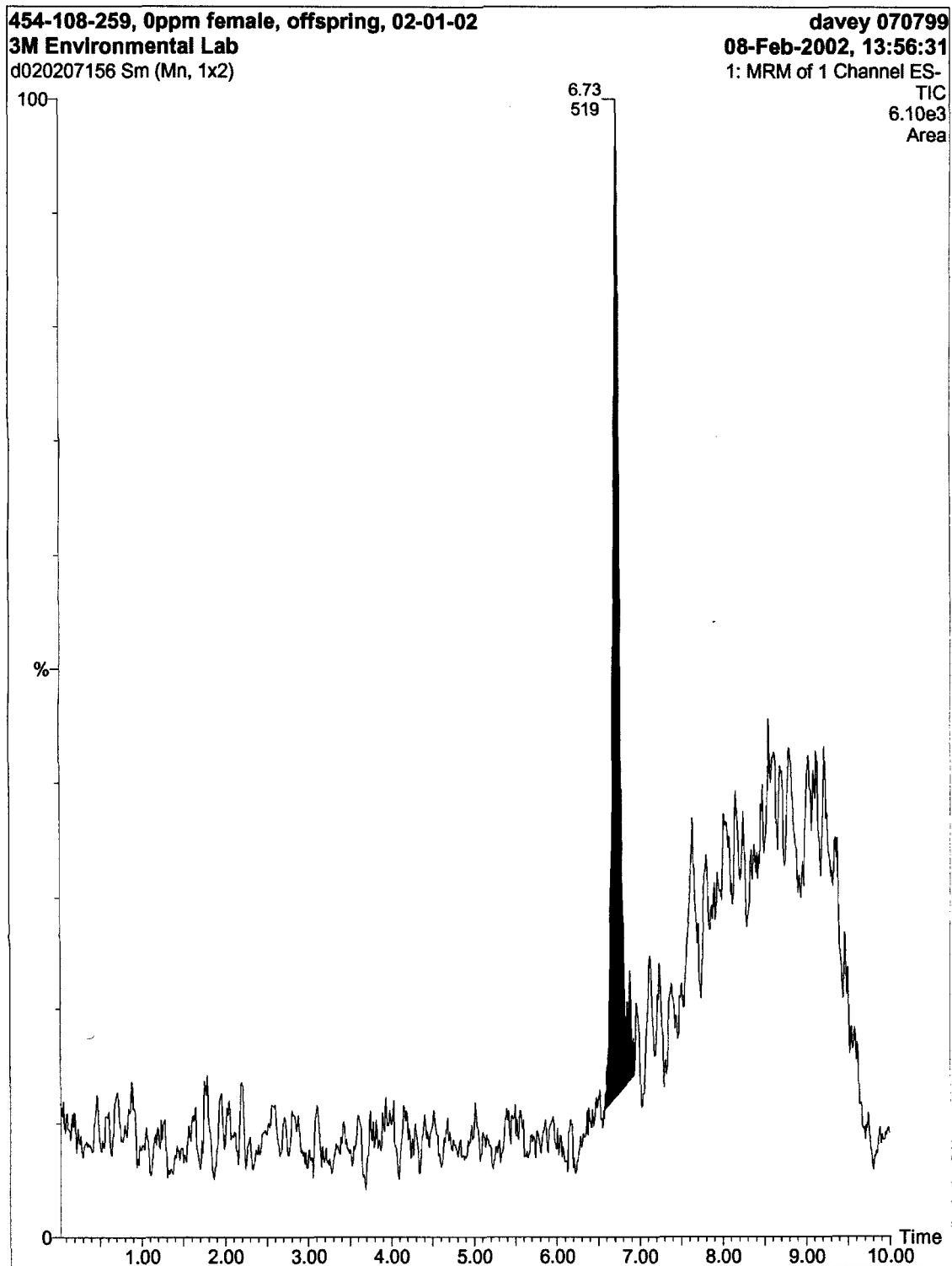
8.47e3

Area



Report E01-1245

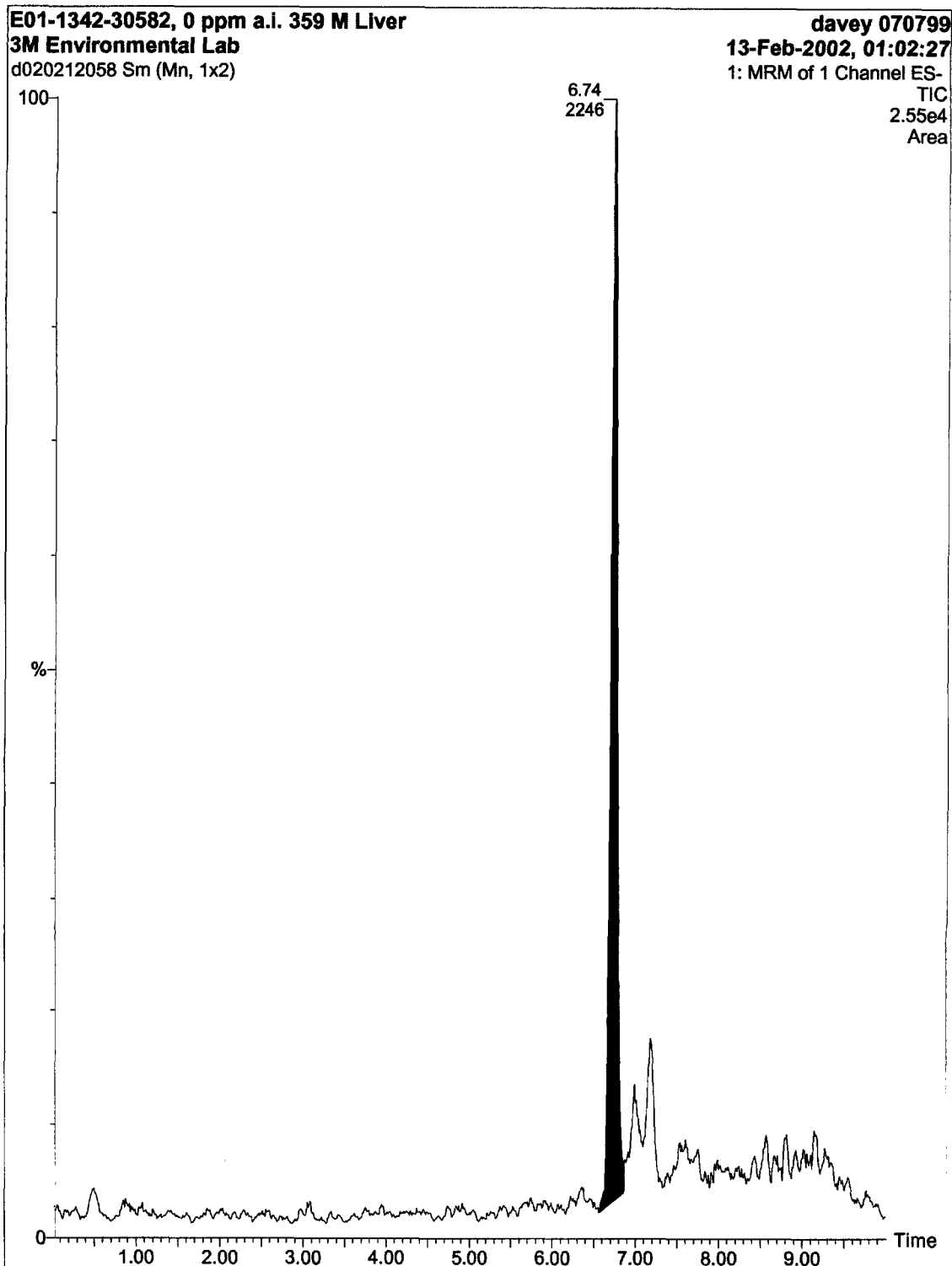
PFOS: A Reproduction Study with the Northern Bobwhite

**Control Group Female Offspring Sera Sample**

Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite

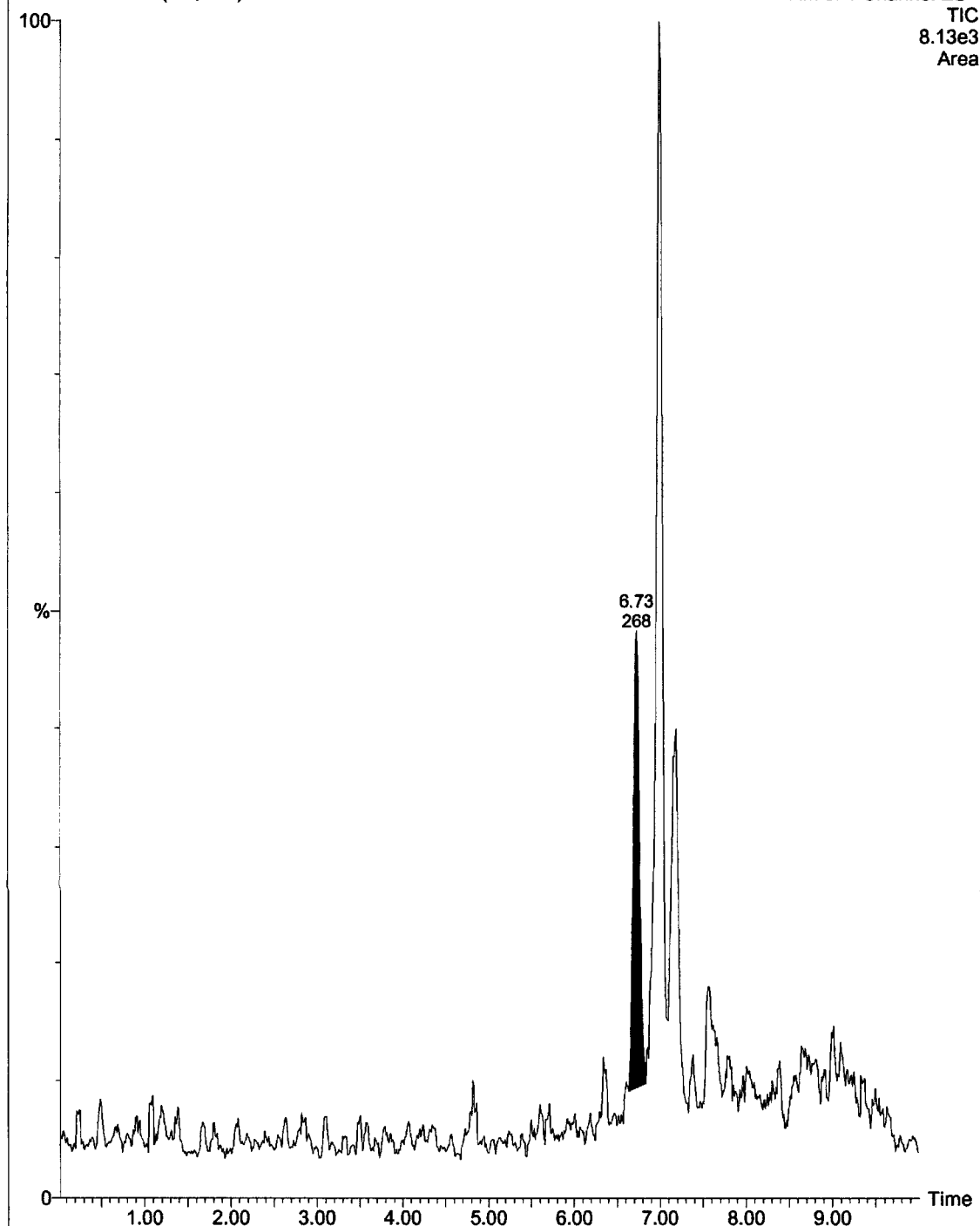
### Control Group Male Liver Sample



**Control Group Female Liver Sample**

E01-1342-30627, 0 ppm a.i. 364 F Liver  
3M Environmental Lab  
d020212063 Sm (Mn, 1x2)

