

AR226-1743

Study Title

ANALYTICAL PHASE REPORT FOR
PFOS: A REPRODUCTION STUDY WITH THE MALLARD

Data Requirement

FIFRA Subdivision E, Section 71-4
OECD Guideline 206

Author

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Analytical Phase Initiation Date

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Analytical Phase Completion Date

At Signing

Performing Laboratory

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Project Identification

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216

Report E01-1256

PFOS: A Reproduction Study with the Mallard

GLP COMPLIANCE STATEMENT

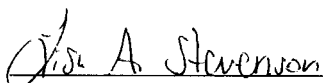
Study Title: Analytical Phase Report for PFOS: A Reproduction Study with the Mallard

Study Identification Number: E01-1256

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:

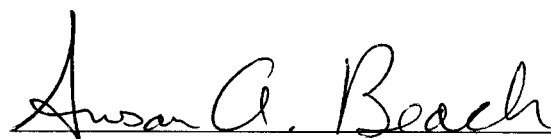
- Automated data collection systems are not validated. The hardcopy printouts are considered as the original raw data.
- The stability of the samples has not been determined.
- Not all data generated were recorded directly or promptly, or initiated and dated on date of entry.



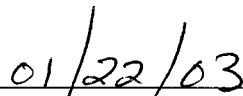
Lisa A. Stevenson, Principal Analytical Investigator



Date



Susan A. Beach, Sponsor Representative



Date

QUALITY ASSURANCE STATEMENT

Study Title: Analytical Phase Report for PFOS: A Reproduction Study with the Mallard

Study Identification Number: E01-1256

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
02/22/02	In-phase	02/22/02	02/22/02
04/03/02-04/08/02, 04/17/02	Data	04/08/02, 05/21/02	04/08/02, 05/21/02
05/13/02-05/15/02	Final Report	05/16/02	05/16/02


Quality Assurance Representative

1/22/03
Date

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STUDY INFORMATION

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Experimental Dates

Sample Analysis Initiation: 19 February 2002
Sample Analysis Completion: 11 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 mallards and their offspring in both a control group and a 10ppm dosed group from Wildlife feeding study #454-109. The following tables summarize the $\text{C}_8\text{F}_{17}\text{SO}_3^-$ concentrations found in the liver and sera of study samples. Extracted-matrix PFOS standards are used to establish the HPLC-ES/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	18	NA	<100 ng/mL (14 of 18) 107-1400 ng/mL range (4 of 18)	20	<50 ng/g	NA
Female Control Group	20	NA	<100 ng/mL (15 of 20) 166-2680 ng/mL range (5 of 20)	20	<50 ng/g	NA
Male 10ppm Group	20	89700 ng/mL	11300-210000 ng/mL	20	60900 ng/g	24200-125000 ng/g
Female 10ppm Group	20	20300 ng/mL	6350-91400 ng/mL	20	10800 ng/g	2480-30700 ng/g
Male Offspring Control Group	3	NA	<100 ng/mL (2 of 3) 103 ng/mL (1 of 3)	3	<50 ng/g	NA
Female Offspring Control Group	7	NA	<100 ng/mL (7 of 7)	7	<50 ng/g	NA
Male Offspring 10ppm Group	3	4410 ng/mL	3670 - 5450 ng/mL	3	3170 ng/g	2200-4640 ng/g
Female Offspring 10ppm Group	7	4960 ng/mL	3420 - 6760 ng/mL	7	3620 ng/g	2240-5930 ng/g

For individual sample values see Tables 10 to 13 and Attachment B.