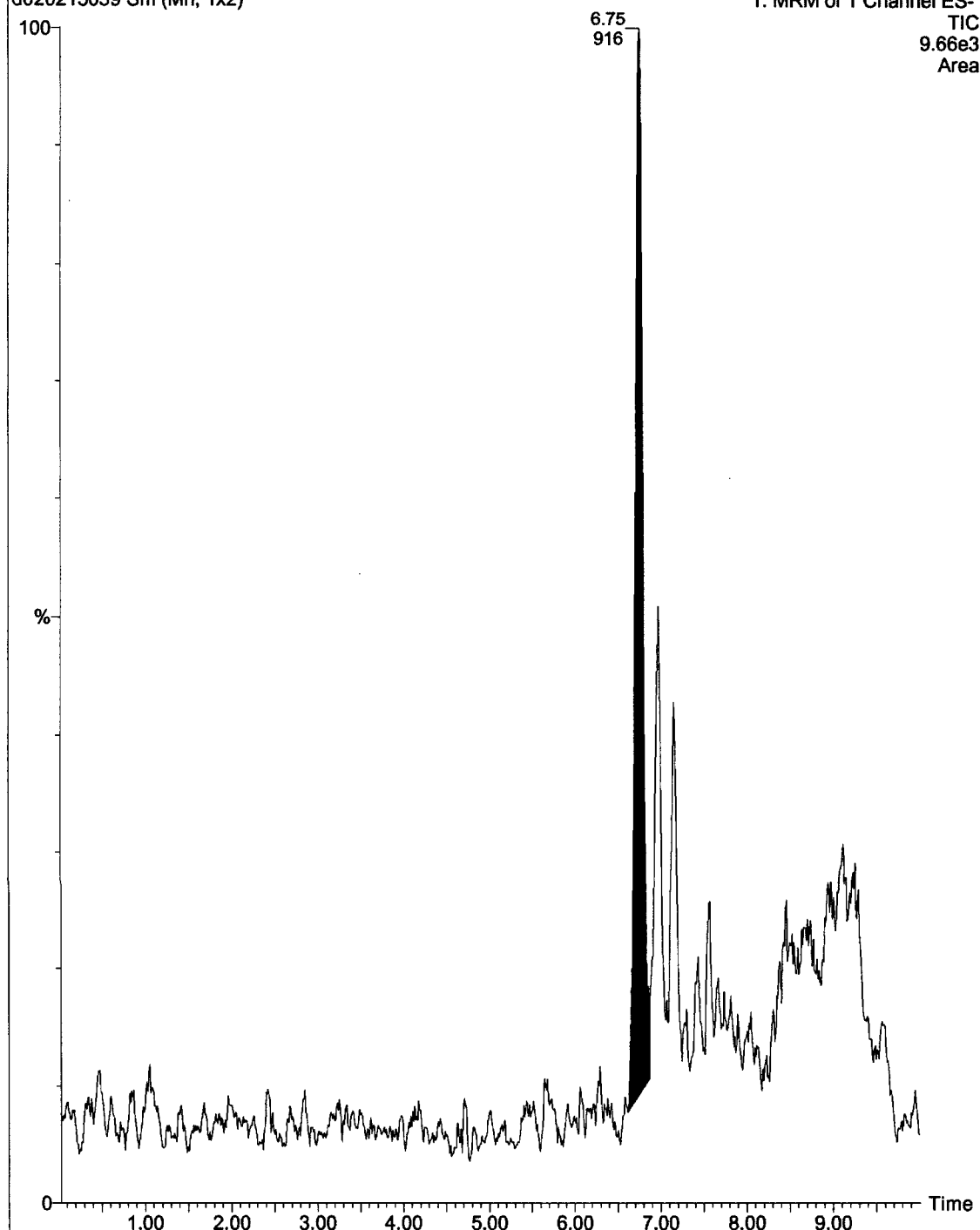
davey 070799  
13-Feb-2002, 01:58:02  
1: MRM of 1 Channel ES-  
TIC  
8.13e3  
Area



**Control Group Male Offspring Liver Sample**

E01-1365-31194, 0 ppm a.i. 253 M Liver, offspring  
3M Environmental Lab  
d020215039 Sm (Mn, 1x2)

davey 070799  
15-Feb-2002, 21:12:32  
1: MRM of 1 Channel ES-  
TIC  
9.66e3  
Area



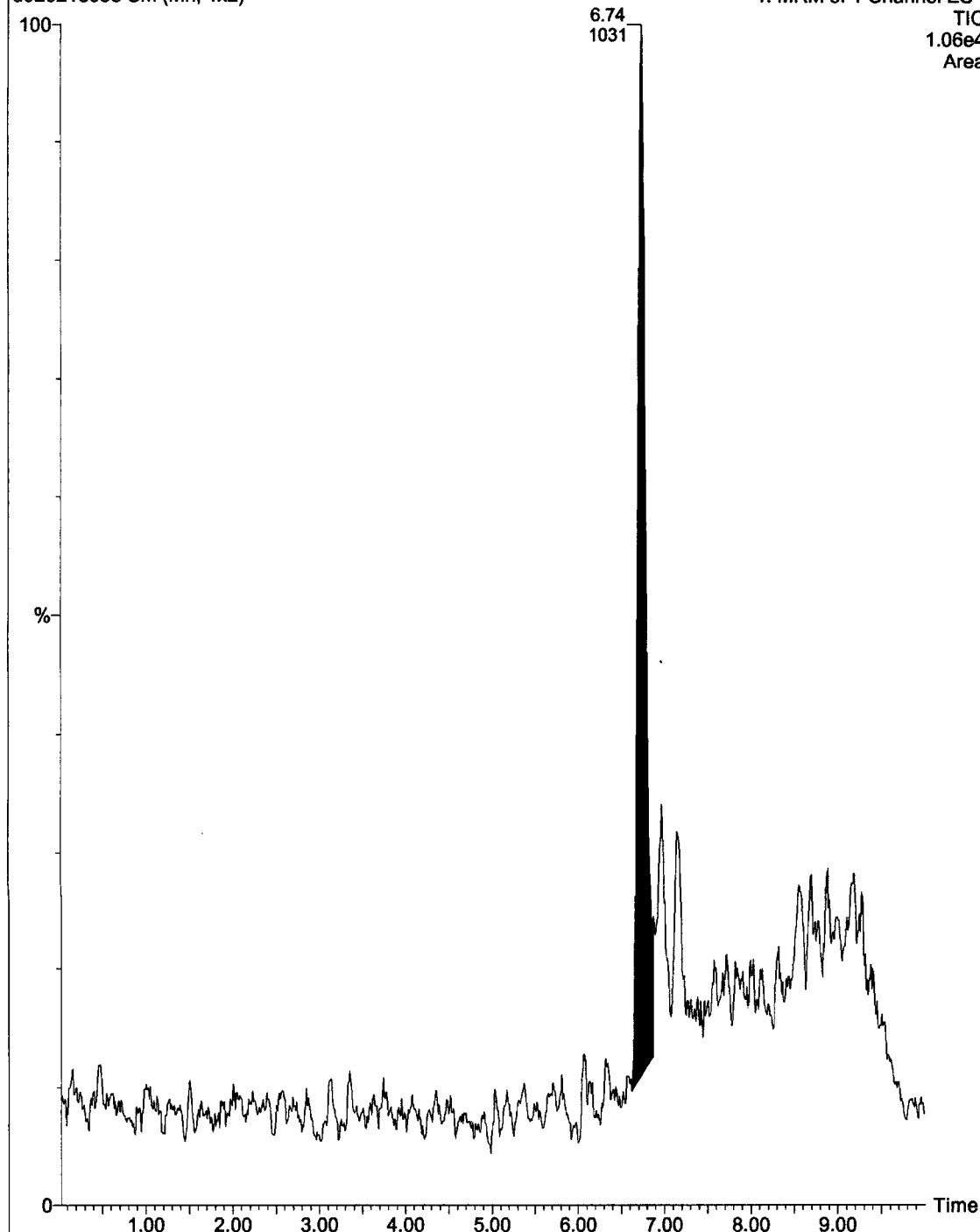
Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite

**Control Group Female Offspring Liver Sample**

E01-1365-31184, 0 ppm a.i. 246 F Liver, offspring  
3M Environmental Lab  
d020215038 Sm (Mn, 1x2)

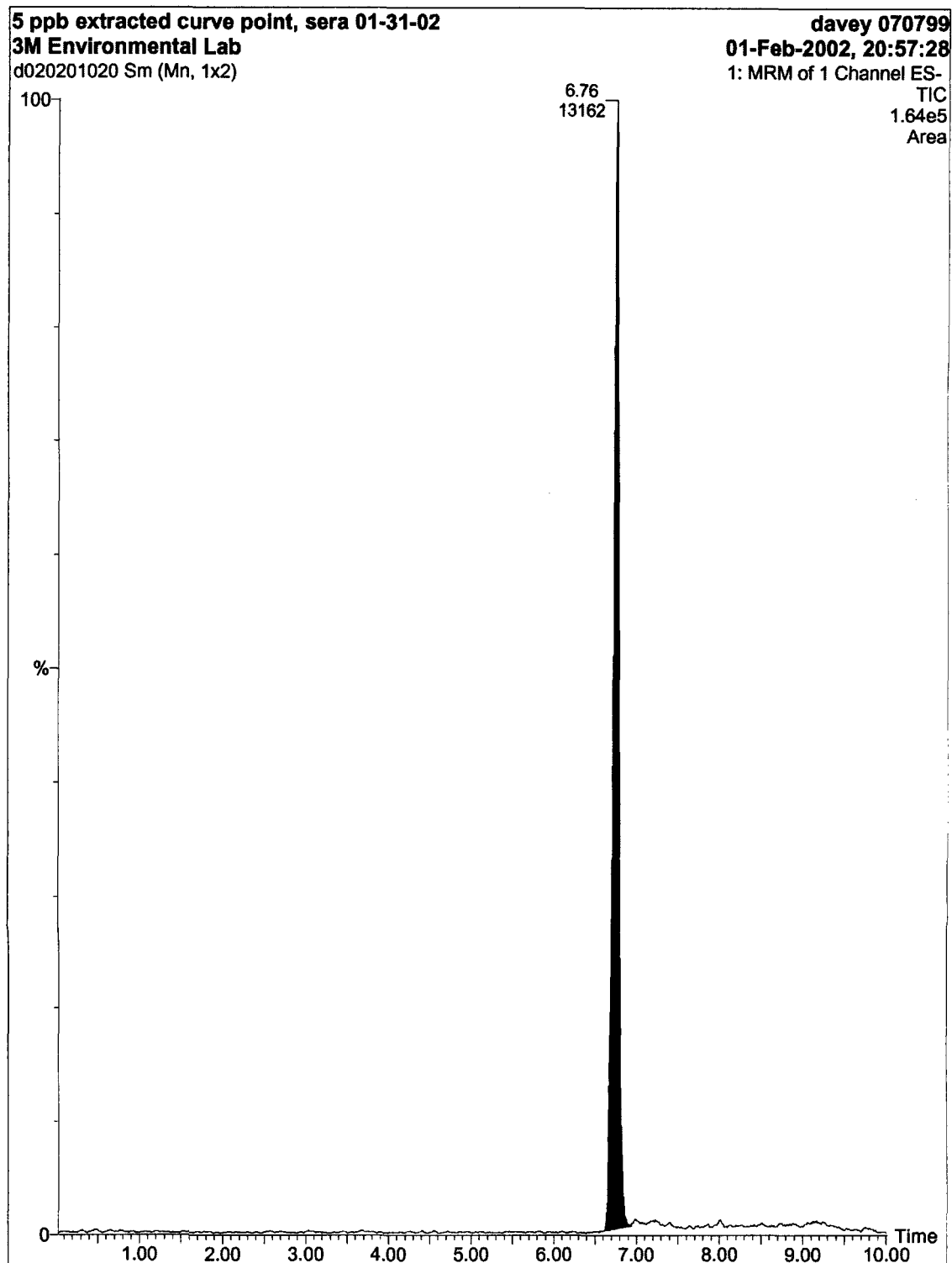
davey 070799  
15-Feb-2002, 21:01:27  
1: MRM of 1 Channel ES-  
TIC  
1.06e4  
Area



Report E01-1245

PFOS: A Reproduction Study with the Northern Bobwhite

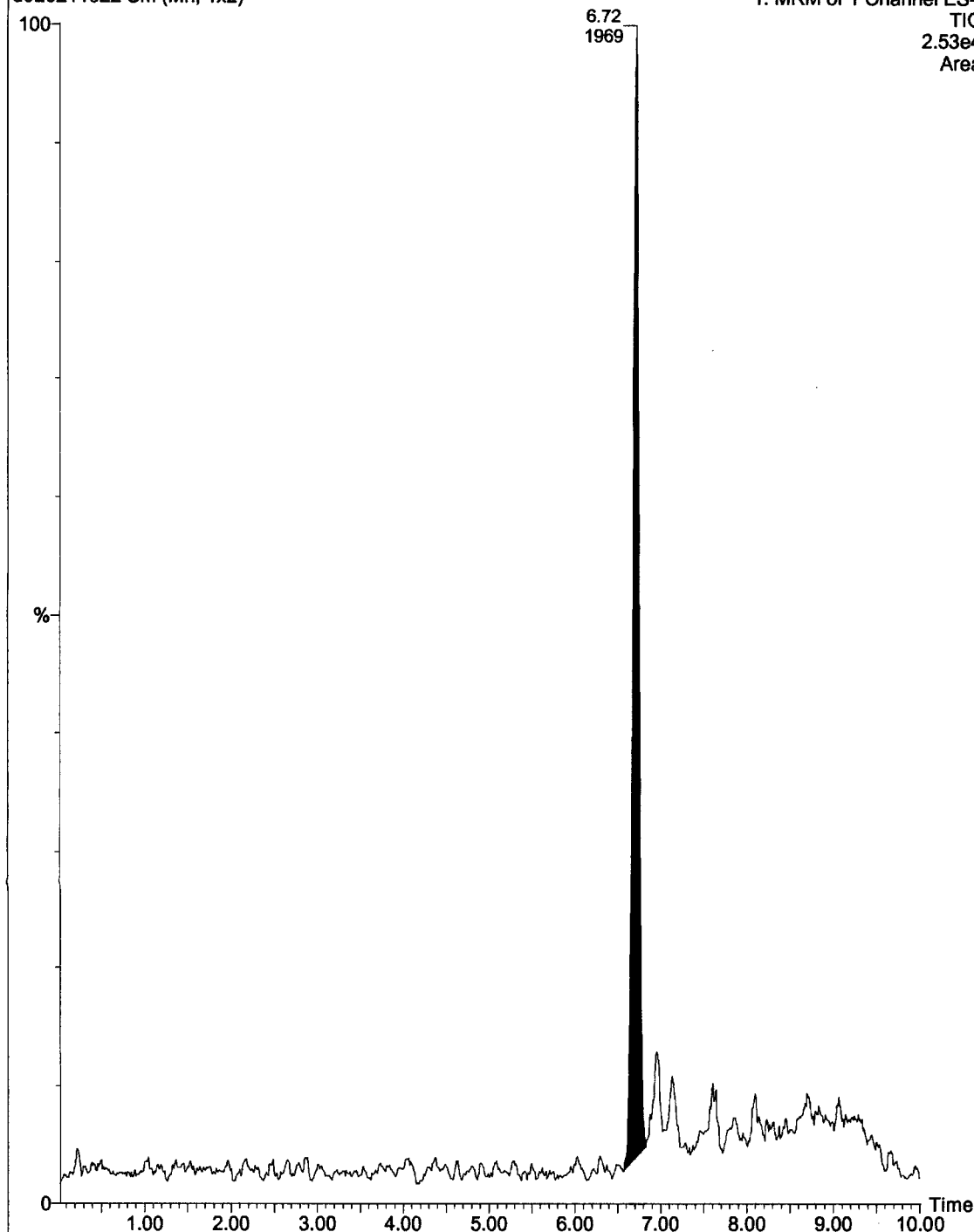
### Extracted Sera Calibration Curve Point

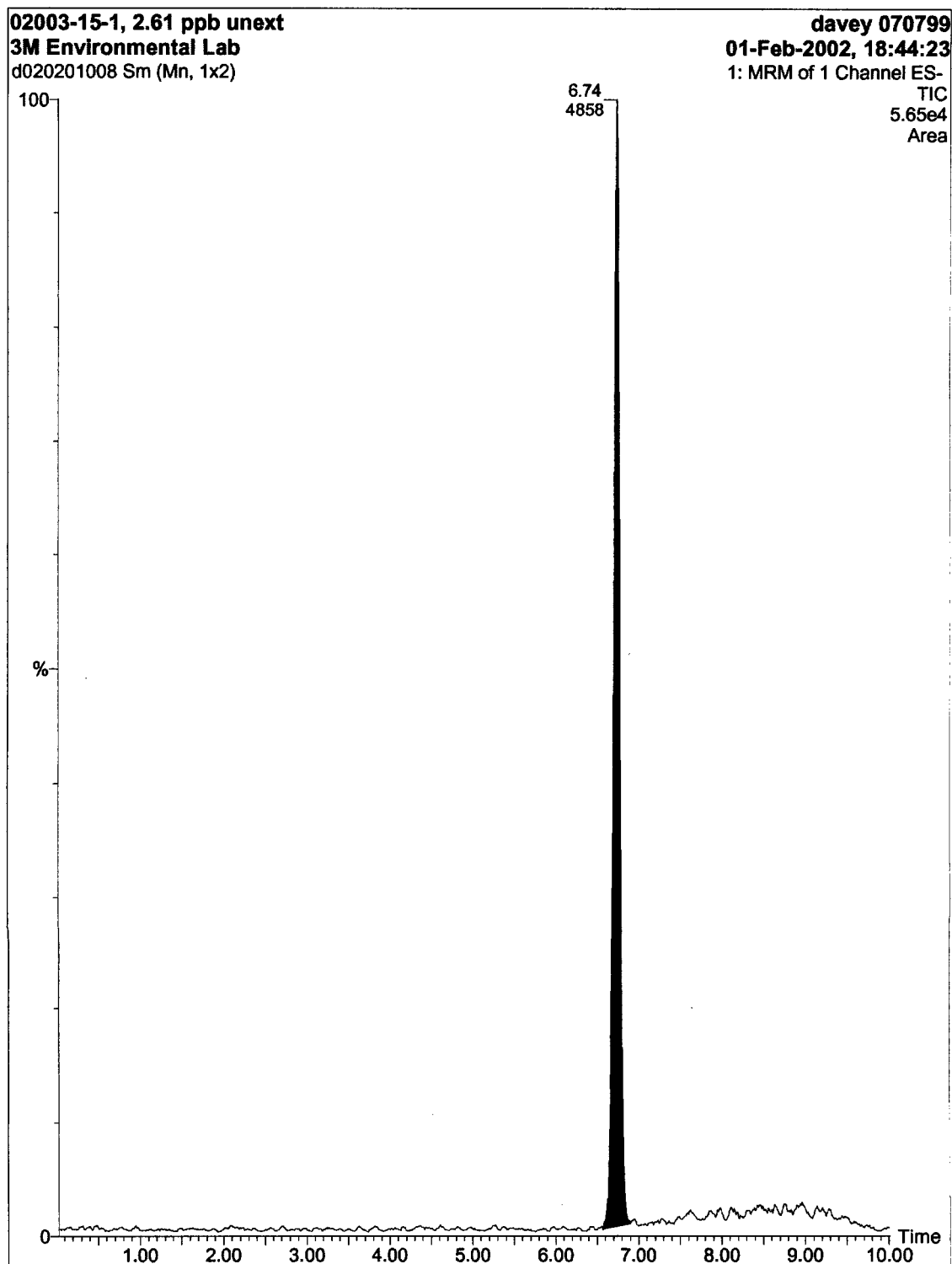


**Extracted Liver Calibration Curve Point**

E01-1342-36007 Curve point-1 ppb in liver extract  
3M Environmental Lab  
d020211022 Sm (Mn, 1x2)

davey 070799  
11-Feb-2002, 20:16:33  
1: MRM of 1 Channel ES-  
TIC  
2.53e4  
Area



**Unextracted Calibration Curve Point**

**Extracted Sera Calibration Curve**

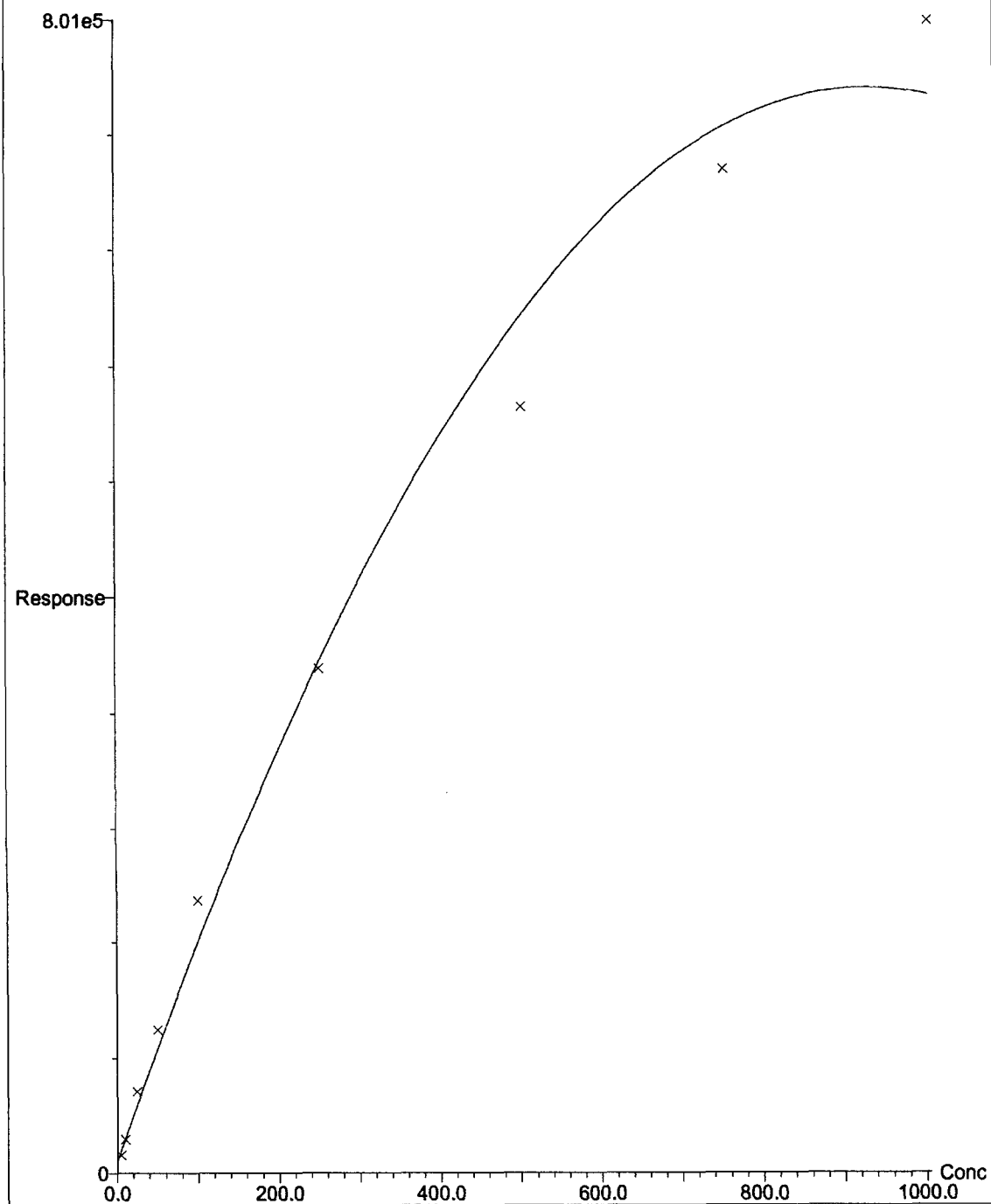
Compound 1 name: pfos (499&gt;99) func 1 Method File: d020201a

Coefficient of Determination: 0.988137

Calibration curve:  $-0.862805 * x^2 + 1603.72 * x + 8692.86$ 

Response type: External Std, Area

Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: None



**Extracted Liver Calibration Curve**

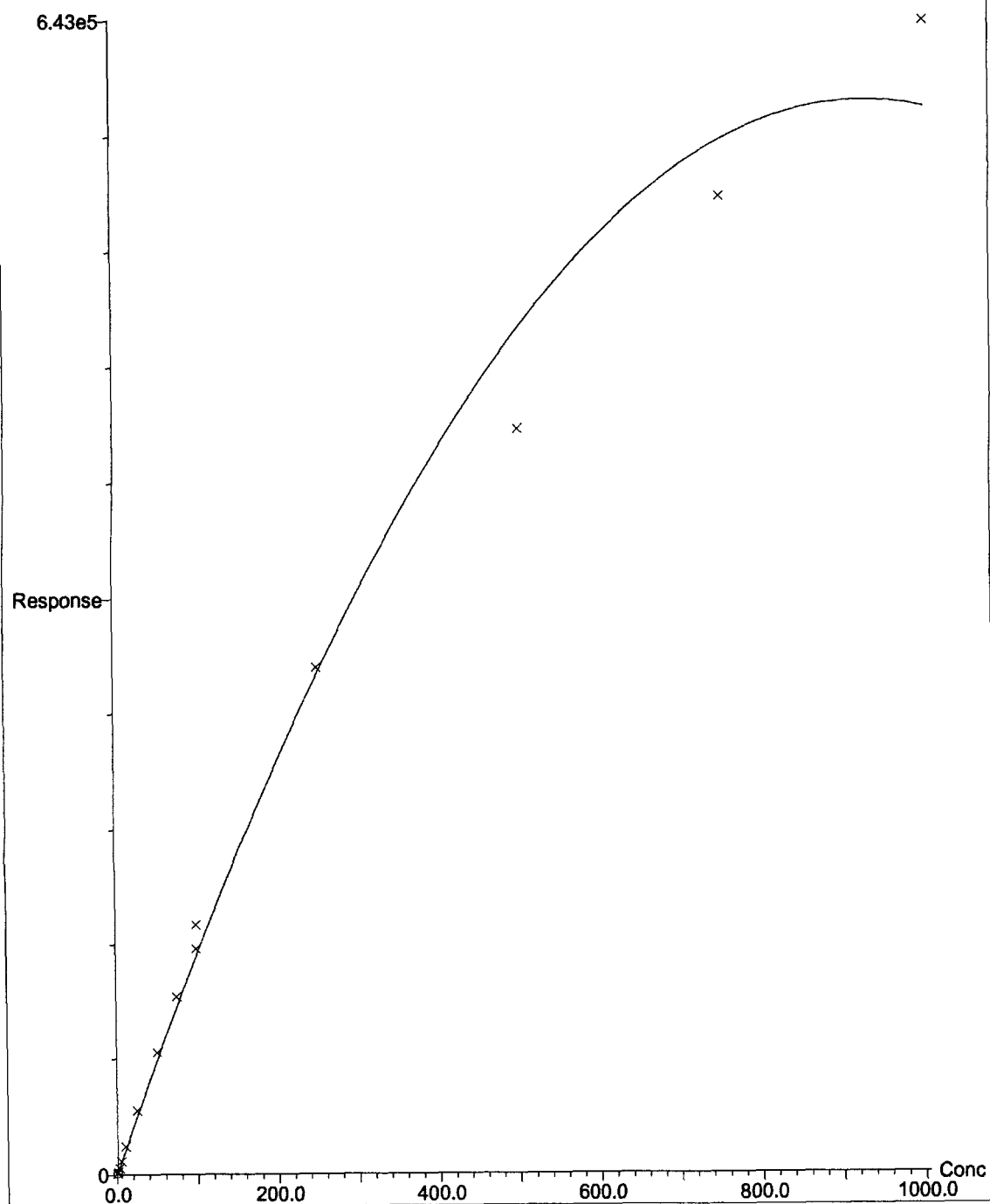
Compound 1 name: pfos (499&gt;99) func 1 Method File: d020215a