Table 2. Summary Table, C₈F₁₇SO₃⁻ Concentration in Sera and Liver with Outliers Removed

SAMPLING EVENT	NUMBER OF SERA SAMPLES	AVERAGE C ₈ F ₁₇ SO ₃ ⁻ CONC. SERA	Range of Results	NUMBER OF LIVER SAMPLES	AVERAGE C ₈ F ₁₇ SO ₃ ⁻ CONC. LIVER	Range of Results
Male Control Group	18*	*	*	20*	*	*
Female Control Group	17	NA	<100 ng/mL (15 of 17) 166-172 ng/mL range (2 of 17)	20*	*	*
Male 10ppm Group	18	87300 ng/mL	68700-108000 ng/mL	20*	*	*
Female 10ppm Group	19	16600 ng/mL	6350-42800 ng/mL	20*	*	*
Male Offspring Control Group	3*	*	*	3*	*	*
Female Offspring Control Group	7*	*	*	7*	*	*
Male Offspring 10ppm Group	3*	*	*	3*	*	*
Female Offspring 10ppm Group	7*	*	*	7*	*	*

* No outliers removed from this data set. Refer to Table 1 for results. For individual sample values see Tables 10 to 13 and Attachment B.

PURPOSE

The purpose of the analytical phase of this study is to quantify C₈F₁₇SO₃⁻ in the liver and sera of both control and dosed mallards and in 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, C₁₀F₁₉O₂H and is used solely to monitor for gross instrument failure and not as an internal standard for quantitation.

SPECIMEN RECEIPT AND STORAGE

Mallard serum and liver samples were obtained from the sacrifice of the test animals upon the conclusion of the in-life phase of the study (#454-109) conducted at Wildlife International, Ltd. and sent to 3M Environmental Laboratory for analysis. The specimens were received at 3M Environmental Laboratory, assigned a laboratory tracking (LIMS) number (E01-1256, E01-1326, or E01-1367), and were stored at -20°C +/- 10°C until prepared for 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. After drying the column, 2.0 mL 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 1.0mL aliquot of the diluted sera or homogenate of liver was extracted by adding 5.0mL 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 volume of sera or weigh portion of liver
2	Dilute sera (1/20) and 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 2.0mL methanol (MeOH).

SAMPLE RECEIPT, PREPARATION 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 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	Aliquoting and preparation of the sera sample homogenates (all groups and dose levels)	19 February 2002
2	Weighing of liver samples (control group and dosed group offspring)	21 February 2002
3	Weighing of liver samples (control group and dosed group adults)	22 February 2002
4	Homogenizing of liver samples (control group and dosed group offspring)	22 February 2002
5	Homogenizing of liver samples (control group and portion of the dosed group adults)	25 February 2002
6	Homogenizing of liver samples (remainder of dosed group adults)	26 February 2002
7	Preparation of method blanks and spikes, calibration curve, qc samples, and sera samples, control group, adults (Day 1 sera samples)	25 February 2002
8	Analysis of Day 1 sera samples	25 February 2002
9	Preparation of method blanks and spikes, and sera samples, control and 10 ppm adults (Day 2 sera samples)	26 February 2002
10	Analysis of Day 2 sera samples	26 February 2002
11	Preparation of method blanks and spikes, and sera samples, 10 ppm adults (Day 3 sera samples)	27 February 2002
12	Analysis of Day 3 sera samples	27 February 2002
13	Preparation of method blanks and spikes, and sera samples, control group, and 10 ppm offspring (Day 4 sera samples)	28 February 2002
14	Analysis of Day 3 and Day 4 sera samples	28 February 2002
15	Analysis of diluted sera samples	01 March 2002
16	Preparation of method blanks and spikes, calibration curve, qc samples, and liver samples, control group, adults (Day 1 liver samples)	04 March 2002
17	Analysis of Day 1 liver samples	04 March 2002
18	Re-analysis of Day 1 liver samples	05 March 2002
19	Preparation of method blanks and spikes, and liver samples, 10ppm dosed group, adults (Day 2 liver samples)	05 March 2002

STEP	PROCEDURE	DATE PERFORMED
20	Analysis of Day 2 liver samples	06 March 2002
21	Preparation of method blanks and spikes, and liver samples, control group and 10ppm dosed group, adults (Day 3 liver samples)	06 March 2002
22	Analysis of Day 3 liver samples	06 March 2002
23	Preparation of method blanks and spikes, and liver samples, control group and 10ppm dosed group, offspring (Day 4 liver samples)	07 March 2002
24	Analysis of Day 4 liver samples	07 March 2002
25	Analysis of female sera dilutions	08 March 2002
26	Analysis of QC liver and sera samples	05 April, 2002
27	Analysis of diluted sera samples	11 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 μ m particle size), a 40°C column compartment temperature, a 10 μ L injection volume and a 0.300 μ L/min pump flow rate. The HPLC gradient is listed below:

TIME, MINUTES	% 2 mM AMMONIUM ACETATE	% METHANOL
0	90	10
1.0	90	10
5.5	5	95
7.5	5	95
8	90	10
10	90	10

- The mass spectrometer used for the study was a Micromass Ultima. The system consists of Z-Spray interface equipped with an electrospray probe operated in negative mode. The instrument was setup to operate in MS/MS mode, passing the $C_8F_{17}SO_3^-$ molecular anion ($m/z = 499$) into the collision cell, where it is collided with inert gas to further fragment to the characteristic ion $m/z = 99$, FSO_3^- . Similarly the PFDA transition

from $m/z = 513$ to $m/z = 299$ was monitored. The collision gas, 95% Argon / 5% Methane, was maintained at a pressure of approximately 3.0×10^{-3} mBar.

ANALYTICAL RESULTS

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

- **Regressions.** Quadratic curve fit, weighted $1/X$, was applied to calibration standards and sample data. Each calibration curve had a coefficient of determination of ≥ 0.985 .
- **Calibration Standards.** Instrument response was calibrated daily prior to sample analysis using matrix-extracted standards ranging in concentration in the final solvent extract from 0.20 to 1000 ng $C_8F_{17}SO_3$ /mL methanol, corresponding to a concentration of 8.00 to 40160 ng/mL of sera or 4.00 to 20080 ng/g liver. The calibration curve analyzed consisted of fourteen matrix-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 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 indeterminant, 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 extracted matrix 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 and the precision of the retention time was equal to or less than 2.5% RSD, meeting the stated data 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
QC-25ppb	1004 ng/mL	145%*	9.8%
QC-250ppb	10040 ng/mL	97.9%	2.9%
QC-4.0ppm	160600 ng/mL	84.7%	6.7%

*These samples were spiked using a 1.0 ul aliquot of spiking solution. Matrix spikes prepared on the same day using 50 ul of a diluted stock meet all QC requirements. Spiking technique of injecting 1.0 ul is suspect.

Table 7. Serum Matrix Spike Summary Table

Serum Matrix Spike Samples*	Concentration in Diluted Serum	Average Recoveries
Four Matrix Spikes	1004 ng/mL	114%
Four Matrix Spikes Duplicates	1004 ng/mL	112%

*Some matrix spike samples were 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-25ppb	497 ng/g	109%*	13%
QC-250ppb	4967 ng/g	94.3%	8.3%
QC-4.0ppm	79470 ng/g	103%	0.72%

*These samples were spiked using a 1.0 ul aliquot of spiking solution. Matrix spikes prepared on the same day using 50 ul of a diluted stock meet all QC requirements. Spiking technique of injecting 1.0 ul is suspect.

Table 9. Liver Matrix Spike Summary Table

Liver Matrix Spike Samples*	Concentration in Diluted Liver	Average Recoveries
Four Matrix Spikes	497 ng/g	111%
Four Matrix Spikes Duplicates	497 ng/g	107%

*Some matrix spike samples were injected in a second analytical batch. Both results are included in the average recoveries.

- The protocol states that the acceptance range is 75-125% but makes allowances for a percentage of results to be outliers. The observed number of outliers for the "QC-25ppb" spikes exceeds that permitted in the protocol but the overall impact on data quality is minimal or none at all. All study samples that were not "Day 0", show analyte levels significantly above the LOQ. Occasionally, Day 0 samples or other types of blanks show positive detections at levels near the LOQ.