Coefficient of Determination: 0.988785

Calibration curve:  $-0.689309 * x^2 + 1283.97 * x + 845.279$ 

Response type: External Std, Area

Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: None



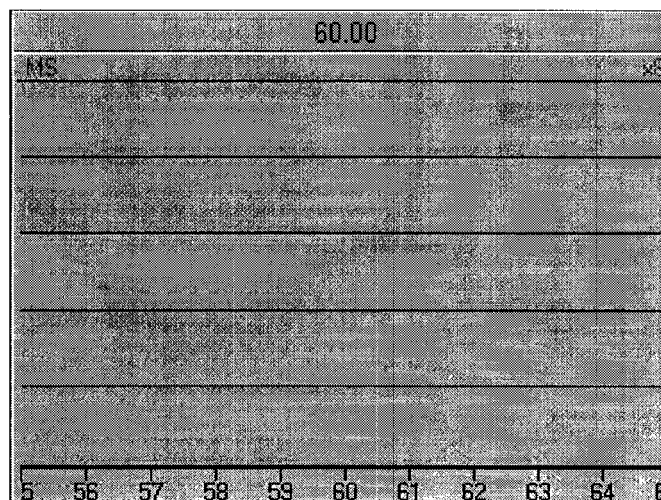
## Mass Spectrometer Tune Settings

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 |
| <b>Pressures</b>   |        |       | <b>Gas Flows</b> | <b>Rdbk</b> |      |
| Analysers Vacuum   | 3.8e-5 |       | Cone Gas         | 150.5       |      |
| Gas Cell           | 2.9e-3 |       | Desolvation      | 701.5       |      |

## 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 | Contact3 |
|---------|---------------|----------|----------|----------|
| Initial | Off           | Off      | Off      | Off      |
| 0.00    | Off           | On       | Off      | On       |
| 0.10    | 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

## Mass Spectrometer Scanning Parameters

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**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

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**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               |            |

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**ATTACHMENT D: SAMPLE PREPARATION SHEETS**

Prep Date: 1/31/2002 1-31-02  
 Analysts initials: OK/HOJ  
 1-31-02

Method Revision: ETS-8-231.0  
 Study Number: E01-1245  
 Matrix: Sera 45

| 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   |
|-----------------------------|---------------------------------------------|--------------------------------|-----------------------------|---------------------------|----------------------------|------------|
| E01-1245-35766              | Blank H <sub>2</sub> O-day 1                | 40+6                           | Waters, 1g, 6ml, lot 1290B2 | NA                        | 2ml of MeOH TN-A-5457      | ② (2-1-02) |
| E01-1245-35767              | 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<br>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              | QUAL RE OK 1-31-02<br>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      | ↓          |

Blank Sera TN-A-5992; Amount of sera aliquoted: 2 ml

Dilute Sera with Kandiyohi water; Amount of water added 30 ml

Aliquot 1ml of sera into 15 ml polypropylene tube

Spike samples accordingly

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

Shake sample for 20 min @ 300 rpm (Shaker 102-0251AL) 1/31/02

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 169G13)

Add 40 ml of Kandiyohi water to 50 ml polypropylene 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 completely 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 ul to extract.

Transfer sample into appropriately marked autovial

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. (A) 1-31-02

② USED 2ml of (Pooled) SERA, DILUTED TO 4ml OF KANDIYOHIC WATER. 1ml WAS THEN USED FOR EXTRACTION. (A) 1-31-02  
 ✓ VISUAL VERIFICATION OF SPIKE PERFORMED BY OK VERIFIED BY HOJ 1-31-02

(AC) Quail Sera } LAS 08/21/02  
 Page 2 of 2 }

Prep Date: 1/31/2002

Analysts initials: OK/HOJ

**Method Revision: ETS-8-231.0**

Study Number: E01-1256 Nov 1-71-02

Matrix:      Sera

[illegible]

Blank Sera TN-A-5992; Amount of sera aliquoted: 2 ml

Dilute Sera with Kandiyoji water; Amount of water added 38 ml

**Aliquot 1 ml of sera into 15 ml polypropylene tube**

**Spike samples accordingly**

Add 5ml of ACN (TN-A-4145) to aliquated sample - Don't (Fe) be a

Shake sample for 20 min @ 300 rpm (Shaker VWR ORBITAL/SIN 04/694)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge # 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-754)

### Wash Column with Kandiyohi Water

Filter sample through conditioned column, discarding filtrate

Allow column to go completely dry

Elute column with indicated amount of solvent ( 100H TN-A- 5754 ) into appropriate 15 ml centrifuge tube

Spike samples with internal standard PFDA standard number 02001-45, conc. 100.7 ppb; added 2ul to extract

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

| <u>SAMPLE VOLUME</u> | H <sub>2</sub> O ADDED |
|----------------------|------------------------|
| 100                  | 0                      |
| 100                  | 10                     |
| 100                  | 20                     |
| 100                  | 30                     |
| 100                  | 40                     |
| 100                  | 50                     |
| 100                  | 60                     |
| 100                  | 70                     |
| 100                  | 80                     |
| 100                  | 90                     |
| 100                  | 100                    |

|        |       |
|--------|-------|
| 500ul  | 1.1mL |
| 1000ul | 1.9mL |
| 2000ul | 3.8mL |
| 3000ul | 5.7mL |
| 4000ul | 7.6mL |

① samples aliquoted by OK.  
② diluted by HOJ. approx

**Study: E01-1245**

**Solvent/TN Number: MeOH/5457 w/ IS PFDA**

**Extraction Date/Analyst: 1/31/02**

**Matrix/Timepoint:** Quail Sera (AC) LAS 11/26/02

[illegible]

1/10 dilution =  $\frac{100 \mu\text{l}}{1000 \mu\text{l}}$  of sample +  $\frac{900 \mu\text{l}}{1000 \mu\text{l}}$  of solvent

1/100 dilution = 10  $\mu$ l of sample + 990  $\mu$ l of solvent

1/1000 dilution = 1  $\mu$ l of sample + 1000  $\mu$ l of solvent

dilutions were done on date of extraction.

Dilution information was confirmed by the autovial sample info. 11/26/02  
Form Completion Verified By: LAS 11/26/02

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<br>Std Conc.<br>ug/mL | PFOS<br>Am't Spiked<br>mL | PFDA<br>Std Conc.<br>ug/mL | PFDA<br>Am't Spiked<br>mL | All<br>Initial Dilution<br>mL/mL | All<br>Final Vol.<br>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  
 US 08/27/02

## Calculated Concentrations of Standards in the sample matrix

| Standard<br>Number       | PFOS<br>Std Conc.<br>ng/mL | PFDA<br>Std Conc.<br>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<br>Number       | PFOS<br>Std Conc.<br>ng/mL | PFDA<br>Std Conc.<br>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: PCW-8/27/02

Prep Date: 2/1/2002

Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0

Study Number: E01-1245

Matrix: Sera

| 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                                    |
|-----------------------------|-----------------------------------|--------------------------------|-----------------------------|---------------------------|----------------------------|---------------------------------------------|
| E01-1245-35825              | Blank H2O-day 2                   | 40+6                           | Waters, 1g, 6ml, lot 1290B2 | NA                        | 2ml of MeOH TN-A-5457      |                                             |
| E01-1245-35826              | Blank mallard sera-day 2          | 40+6                           | Waters, 1g, 6ml, lot 1290B2 | NA                        | 2ml of MeOH TN-A-5457      | USED 1ml of DCM that was present on 1-31-02 |
| E01-1245-35827              | Mallard Sera MS-day 2             | 40+6                           | Waters, 1g, 6ml, lot 1290B2 | 10ul of 02001-53          | 2ml of MeOH TN-A-5457      |                                             |
| E01-1245-35828              | 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                                       |
| E01-1245-28464              | 454-108-352, 0 PPM, Female, Adult | 40+6                           | Waters, 1g, 6ml, lot 1290B2 |                           | 2ml of MeOH TN-A-5457      | 400ul                                       |
| E01-1245-28465              | 454-108-353, 0 PPM, Male, Adult   | 40+6                           | Waters, 1g, 6ml, lot 1290B2 |                           | 2ml of MeOH TN-A-5457      | 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      | 30ul                                        |
| 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      | 20ul                                        |
| 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 EXTRA SAMPLE FOR THIS NUMBER. ONLY RECORD.  
2-1-02 OK

Spike samples accordingly

Add 5ml of ACN (TN-A-4145) 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 polypropylene 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 completely 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

Transfer sample into appropriately marked autovial

FOR THE ABOVE SAMPLE VOLUMES THE FOLLOWING  
Amount of KANDIYOHI WATER WAS ADDED TO  
Activate-A 1:20 DILUTION

250ul - 1.4ul

200ul - 1.4ul

100ul - 1.4ul

200ul - 3.8ul

300ul - 5.7ul

400ul - 7.6ul

500ul - 9.5ul

60ul = 1.2ul

80ul = 1.6ul

90ul = 1.8ul

Prep Date: 2/1/2002 2:02 OK  
Analysts initials: OK/HOJ  
OK 2/1-02Method Revision: ETS-8-231.0  
Study Number: E01-1245  
Matrix: Sera

| 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 |
|-----------------------------|------------------------------------|--------------------------------|-----------------------------|---------------------------|----------------------------|----------|
| E01-1245-28476              | 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      | 100uL    |
| 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      | 70uL     |
| 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    |

Blank Sera TN-A- 5752; Amount of sera aliquoted: 1/4 ml

Dilute Sera with Kandiyohi water; Amount of water added 1/4 ml

Aliquot 1ml of sera into 15 ml polypropylene tube

Spike samples accordingly

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

Shake sample for 20 min @ 300 rpm (Shaker 1/4 088 rpm SINCO 4164)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 2/4 769 617)

Add 40 ml of Kandiyohi water to 50 ml polypropylene 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 completely dry

Elute column with indicated amount of solvent (1/4000 TN-A- 5754 ) into appropriate 15 ml centrifuge tube

Spike samples with internal standard PFDA standard number 02001-45, conc. 10.71M, amount added 2uL to 5 tubes.

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

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

| 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                           |
|-----------------------------|---------------------------------------|--------------------------------|-----------------------------|---------------------------|----------------------------|------------------------------------|
| E01-1245-28498              | 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      | 30uL                               |
| 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      | 30uL                               |
| E01-1245-28508              | 454-108-398, 10 PPM, Female, Adult    | 40+6                           | Waters, 1g, 6ml, lot 1290B2 | NA                        | 2ml of MeOH TN-A-5457      | 10uL                               |
| 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      | 20uL                               |
| 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      | 10uL                               |
| E01-1245-28515              | 454-108-406, 10 PPM, Female, Adult    | 40+6                           | Waters, 1g, 6ml, lot 1290B2 | NA                        | 2ml of MeOH TN-A-5457      | 20uL                               |
| 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      | 20uL                               |
| 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 (1 ml H <sub>2</sub> O added) |

Blank Sera TN-A- 5592 ; Amount of sera aliquoted: 11A ml

Dilute Sera with Kandiyohi water; Amount of water added 11A ml

Aliquot 1ml of sera into 15 ml polypropylene tube

Spike samples accordingly

Add 5ml of ACN (TN-A- 445 ) to aliquated sample

Shake sample for 20 min @300 rpm (Shaker VAN ORN 510241694)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 7A 769613)

Add 40 ml of Kandiyohi water to 50 ml polypropylene 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 completely 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 82001-45, conc. 100.744, amount added 2uL TO EXTRACT

samples were aliquoted  
 by OK & diluted by  
 HOJ. (AC) OK 8802

① (AC) OK 8-2802

Prep Date: 2/1/2002 2-1-02  
Analysts initials: OK/HOJ 016  
2-1-02

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

| 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 |
|-----------------------------|----------------------------------------|--------------------------------|-----------------------------|---------------------------|----------------------------|----------|
| E01-1245-28520              | 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      | 20uL     |
| 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    |
|                             |                                        |                                |                             |                           |                            |          |
|                             |                                        |                                |                             |                           |                            |          |
|                             |                                        |                                |                             |                           |                            |          |
|                             |                                        |                                | UAS 08/22/02                |                           |                            |          |

Blank Sera TN-A-5992; Amount of sera aliquoted: N/A ml

Dilute Sera with Kandiyohi water; Amount of water added N/A ml

**Aliquot 1ml of sera into 15 ml polypropylene tube**

**Spike samples accordingly**

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

Shake sample for 20 min @ 300 rpm (Shaker *VAR ORIGINAL SIN 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 Kandiyoji Water

**Filter sample through conditioned column, discarding filtrate**

Allow column to go completely dry

Elute column with indicated amount of solvent ( 100% MeOH TN-A- 5754 ) into appropriate 15 ml centrifuge tube

Spike samples with internal standard PFDA standard number 02001-45, conc. 100.7114 amount added 2 µL TO E.F. 100

⑦ AC 1 ml of  $H_2O$   
added to these  
samples.  
4.9.02 OK

- All samples aliquoted by PK & diluted by H<sub>2</sub>O.

✓ RF OK 82802

## 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 #:                   | TN-A-4145 |

|                 | Mass of solvent (g) | Density <sup>1</sup><br>(g/ml) | Volume of Solvent |
|-----------------|---------------------|--------------------------------|-------------------|
| 1               | 3.9368              | 0.7857                         | 5.0106            |
| 2               | 3.9413              | 0.7857                         | 5.0163            |
| 3               | 3.9382              | 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,<br>(mL)            | Accuracy,<br>(%) | C.V.,<br>(%) |
| 1 to 10                    | 98.0 - 102 %     | ± 1.0 %      |

|                          |
|--------------------------|
| Corrective Actions:      |
| None taken. 2-1-02<br>OK |

<sup>1</sup>Density values taken from the Merck Index, 12<sup>th</sup> Edition, Copyright 1996

$d^y_x$  = specific gravity at y<sup>degrees C</sup> referred to water at x<sup>degrees C</sup>

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

Prep Date: 27-02/28-02

Analysts initials: CK/HOJ

Method Revision: 27-8-231.1

Study Number: E01-1245 28-02

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       |                                |                             |                           |                            | 1.0052g                     | NA       |
| E01-1342-30321              | 0 ppm a.i. 330 F Liver       |                                |                             |                           |                            | 1.0749g                     |          |
| 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       |                                |                             |                           |                            | 1.0183g                     |          |
| E01-1342-30384              | 0 ppm a.i. 337 M Liver       |                                |                             |                           |                            | 1.0269g                     |          |
| E01-1342-30393              | 0 ppm a.i. 338 F Liver       |                                |                             |                           |                            | 1.0543g                     |          |
| E01-1342-30402              | 0 ppm a.i. 339 M Liver       |                                |                             |                           |                            | 1.0886g                     |          |
| E01-1342-30411              | 0 ppm a.i. 340 F Liver       |                                |                             |                           |                            | 1.082g                      |          |
| 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       |                                |                             |                           |                            | 1.0998g                     |          |
| 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 polypropylene 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 polypropylene 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 completely 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 appropriately marked autovial

samples were weighed out by CK 3802

@@ CK 2802

Prep Date: 2-7-02/2802  
Analysts initials: OK/HOJ

Method Revision: 675-8-231.1

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 |
|-----------------------------|------------------------------|--------------------------------|-----------------------------|---------------------------|----------------------------|-----------------------------|----------|
| E01-1342-30510              | 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       |                                |                             |                           |                            | 1.0382g                     |          |
| 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 polypropylene 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 polypropylene 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 completely 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 appropriately marked autovial

3802  
OK

Samples were weighed out by OK & homogenized by HOJ. ACN 8802 OK

Prep Date: 2.7.02 / 2.8.02  
Analysts initials: OK / H.O.J

Method Revision: 675-8: 231.1

Study Number: 501-1245

Matrix: Liver 401 2.8.07

| 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      |                                |                             |                           |                            | 1.1008g                     |          |
| 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      |                                |                             |                           |                            | 1.0826g                     |          |
| E01-1342-30780              | 10 ppm a.i. 381 M Liver      |                                |                             |                           |                            | 1.0501g                     |          |
| E01-1342-30789              | 10 ppm a.i. 382 F Liver      |                                |                             |                           |                            | 1.022g                      |          |
| 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      |                                |                             |                           |                            | 1.0678g                     |          |
| E01-1342-30838              | 10 ppm a.i. 386 F Liver      |                                |                             |                           |                            | 1.1215g                     |          |
| E01-1342-30847              | 10 ppm a.i. 387 M Liver      |                                |                             |                           |                            | 1.0078g                     | 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                     |          |

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

Homogenize liver with Kandiyohi water; Amount of water added

Aliquot 1 ml of liver into 15 ml polypropylene 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 Kandiyohei Water

Filter sample through conditioned column, discarding filtrate

Allow column to go completely 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 appropriately marked autovial

Samples were weighed out by OK  
& homogenized by H.O.J. ~~(KOP)~~ OK  
① 8.8.02

AC TRUE FOR  
ALL BLANKS IN  
THIS STUDY

2002 All samples were <sup>THIS STUDY</sup> weighed out & homogenized on 2.802 & 2.702 by OK & HJ, including blanks.

Prep Date: 9  
Analysts initials:

Method Revision: 675.E.231.1  
Study Number: 601.1245  
Matrix: 61089

Matrix: *2015*

[illegible]

Blank Liver TN-A-\_\_\_\_\_; Amount of liver: \_\_\_\_\_g  
Homogenize liver with Kandiyohi water; Amount of water added \_\_\_\_\_ ml

**Aliquot 1 ml of liver into 15 ml polypropylene 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 completely 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 appropriately marked autovial

samples were weighed out by OK  
& homogenized by H.O.J. (AN) 8802

AC

Blank Liver }  
Page 5 of 5

LAs 08/22/02

Prep Date: 2-7-02/28-02  
Analysts initials: DK/HOJ

Method Revision: 675.8-231.1

Study Number: E01-1245

Matrix: GUM

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       |                                |                             |                           |                            | 0.5004g                     |          |
| E01-1365-31224              | 0 ppm a.i. 277 F Liver       |                                |                             |                           |                            | 0.4326g                     |          |
| E01-1365-31229              | 0 ppm a.i. 283 M Liver       |                                |                             |                           |                            | 0.6066g                     |          |
| E01-1365-31234              | 0 ppm a.i. 291 M Liver       |                                |                             |                           |                            | 0.6072g                     |          |
| E01-1365-31240              | 10 ppm a.i. 517 F Liver      |                                |                             |                           |                            | 0.6570g                     |          |
| 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      |                                |                             |                           |                            | 0.6903g                     |          |
| 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 polypropylene 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 polypropylene 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 completely 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 appropriately marked autovial

SAMPLE WEIGHTS WERE ROUNDED TO THE NEAREST 0.1g AND HAD KANDIYOHNI WATER ADDED AS SUMMARIZED BELOW: 2-8-02

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

(AC) THIS IS TRUE FOR ALL THE LIVERS WEIGHED IN THIS STUDY.  
 4-9-02 OK.

(AC) 8-8-02 OK

3 samples were weighed out by OK; homogenized by HOJ. and is true for all samples in this study.

Prep Date: 2/11/2002

Analysts initials: OK.HOJ

2-11-02  
OK

Method Revision: ETS-8-231.0

Study Number: E01-1342-1365-1245 (20) 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<br>TN-A-5754      | NA       |
| E01-1342-36001              | Blank female liver-day 1               | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36002              | Blank male liver-day 1                 | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36003              | Female liver MS-day 1                  | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 50ul of 02001-66          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36004              | Female liver MSD-day 1                 | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 50ul of 02001-66          | 2ml MeOH<br>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<br>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<br>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<br>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<br>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<br>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<br>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<br>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<br>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<br>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<br>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<br>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<br>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<br>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<br>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<br>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 polypropylene tube

Spike samples accordingly

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

Shake sample for 20 min @300 rpm (Shaker VWR SHAKER 3N: 041694)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 34# 769613)

Add 40 ml of Kandiyohi water to 50 ml polypropylene 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 completely 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 appropriately marked autovial

Entire extraction done by OK. 2-8-02 OK

## SPE Columns Extraction Worksheet

(AC)

Quail Liver } LAs 08/22/02  
Page 2 of 2Prep Date: 2-11-02  
Analysts initials: OK/HOJ

Method Revision: FTS-8-231.0

Study Number: E01-1342/1305 (R)

Matrix: LIVER 1245 16 2.11.02

| 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 | Comments |
|-----------------------------|------------------------------|--------------------------------|-----------------------------|---------------------------|----------------------------|----------|
| E01-1342-36017              | QC@10 ppb-1                  | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 4ul of 02001-52           | 2ml MeOH<br>TN-A-5754      | NA       |
| E01-1342-36018              | QC@10 ppb-2                  | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 4ul of 02001-52           | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36019              | QC@10 ppb-3                  | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 4ul of 02001-52           | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36020              | QC@75 ppb-1                  | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 30ul of 02001-52          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36021              | QC@75 ppb-2                  | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 30ul of 02001-52          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36022              | QC@75 ppb-3                  | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 30ul of 02001-52          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36023              | QC@150 ppb-1                 | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 60ul of 02001-52          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36024              | QC@150 ppb-2                 | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 60ul of 02001-52          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36025              | QC@150 ppb-3                 | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 60ul of 02001-52          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30312              | 0 ppm a.i. 329 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30321              | 0 ppm a.i. 330 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30330              | 0 ppm a.i. 331 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30339              | 0 ppm a.i. 332 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30348              | 0 ppm a.i. 333 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30357              | 0 ppm a.i. 334 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30366              | 0 ppm a.i. 335 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30375              | 0 ppm a.i. 336 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30384              | 0 ppm a.i. 337 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30393              | 0 ppm a.i. 338 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
|                             |                              |                                |                             |                           |                            |          |
|                             |                              |                                |                             |                           |                            |          |

Blank Liver TN-A- 5143 ; Amount of liver: 3.0353 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 polypropylene tube

Spike samples accordingly

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

Shake sample for 20 min @300 rpm (Shaker VWR 5/1041694

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 3M 769013

Add 40 ml of Kandiyohi water to 50 ml polypropylene 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 completely 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 2ul

Transfer sample into appropriately marked autovial

- 1.00 extraction performed by OK. (R) 2.11.02

### 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

| Actual Concentrations of Standards in the LC-MIX |                            |                          |                            |                         |
|--------------------------------------------------|----------------------------|--------------------------|----------------------------|-------------------------|
| Standard Number                                  | PFOS<br>Std Conc.<br>ug/mL | PFOS<br>Amt Spiked<br>mL | PFDA<br>Std Conc.<br>ug/mL | All<br>Amt Spiked<br>mL |
| E01-1342-36005                                   | 1.004                      | 0.0004                   | 100.7                      | 0.002                   |
| E01-1342-36006                                   | 1.004                      | 0.0010                   | 100.7                      | 0.002                   |
| E01-1342-36007                                   | 1.004                      | 0.0020                   | 100.7                      | 0.002                   |
| E01-1342-36008                                   | 1.004                      | 0.0050                   | 100.7                      | 0.002                   |
| E01-1342-36009                                   | 1.004                      | 0.0100                   | 100.7                      | 0.002                   |
| E01-1342-36010                                   | 1.004                      | 0.0200                   | 100.7                      | 0.002                   |
| E01-1342-36011                                   | 1.004                      | 0.0500                   | 100.7                      | 0.002                   |
| E01-1342-36012                                   | 1.004                      | 0.1000                   | 100.7                      | 0.002                   |
| E01-1342-36013                                   | 1.004                      | 0.1500                   | 100.7                      | 0.002                   |
| E01-1342-36014                                   | 1.004                      | 0.2000                   | 100.7                      | 0.002                   |
| E01-1342-36062                                   | 50.20                      | 0.0040                   | 100.7                      | 0.002                   |
| E01-1342-36015                                   | 50.20                      | 0.0100                   | 100.7                      | 0.002                   |
| E01-1342-36016                                   | 50.20                      | 0.0200                   | 100.7                      | 0.002                   |
| E01-1342-36060                                   | 50.20                      | 0.0300                   | 100.7                      | 0.002                   |
| E01-1342-36061                                   | 50.20                      | 0.0400                   | 100.7                      | 0.002                   |
| QC-10 ppb                                        | 5.020                      | 0.0040                   | 100.7                      | 0.002                   |
| QC-75 ppb                                        | 5.020                      | 0.0300                   | 100.7                      | 0.002                   |
| QC-150 ppb                                       | 5.020                      | 0.0600                   | 100.7                      | 0.002                   |
| Quail Liver-MS/MSD-Day 1                         | 1.004                      | 0.0500                   | 100.7                      | 0.002                   |
| Quail Liver-MS/MSD-Day 2                         | 1.004                      | 0.0500                   | 100.7                      | 0.002                   |
| Quail Liver-MS/MSD-Day 3                         | 1.004                      | 0.0500                   | 100.7                      | 0.002                   |
| Quail Liver-MS/MSD-Day 4                         | 1.004                      | 0.0500                   | 100.7                      | 0.002                   |

[illegible]

Calculated Concentrations of Standards in the sample matrix

| Calculated Concentrations of Standards in the sample matrix |                   |                   |
|-------------------------------------------------------------|-------------------|-------------------|
| Standard Number                                             | PPOS<br>Std Conc. | PFDA<br>Std Conc. |
|                                                             | ng/g              | 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

| Calculated Concentrations of standards in the final solvent |                            |                            |
|-------------------------------------------------------------|----------------------------|----------------------------|
| Standard Number                                             | PFOS<br>Std Conc.<br>ng/mL | PFDA<br>Std Conc.<br>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:

UAS 11/26/02

## BOTTLE-TOP DISPENSER DAILY CALIBRATION CHECK

Manufacturer *VWR*  
 and Model: *5mL*  
 Date: *02-11-02*  
 Initials: *HQJ*  
 Dispenser ID: *0799*  
 Balance ID: *900*  
 Volume: *5mL*  
 Solvent: *Acn*  
 ID #: *TW-A-2145*

|                 | Mass of solvent (g) | Density <sup>1</sup><br>(g/ml) | Volume of Solvent |
|-----------------|---------------------|--------------------------------|-------------------|
| 1               | <i>3.9525</i>       | <i>0.7857</i>                  | <i>5.0305</i>     |
| 2               | <i>3.9511</i>       |                                | <i>5.0286</i>     |
| 3               | <i>3.9585</i>       |                                | <i>5.0382</i>     |
| 4               | <i>3.9555</i>       |                                | <i>5.0344</i>     |
| 5               | <i>3.9573</i>       |                                | <i>5.0367</i>     |
| Average Volume: |                     |                                | <i>5.0377</i>     |
| %Accuracy:      |                     |                                | <i>100.7</i>      |
| Std. Dev.:      |                     |                                | <i>0.003987</i>   |
| %C.V.:          |                     |                                | <i>0.0792</i>     |
| Pass/Fail:      |                     |                                | <i>(P) / F</i>    |

= SD/Avg \* 100

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

Corrective Actions:

*None taken* *ASJ 2-11-02*

<sup>1</sup>Density values taken from the Merck Index, 12<sup>th</sup> Edition, Copyright 1996 $d_x^y$  = specific gravity at y<sup>degrees C</sup> referred to water at x<sup>degrees C</sup>n-Hexane  $d_4^{20}$  = 0.660Acetone  $d_0^{25}$  = 0.788Methanol  $d_4^{25}$  = 0.7866Methylene Chloride  $d_4^{20}$  = 1.3255

Prep Date: 2/12/2002  
 Analysts initials: OK/HOJ 2-12-02

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 |
|-----------------------------|------------------------------|--------------------------------|-----------------------------|---------------------------|----------------------------|----------|
| E01-1342-36026              | Blank H2O-day 2              | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      | NA       |
| E01-1342-36027              | Blank female liver-day 2     | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36028              | Blank male liver-day 2       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36029              | Female liver MS-day 2        | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 50ul of 02001-66          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36030              | Female liver MSD-day 2       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 50ul of 02001-66          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30402              | 0 ppm a.i. 339 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30411              | 0 ppm a.i. 340 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30420              | 0 ppm a.i. 341 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30429              | 0 ppm a.i. 342 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30438              | 0 ppm a.i. 343 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30447              | 0 ppm a.i. 344 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30456              | 0 ppm a.i. 345 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30465              | 0 ppm a.i. 346 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30474              | 0 ppm a.i. 347 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30483              | 0 ppm a.i. 348 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30492              | 0 ppm a.i. 349 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30501              | 0 ppm a.i. 350 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30510              | 0 ppm a.i. 351 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30519              | 0 ppm a.i. 352 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30528              | 0 ppm a.i. 353 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>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 ml Amount of water added 9 ml

Aliquot 1ml of liver into 15 ml polypropylene 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 # 769613

Add 40 ml of Kandiyohi water to 50 ml polypropylene 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 completely 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 appropriately marked autovial

extraction done by OK. (AC) OK  
 0 8-802  
 ① RE OK 82802

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

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

Matrix: Liver

[illegible]

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 polypropylene 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 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 completely 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 appropriately marked autovial

extraction done by OK. (AC) (P) OK  
O J.P.02

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**BOTTLE-TOP DISPENSER DAILY CALIBRATION CHECK**

|                                |                    |
|--------------------------------|--------------------|
| <b>Manufacturer and Model:</b> | VWR, 5ml DISPENSER |
| <b>Date:</b>                   | 2.12.02            |
| <b>Initials:</b>               | OK                 |
| <b>Dispenser ID:</b>           | 0799               |
| <b>Balance ID:</b>             | 900                |
| <b>Volume:</b>                 | 5 ml               |
| <b>Solvent:</b>                | ACN                |
| <b>ID #:</b>                   | TN-A-4145          |

|   | Mass of solvent (g) | Density <sup>1</sup><br>(g/ml) | Volume of Solvent<br><i>ml</i> |
|---|---------------------|--------------------------------|--------------------------------|
| 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:                |
|   |                     | %Accuracy:                     | 100.7                          |
|   |                     | Std. Dev.:                     | 0.0283                         |
|   |                     | %C.V.:                         | 0.5626                         |
|   |                     | Pass/Fail:                     | (P) F                          |

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

|                                                                                                       |
|-------------------------------------------------------------------------------------------------------|
| <p align="center"><b>Corrective Actions:</b></p> <p align="center">none taken!<br/>2.12.02<br/>OK</p> |
|-------------------------------------------------------------------------------------------------------|

<sup>1</sup>Density values taken from the Merck Index, 12<sup>th</sup> Edition, Copyright 1996

$d^y_x$  = specific gravity at y<sup>degrees C</sup> referred to water at x<sup>degrees C</sup>

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



## Bottle-Top Dispenser Daily Calibration Check

EXACT COPY OF  
THE ORIGINAL  
38-020K

|                         |                   |
|-------------------------|-------------------|
| 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         |

|                 | Mass of solvent (g) | Density <sup>1</sup><br>(g/ml) | Volume of<br>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                |
| Average Volume: |                     |                                | 5.052                |
| %Accuracy:      |                     |                                | 101.03               |
| Std. Dev.:      |                     |                                | 0.0169               |
| %C.V.:          |                     |                                | 0.33                 |
| Pass/Fail:      |                     |                                | PASS                 |

PASS

PASS

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

|                        |
|------------------------|
| Corrective Actions:    |
| NONE TAKEN! OK 2-13-02 |

<sup>1</sup>Density values taken from the Merck Index, 12<sup>th</sup> Edition, Copyright 1996

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

n-Hexane  $d^{20}_4 = 0.660$

Acetone  $d^{25}_0 = 0.788$

Methanol  $d^{25}_4 = 0.7866$

Methylene Chloride  $d^{20}_4 = 1.3255$

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

Method Revision: ETS-8-231.0

Study Number: E01-1342/1365-1245 OK 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<br>TN-A-5754      | NA       |
| E01-1342-36032              | Blank female liver-day 3     | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36033              | Blank male liver-day 3       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36034              | Female liver MS-day 3        | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 50ul of 02001-66          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36035              | Female liver MSD-day 3       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 50ul of 02001-66          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30672              | 10 ppm a.i. 369 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30681              | 10 ppm a.i. 370 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30690              | 10 ppm a.i. 371 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30699              | 10 ppm a.i. 372 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30708              | 10 ppm a.i. 373 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30717              | 10 ppm a.i. 374 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30726              | 10 ppm a.i. 375 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30735              | 10 ppm a.i. 376 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30744              | 10 ppm a.i. 377 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30753              | 10 ppm a.i. 378 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30762              | 10 ppm a.i. 379 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30771              | 10 ppm a.i. 380 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30780              | 10 ppm a.i. 381 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30789              | 10 ppm a.i. 382 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30798              | 10 ppm a.i. 383 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>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 40 40 45 ml

Amount of water added 9ml

Aliquot 1ml of liver into 15 ml polypropylene tube

Spike samples accordingly

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

Shake sample for 20 min @300 rpm (Shaker VWR 510 041694)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 311 769613)

Add 40 ml of Kandiyohi water to 50 ml polypropylene 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 completely 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 appropriately marked autovial

EXTRACTION DONE BY OK. (ACCP) OK 8.8.02

OK 8.8.02

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<br>TN-A-5754      | NA       |
| E01-1342-30829              | 10 ppm a.i. 385 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30838              | 10 ppm a.i. 386 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30847              | 10 ppm a.i. 387 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30856              | 10 ppm a.i. 388 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30865              | 10 ppm a.i. 389 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30874              | 10 ppm a.i. 390 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30883              | 10 ppm a.i. 391 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30892              | 10 ppm a.i. 392 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30901              | 10 ppm a.i. 393 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30910              | 10 ppm a.i. 394 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30919              | 10 ppm a.i. 395 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30928              | 10 ppm a.i. 396 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30937              | 10 ppm a.i. 397 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30946              | 10 ppm a.i. 398 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
|                             |                              |                                |                             |                           |                            |          |
|                             |                              |                                |                             |                           |                            |          |
|                             |                              |                                |                             |                           |                            |          |
|                             |                              |                                |                             |                           |                            |          |
|                             |                              | LAS 08/22/02                   |                             |                           |                            |          |
|                             |                              |                                |                             |                           |                            |          |

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 ml

Amount of water added 9ml

Aliquot 1 ml of liver into 15 ml polypropylene tube

Spike samples accordingly

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

Shake sample for 20 min @300 rpm (Shaker WR 51N) 041694

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 34 #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 Kandiyohei Water

Filter sample through conditioned column, discarding filtrate

Allow column to go completely 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 appropriately marked autovial

Extraction done by OK. (1) OK  
0 88.02

### Bottle-Top Dispenser Daily Calibration Check

|                                |                   |               |
|--------------------------------|-------------------|---------------|
| <b>Manufacturer and Model:</b> | VWR 5ml dispenser |               |
| <b>Date:</b>                   | 2/13/2002         | OK<br>2-13-02 |
| <b>Initials:</b>               | OK                |               |
| <b>Dispenser ID:</b>           | 0799              |               |
| <b>Balance ID:</b>             | 900               |               |
| <b>Volume (mL):</b>            | 5                 |               |
| <b>Solvent:</b>                | ACN               |               |
| <b>ID #:</b>                   | TN-A-4145         |               |

|                        | Mass of solvent (g) | Density <sup>1</sup><br>(g/ml) | Volume of<br>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                |
| <b>Average Volume:</b> |                     |                                | 5.052                |
| <b>%Accuracy:</b>      |                     |                                | 101.03               |
| <b>Std. Dev.:</b>      |                     |                                | 0.0169               |
| <b>%C.V.:</b>          |                     |                                | 0.33                 |
| <b>Pass/Fail:</b>      |                     |                                | PASS                 |

PASS

PASS

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

|                                                                                                                     |
|---------------------------------------------------------------------------------------------------------------------|
| <b>Corrective Actions:</b><br><br><div style="font-family: cursive; font-size: 1.2em;">NONE TAKEN! OK 2-13-02</div> |
|---------------------------------------------------------------------------------------------------------------------|

<sup>1</sup>Density values taken from the Merck Index, 12<sup>th</sup> Edition, Copyright 1996

$d^y_x$  = specific gravity at y<sup>degrees C</sup> referred to water at x<sup>degrees C</sup>

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<br>or Description | Dilutions |       |    |    |    |    |    | Comments     | Verified By: |
|---------------------------------|-----------|-------|----|----|----|----|----|--------------|--------------|
|                                 | 1/10      | 1/100 | 1/ | 1/ | 1/ | 1/ | 1/ |              |              |
| E01-1245-28480                  | NA        | x     |    |    |    |    |    |              | OK 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 (P) 90.

OK  
8202

Form Completion Verified By: OK 4.4.02

## Attachment D: Dilutions Summary Worksheet

Study: E01-1342

Dilution Date/Analyst:

Box Number: E01-1342

Solvent/TN Number: MeOH/5457 W/IS PFDA

Extraction Date/Analyst: 2/13/02

Matrix/Timepoint: Quail liver

| Sample Number<br>or Description | Dilutions |       |    |    |    |    |    | Comments | Verified By: |
|---------------------------------|-----------|-------|----|----|----|----|----|----------|--------------|
|                                 | 1/10      | 1/100 | 1/ | 1/ | 1/ | 1/ | 1/ |          |              |
| E01-1342-30672                  | x         |       |    |    |    |    |    |          | OK 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         |       |    |    |    |    |    |          |              |
| 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 ~~E~~ AC  
OK 4-28-02

Form Completion Verified By: OK 4-11-02

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

Method Revision: ETS-8-231.0

Study Number: E01-1342/1365 (25) 1245 643

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<br>TN-A-5754      | NA       |
| E01-1342-36037              | Blank female liver-day 4     | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36038              | Blank male liver-day 4       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36039              | Female liver MS-day 4        | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 50ul of 02001-66          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-36040              | Female liver MSD-day 4       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | 50ul of 02001-66          | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30955              | 10 ppm a.i. 399 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30964              | 10 ppm a.i. 400 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30973              | 10 ppm a.i. 401 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30982              | 10 ppm a.i. 402 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-30991              | 10 ppm a.i. 403 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-31000              | 10 ppm a.i. 404 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-31009              | 10 ppm a.i. 405 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-31018              | 10 ppm a.i. 406 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-31027              | 10 ppm a.i. 407 M Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1342-31036              | 10 ppm a.i. 408 F Liver      | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1365-31184              | 0 ppm a.i. 246 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1365-31194              | 0 ppm a.i. 253 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1365-31199              | 0 ppm a.i. 256 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1365-31204              | 0 ppm a.i. 259 F Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>TN-A-5754      |          |
| E01-1365-31209              | 0 ppm a.i. 266 M Liver       | 40+6                           | C18, 1g, 6cc, lot W2025B1   | NA                        | 2ml MeOH<br>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 polypropylene 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 polypropylene 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 completely 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 3ul

Transfer sample into appropriately marked autovial

Extraction done by OK. AC OK 2/15/02

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

Method Revision: ETS-8-231.0

Study Number: E01-1342/1365

Matrix:

[illegible]

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 polypropylene 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 completely 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  $\mu\text{g/mL}$ , amount added 2  $\mu\text{L}$

Transfer sample into appropriately marked autovial

Extraction done by OK ~~AC~~ ~~OK~~ ~~RS~~ ~~OK~~ 82802

## Bottle-Top Dispenser Daily Calibration Check

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

|                 | Mass of solvent (g) | Density <sup>1</sup><br>(g/ml) | Volume of<br>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               |
| Average Volume: |                     |                                | 5.0249               |
| %Accuracy:      |                     |                                | 100.50               |
| Std. Dev.:      |                     |                                | 0.04202              |
| %C.V.:          |                     |                                | 0.84                 |
| Pass/Fail:      |                     |                                | PASS                 |

PASS

PASS

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

|                     |
|---------------------|
| Corrective Actions: |
|---------------------|

<sup>1</sup>Density values taken from the Merck Index, 12<sup>th</sup> Edition, Copyright 1996

$d_x^y$  = specific gravity at y<sup>degrees C</sup> referred to water at x<sup>degrees C</sup>

n-Hexane  $d_4^{20}$  = 0.660

Acetone  $d_0^{25}$  = 0.788

Methanol  $d_4^{25}$  = 0.7866

Methylene Chloride  $d_4^{20}$  = 1.3255

info entered into form directly since  
computer was available in prep lab.

---

**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.

JAN. 29. 2002 11:34AM

ENVIRONMENTAL LAB 2 3E 09

NO. 6313 P. 3

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-108,  
E01-1245

Séan P. Gallagher  
Sean P. Gallagher, Study Director

1/29/02  
Date

Joann B. Beavers  
Joann B. Beavers, Wildlife International, LTD  
Laboratory Management

1/29/02  
Date

Harold O. Johnson  
Harold O. Johnson, Principal Analytical Investigator

4.4.02  
Date

William K. Reagen  
William K. Reagen, 3M Environmental Laboratory,  
Laboratory Management

04/04/02  
Date

Rochelle K. Robideau  
Rochelle K. Robideau, Sponsor's Representative

1/31/02  
Date

WILDLIFE INTERNATIONAL, LTDPROJECT NO.: 454-108,  
E01-1245Sean P. Gallagher, Study DirectorDateJoann B. Beavers, Wildlife International, LTD  
Laboratory ManagementDateHarold O. Johnson, Principal Analytical InvestigatorDateWilliam K. Reagen, 3M Environmental Laboratory,  
Laboratory ManagementDateRochelle R. Robideau, Sponsor's RepresentativeDate



## **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  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$  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 ( $\text{C}_8\text{F}_{17}\text{SO}_3\text{K}$ ). Note: The molecular weight of the anion (PFOS) is 499 ( $\text{C}_8\text{F}_{17}\text{SO}_3^-$ ).

**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 may 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 Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC Electrospray/Mass Spectrometry" and "Extraction of Potassium

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

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

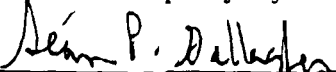
EFFECTIVE DATE: January 30, 2002

AMENDMENT:

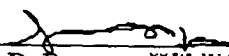
This amendment includes an attachment (Attachment A) which provides updated information necessary to complete the analytical phase of this study. This attachment A replaces the attachment A in amendment 9.

REASON:

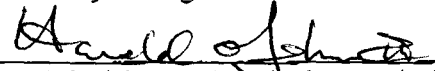
The amendment 9 attachment A was updated to include a different extraction and analytical method, to update the data quality objectives, and to change the liver and sera extraction location.

  
Sean P. Gallagher, Study Director

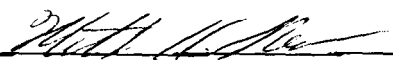
1/30/02  
Date

  
Joann B. Beavers, Wildlife International, LTD,  
Laboratory Management

1/30/02  
Date

  
Harold O. Johnson, Principal Analytical Investigator

01/31/02  
Date

  
William K. Reagen, 3M Environmental Laboratory,  
Laboratory Management

01/31/02  
Date

  
Rochelle R. Robideau, Sponsor's Representative

01/31/02  
Date

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WILDLIFE INTERNATIONAL, LTDPROJECT NO.: 454-108,  
E01-1245

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**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

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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.

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Sean P. Gallagher, Study Director

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Date

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Joann B. Beavers, Wildlife International, LTD,  
Laboratory Management

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Date

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Harold O. Johnson, Principal Analytical Investigator

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Date

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William K. Reagen, 3M Environmental Laboratory,  
Laboratory Management

---

Date

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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<sub>8</sub>F<sub>17</sub>SO<sub>3</sub>K). Note: The molecular weight of the anion (PFOS) is 499 (C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup>).

**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.

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.: 11

SPONSOR: 3M Corporation

PROJECT NO.: 454-108, E01-1245

EFFECTIVE DATE: March 27, 2002

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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  $\geq 0.985$ .

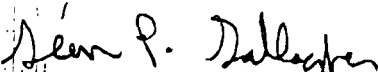
REASON: No coefficient of determination is included in the protocol. It was inadvertently removed prior to printing of the final copy.

04/01/02 19:29 ASD INF TECH 2-2E-01 → \*84108228915

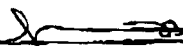
NO.905 P007

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-108,  
E01-1245

  
Sean P. Gallagher, Study Director

4/2/02  
Date

  
Joann B. Beavers, Wildlife International, LTD  
Laboratory Management

4/2/02  
Date

Harold O. Johnson, Principal Analytical Investigator

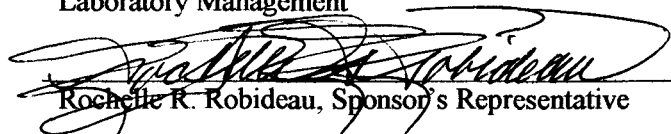
Date

William K. Reagen, 3M Environmental Laboratory,  
Laboratory Management

Date

  
Rochelle K. Robideau, Sponsor's Representative

4/1/02  
Date

WILDLIFE INTERNATIONAL, LTDPROJECT NO.: 454-108,  
E01-1245Sean P. Gallagher, Study DirectorDateJoann B. Beavers, Wildlife International, LTD  
Laboratory ManagementDate  
Harold O. Johnson, Principal Analytical Investigator4-2-02Date  
William K. Reagen, 3M Environmental Laboratory,  
Laboratory Management04-02-02  
Date  
Rochelle R. Robideau, Sponsor's Representative4/1/02  
Date

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WILDLIFE INTERNATIONAL, LTDPROJECT NO.: 454-108,  
E01-1245

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**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

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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:

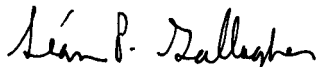
Change: Harold Johnson is the Principal Analytical Investigator.

To: Lisa A. Stevenson is the Principal Analytical Investigator.

## REASON:

To change role of the Principal Analytical Investigator.

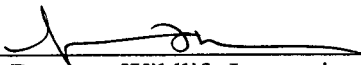
WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-108,  
E01-1245

Sean P. Gallagher, Study Director

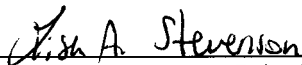
9/25/02

Date

Joann B. Beavers, Wildlife International, LTD,  
Laboratory Management

9/25/02

Date



Lisa A. Stevenson, Principal Analytical Investigator

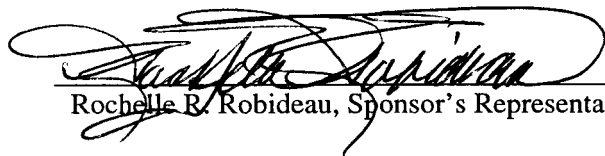
08/20/02

Date

William K. Reagen, 3M Environmental Laboratory,  
Laboratory Management

08/20/02

Date



Rochelle R. Robideau, Sponsor's Representative

8/20/02

Date

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

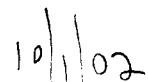
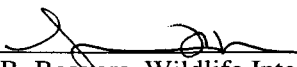
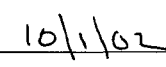
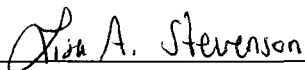
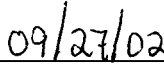
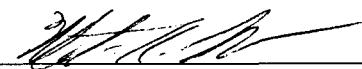
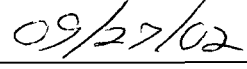
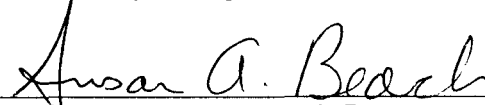
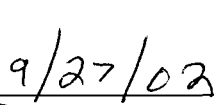
## AMENDMENT:

Change: Rochelle R. Robideau is the Sponsor Representative.

To: Susan A. Beach is the Sponsor Representative.

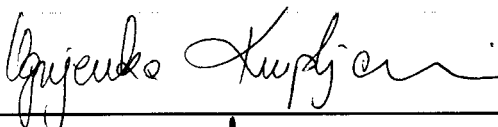
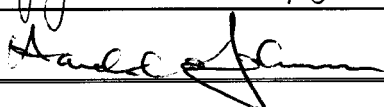
## REASON:

To change role of the Sponsor Representative.

  
Sean P. Gallagher, Study Director  
Date  
Joann B. Beavers, Wildlife International, LTD,  
Laboratory Management  
Date  
Lisa A. Stevenson, Principal Analytical Investigator  
Date  
William K. Reagen, 3M Environmental Laboratory,  
Laboratory Management  
Date  
Susan A. Beach, Sponsor's Representative  
Date

3M Confidential

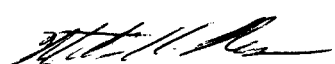
## Record of Deviation

| <b>I. Identification</b>                                                                                                                                                                                                                                                                                                    |                                                                                                                                               |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Study / Project No.<br>E01-1245                                                                                                                                                                                                                                                                                             |                                                                                                                                               |
| Deviation type <input checked="" type="checkbox"/> SOP <input type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure<br>(Check one) <input type="checkbox"/> Protocol <input type="checkbox"/> Other:                                                                                                        |                                                                                                                                               |
| Document number ETS-4-4.0 <div style="float: right; text-align: right;">             Date(s) of occurrence<br/>             1/31/02, 2/1/02, 2/13/02, 2/7/02, 2/8/02, 2/11/02,<br/>             2/12/02, 2/13/02, 2/15/02           </div>                                                                                  |                                                                                                                                               |
| <b>II. Description</b>                                                                                                                                                                                                                                                                                                      |                                                                                                                                               |
| <b>Required procedure/process:</b><br>ETS-4-4.0 Internal chain of custody<br>The internal chain of custody form to be used to track all movement of samples<br>Within the lab. The form should be filed with study data or other relevant sample documents.                                                                 |                                                                                                                                               |
| <b>Actual procedure/process:</b><br>Internal chains of custodies were not used to track samples and sample extracts. Since receipt samples were<br>Stored in freezer F1. After homogenizing liver and aliquating sera samples were stored in Freezer F8<br>Until extraction date. Samples remained frozen until extraction. |                                                                                                                                               |
| <b>III. Actions Taken</b>                                                                                                                                                                                                                                                                                                   |                                                                                                                                               |
| (such as amendment issued, SOP revision, etc.)                                                                                                                                                                                                                                                                              |                                                                                                                                               |
| Deviation to SOP written.                                                                                                                                                                                                                                                                                                   |                                                                                                                                               |
| Recorded by                                                                                                                                                                                                                                                                                                                 | <br><div style="text-align: right;">Date<br/>4-9-02</div>  |
| Authorized by                                                                                                                                                                                                                                                                                                               | <br><div style="text-align: right;">Date<br/>4-10-02</div> |

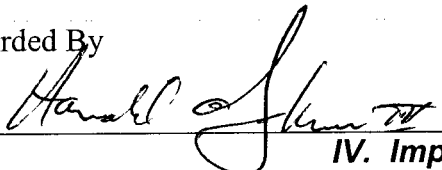
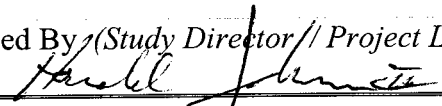
Deviation No. 1

(assigned by Study Director or Project Lead at the end of study or project)

Study Director: Sean P. Gallagher 9/24/02

Laboratory Management:  08/20/02

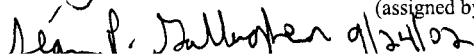
## Record of Deviation

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                           |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>I. Identification</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                           |
| Study / Project No.E01-1245                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                           |
| Deviation Type<br>(Check one)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <input type="checkbox"/> SOP <input type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure<br><input checked="" type="checkbox"/> Protocol <input type="checkbox"/> Other: |
| Document Number:Protocol #:<br>454/120700/QR/SUB454,<br>amendment #: 10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Date(s) of occurrence: :1/31/02, 2/1/02, 2/2/02, 2/7/02,<br>2/11/02, 2/12/02, 2/13/02, 2/14/02, 2/18/02, 4/11/02,<br>4/16/02                                                              |
| <b>II. Description:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                           |
| <p><b>Required Procedure/process:</b> Section 11.1, System Suitability, second sentence says that a mid-level unextracted calibration standard will be analyzed.</p> <p><b>Actual Procedure/process:</b><br/>           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.</p> |                                                                                                                                                                                           |
| <b>III. Actions Taken:</b><br>(such as amendment issued, SOP revision, etc.)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                           |
| Issuing of this protocol deviation.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                           |
| Recorded By<br>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Date<br>4.19.02                                                                                                                                                                           |
| <b>IV. Impact on Study / Project</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                           |
| 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)<br>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Date<br>4.19.02                                                                                                                                                                           |

3M Environmental Laboratory  
Form ETS-4-8.0

Deviation No. 2

Lab. Director:

 9/24/02

(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                      ☒ SOP                      ☐ Method                      ☐ Equipment Procedure  
(Check one)                      ☐ 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...

$(20\mu\text{L}) (1.00\mu\text{gPFOS}/\text{mL MeOH}) (1.0\text{mL}/1000\mu\text{L}) (1000\text{ng}/1.0\mu\text{g}) = 20\text{ng PFOS}^- \text{ onto SPE column}$

$20\text{ng PFOS}^- / 2.0\text{mL MeOH} = 10\text{ng PFOS}^-/\text{mL MeOH}.$

(Since this standard is recorded as 10ppb, the correct units are 10ngPFOS<sup>-</sup>/mL of MeOH.)

These are therefore the units being measured and reported on the chromatographic system.

Similarly, the following statement is true.

$(20\text{ng PFOS}^- / 1.0\text{mL homogenate}) (1.0\text{mL homogenate} / 0.1\text{g liver}) = 200\text{ng PFOS}^- / \text{g liver}$

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:

$(10.76\text{ng PFOS}^- / \text{mL MeOH}) (2\text{mL MeOH} / 0.1033 \text{ g liver}) = 208.4 \text{ ng PFOS}^- / \text{g liver}$

Sample Calculation:

Sample "E01-1342-36017, QC@10ppb-1": This QC sample is prepared by adding 4uL of 02001-53 (5.020ug PFOS<sup>-</sup> / mL MeOH) spiked into 1mL liver homogenate.

$((4\mu\text{L}) (5.020\mu\text{g}/\text{mL}) (1\text{mL}/1000\mu\text{L}) (1000\text{ng}/\mu\text{g})) / (0.1\text{g liver}) = 200.8\text{ng PFOS}^- / \text{g liver}$

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<sup>-</sup> / g liver, the recovery is  $(271.8/200.8)(100)=135.4\%$ .

**Record of Deviation**

|                                                                                                                                     |                 |
|-------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| <b>III. Actions Taken:</b><br>(such as amendment issued, SOP revision, etc.)<br>Issuing of this SOP deviation and a note to file.   |                 |
| Recorded By<br>                                    | Date<br>4.19.02 |
| <b>IV. Impact on Study / Project</b><br>There will be no impact on the study as this is for clarification purposes.                 |                 |
| Authorized By (Study Director / Project Lead)<br> | Date<br>4.19.02 |

Study Director: Sean P. Gallagher 4/24/02

Laboratory Management:  04/20/02

## Record of Deviation

| <b>I. Identification</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                             |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| Study / Project No. E01-1245                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                             |
| Deviation Type <span style="margin-left: 50px;"><input type="checkbox"/> SOP</span> <span style="margin-left: 50px;"><input type="checkbox"/> Method</span> <span style="margin-left: 50px;"><input type="checkbox"/> Equipment Procedure</span><br>(Check one) <span style="margin-left: 50px;"><input checked="" type="checkbox"/> Protocol</span> <span style="margin-left: 50px;"><input type="checkbox"/> Other:</span>                                                                                                                                                                                                                                                                                                                                                                                                |                                             |
| Document Number: ETS-8-231.1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Date(s) of occurrence: Throughout the study |
| <b>II. Description:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                             |
| <b>Required Procedure/process:</b><br>Section 11.3, Sentence 5 states: Any level outside 75% - 125% of nominal value must be deactivated, and regression recalculated...                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                             |
| <b>Actual Procedure/process:</b><br>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.<br>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. |                                             |
| <b>III. Actions Taken:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                             |
| <i>(such as amendment issued, SOP revision, etc.)</i><br>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<br>Lisa A. Stevenson <i>Lisa A. Stevenson</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Date<br>08/16/02<br><i>08/16/02</i>         |
| Authorized By (Study Director / Project Lead)<br>Study Director: <i>Sean P. Gallagher</i> <i>9/24/02</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Date<br><i>8/20/02</i>                      |
| Sponsor Representative: <i>[Signature]</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                             |

## Record of Deviation

| <b>I. Identification</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| Study / Project No. E01-1245                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                     |
| Deviation Type <span style="margin-left: 50px;"><input checked="" type="checkbox"/> SOP</span> <span style="margin-left: 20px;"><input type="checkbox"/> Method</span> <span style="margin-left: 20px;"><input type="checkbox"/> Equipment Procedure</span><br>(Check one) <span style="margin-left: 50px;"><input type="checkbox"/> Protocol</span> <span style="margin-left: 20px;"><input type="checkbox"/> Other:</span>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                     |
| Document Number: ETS-8-231.1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Date(s) of occurrence: Entire Study |
| <b>II. Description:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                     |
| <b>Required Procedure/process:</b><br>Section 12.1, Calculations: If other calculations are used than those listed, they will be documented in the raw data.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                     |
| <b>Actual Procedure/process:</b><br>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...<br><br>$(5.020\text{ug PFOS/mL MeOH}) * (4\text{ul}) * (1.0\text{mL}/1000\text{uL}) * (1000\text{ng}/1.0\text{ug}) = 20\text{ng PFOS onto SPE column}$<br><br>$20\text{ng PFOS} / 2.0\text{mL MeOH} = 10\text{ng PFOS/mL MeOH.}$ (Since this standard is recorded as 10ppb, the correct units are <u>10ng PFOS/mL MeOH.</u> )<br>These are therefore the units being measured and reported on the chromatographic system.<br><br>Similarly, the following statement is true.<br>$(20\text{ng PFOS} / 1.0\text{mL diluted sera}) * (40.0\text{mL diluted sera} / 2.0\text{mL initial sera}) = 400\text{ng PFOS/ mL Sera}$<br><br>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.<br><br><b>Sample Calculation:</b><br>"E01-1245-28481, 454-108-370, 10 ppm female, adult, 02-01-02<br>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 38.0mL water, and 1mL is used for the entire SPE procedure, the calculation is as follows:<br>$229.2\text{ng PFOS-} / 1.0\text{mL MeOH}) * (2.0\text{mL MeOH} / 0.05\text{mL Sera}) = 9170\text{ng PFOS-} / \text{mL Sera}$<br><br><b>Sample Calculation:</b><br>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).<br><br>$((5.02\text{ug/mL}) * (2\text{ul}) * (1\text{mL}/1000\text{uL}) * (1000\text{ng}/\text{ug})) / (0.05 \text{ mL Sera}) = 200.8 \text{ ng PFOS} / \text{mL Sera}$<br><br>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 $(246.8/200.8)*(100)=122.9\%$ . |                                     |
| <b>III. Actions Taken:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                     |
| (such as amendment issued, SOP revision, etc.)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                     |
| This deviation was written and a note to file was written.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                     |
| Recorded By<br>Lisa A. Stevenson <i>Lisa A. Stevenson</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Date<br>08/16/02 <i>08/16/02</i>    |
| Authorized By (Study Director / Project Lead)<br>Study Director: <i>Sean P. Gallagher</i> <i>9/24/02</i> <i>11/25/02</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                     |
| Laboratory Management: <i>[Signature]</i> <i>08/20/02</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                     |
| 3M Environmental Laboratory<br>Form ETS-4.8.0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                     |
| Deviation No. <u>5</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                     |

(assigned by Study Director or Project Lead at the end of study or project)

## Record of Deviation

| <b>I. Identification</b>                                                                                                                                                                                                      |                                                                                                                                                                                           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Study / Project No. E01-1245                                                                                                                                                                                                  |                                                                                                                                                                                           |
| Deviation Type<br>(Check one)                                                                                                                                                                                                 | <input type="checkbox"/> SOP <input checked="" type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure<br><input type="checkbox"/> Protocol <input type="checkbox"/> Other: |
| Document Number: ETS-8-231.1                                                                                                                                                                                                  | Date(s) of occurrence: Throughout the study                                                                                                                                               |
| <b>II. Description:</b>                                                                                                                                                                                                       |                                                                                                                                                                                           |
| <b>Required Procedure/process:</b><br>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%.          |                                                                                                                                                                                           |
| <b>Actual Procedure/process:</b><br>All 25 ppb quail liver quality control sample recoveries were >125%.<br>10 RE UAS 03/11/03<br>7/14 03/12/03<br>123 3/14/03                                                                |                                                                                                                                                                                           |
| <b>III. Actions Taken:</b>                                                                                                                                                                                                    |                                                                                                                                                                                           |
| (such as amendment issued, SOP revision, etc.)<br>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<br>Lisa A. Stevenson                                                                                                                                                                                              | Date<br>08/26/02 8/26/02<br><i>Lisa A. Stevenson</i>                                                                                                                                      |
| Authorized By (Study Director / Project Lead)                                                                                                                                                                                 | Date                                                                                                                                                                                      |
| Study Director: <i>Sean P. Gallagher</i>                                                                                                                                                                                      | 10/1/02                                                                                                                                                                                   |
| Laboratory Management: <i>[Signature]</i> 08/26/02                                                                                                                                                                            |                                                                                                                                                                                           |

## Record of Deviation

| <b>I. Identification</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                           |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Study / Project No.E01-1245                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                           |
| Deviation Type<br>(Check one)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | <input checked="" type="checkbox"/> SOP <input type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure<br><input type="checkbox"/> Protocol <input type="checkbox"/> 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                                                                      |
| <b>II. Description:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                           |
| <b>Required Procedure/process:</b> Section 12.1, Calculations: If other calculations are used than those listed, they will be documented in the raw data.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                           |
| <b>Actual Procedure/process:</b><br>This deviation supersedes deviation #3 explaining the liver calculation:<br><br>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...<br><br>$(20 \text{ uL}) * (1.000 \text{ ug PFOS/mL MeOH}) * (1.0 \text{ mL}/1000 \text{ uL}) * (1000 \text{ ng}/1.0 \text{ ug}) = 20.0 \text{ ng PFOS onto SPE column}$ $20.0 \text{ ng PFOS} / 2.0 \text{ mL MeOH} = 10.0 \text{ ng PFOS/mL MeOH.}$ (Since this standard is recorded as 10ppb, the correct units are <u>10.0 ng PFOS/mL of MeOH.</u> )<br>These are the units being measured and reported on the chromatographic system.<br><br>Similarly, the following statement is true.<br><br>$(20.0\text{ng PFOS} / 1.0\text{mL homogenate}) * (1.0\text{mL homogenate} / 0.100635 \text{ g liver}) = 199\text{ng PFOS} / \text{g liver}$ |                                                                                                                                                                                           |
| <u>Sample Calculation:</u><br>"E01-1342-30348, 0ppm a.i. 333 M liver"<br><br>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:<br><br>$1.0338 \text{ g} / (9.0 \text{ mL water} + 1.0338 \text{ g liver}) = 0.103032 \text{ g of liver in } 1.0 \text{ mL of homogenate removed for extraction.}$ $(10.76 \text{ ng PFOS-/ mL MeOH}) * (2.0 \text{ mL MeOH} / 0.103032 \text{ g liver}) = 209 \text{ ng PFOS- / g liver}$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                           |
| <u>Sample Calculation:</u><br>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.<br><br>$((4\text{ul}) * (5.020\text{ug/mL}) * (1\text{mL}/1000\text{uL}) * (1000\text{ng/ug})) / (0.100635\text{g liver}) = 200 \text{ ng PFOS} / \text{g liver}$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                           |
| 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 $(270/200) * (100) = 135.0\%$ .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                           |
| <b>III. Actions Taken:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                           |
| (such as amendment issued, SOP revision, etc.)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                           |
| Issuing of this SOP deviation.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                           |
| Recorded By<br>Lisa A. Stevenson <i>Lisa A. Stevenson</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Date<br>12/01/02 <i>12/01/02</i>                                                                                                                                                          |
| Authorized By (Study Director / Project Lead)<br>Study Director: Sean Gallagher <i>Sean P. Gallagher</i><br>Laboratory Management: William Reagen <i>William Reagen</i><br>3M Environmental Laboratory                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Date<br>12/3/02 <i>12/3/02</i>                                                                                                                                                            |
| <b>Deviation No.</b> <u>7</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                           |

(assigned by Study Director or Project Lead at the end of study or project)

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## ATTACHMENT F: EXAMPLE CALCULATIONS

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### FORMULA USED FOR SERA ANALYSES IN STUDY E01-1245

$$\text{AR (ng/mL MeOH)} \times \text{DF} \times \text{Dilution(diluted sera)} = \text{ng/mL Sera}$$

**Calculation Used for E01-1245-28481, 454-108-370, 10 ppm Female, adult (initial volume = 0.3 mL)**

$$229.24 \text{ ng/mL MeOH} \times 1 \times 40 = 9170 \text{ ng/mL Sera}$$

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### 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)**

$$393.21 \text{ ng/mL MeOH} \times 10 \times \frac{2.0 \text{ mL MeOH}}{0.100090 \text{ g Liver}} = 78600 \text{ ng/g Liver}$$

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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)

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#### 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

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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