- **Absolute Recoveries.** The absolute recovery of the QC samples is determined by comparing the results of the extracted quality control spikes quantitated versus an extracted matrix 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 11.1% higher, the medium spikes 8.3% higher, and the high spikes 8.3% higher. The 9.5% average higher quantitation of the three levels of spikes using a solvent curve versus an extracted matrix curve demonstrates that it is appropriate to use matrix-extracted standards. The same trend is observed for liver samples. The extracted liver matrix curves are 12.2% higher, 7.8% higher, and 5.6% higher respectively for the low, medium and high spikes of the liver matrix, again demonstrating that it is appropriate to use matrix-extracted standards.
- **Internal Standard:** The internal standard (PFDA) was added to all samples and standards. PFDA was not used for quantitation, but was used to monitor for gross instrument failure.
- **Instrument Response Calibration.** HPLC-ES/MS/MS instrument response was calibrated using the concentration of the $C_8F_{17}SO_3^-$ anion of perfluorooctane sulfonate, potassium salt (PFOS) that was adjusted for purity and the potassium ion contribution to the mass of PFOS used to prepare stock solutions. Any future calculations requiring concentrations to be expressed as a percentage of dietary in-take must adjust the dosed amounts as well.

DATA SUMMARY

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

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

3M SAMPLE NUMBER	SAMPLE DESCRIPTION	AMOUNT OF SERA USED (ML)	ADDITIONAL DILUTION	CONC. OF $C_8F_{17}SO_3^-$ IN SAMPLE (NG/ML OF SERA)
E01-1256-28663	454-109-3218, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28665	454-109-3220, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28667	454-109-3222, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28669	454-109-3224, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28670	454-109-3226, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28672	454-109-3228, 0 PPM, Female, Adult	0.5	1	2680 ●
E01-1256-28674	454-109-3230, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28676	454-109-3232, 0 PPM, Female, Adult	0.5	1	172
E01-1256-28678	454-109-3234, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28679	454-109-3236, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28681	454-109-3238, 0 PPM, Female, Adult	0.5	1	166
E01-1256-28683	454-109-3240, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28685	454-109-3242, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28687	454-109-3244, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28689	454-109-3246, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28691	454-109-3248, 0 PPM, Female, Adult	0.5	1	398 ●
E01-1256-28693	454-109-3250, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28695	454-109-3252, 0 PPM, Female, Adult	0.5	1	757 ●

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)
E01-1256-28697	454-109-3254, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28699	454-109-3256, 0 PPM, Female, Adult	0.5	1	<100
E01-1256-28701	454-109-3258, 10 PPM, Female, Adult	0.5	1	13600
E01-1256-28703	454-109-3260, 10 PPM, Female, Adult	0.5	1	9420
E01-1256-28705	454-109-3262, 10 PPM, Female, Adult	0.5	1	17000
E01-1256-28707	454-109-3264, 10 PPM, Female, Adult	0.5	1	6350
E01-1256-28709	454-109-3266, 10 PPM, Female, Adult	0.5	1	7900
E01-1256-28711	454-109-3268, 10 PPM, Female, Adult	0.5	1	6730
E01-1256-28713	454-109-3270, 10 PPM, Female, Adult	0.5	10	91400●
E01-1256-28715	454-109-3272, 10 PPM, Female, Adult	0.5	1	7710
E01-1256-28717	454-109-3274, 10 PPM, Female, Adult	0.5	10	29400
E01-1256-28719	454-109-3276, 10 PPM, Female, Adult	0.5	10	40600
E01-1256-28721	454-109-3278, 10 PPM, Female, Adult	0.5	1	9840
E01-1256-28723	454-109-3280, 10 PPM, Female, Adult	0.2	10	32200
E01-1256-28725	454-109-3282, 10 PPM, Female, Adult	0.5	1	10800
E01-1256-28727	454-109-3284, 10 PPM, Female, Adult	0.5	1	6570
E01-1256-28729	454-109-3286, 10 PPM, Female, Adult	0.5	10	28900
E01-1256-28731	454-109-3288, 10 PPM, Female, Adult	0.5	10	42800
E01-1256-28733	454-109-3290, 10 PPM, Female, Adult	0.5	1	16000
E01-1256-28735	454-109-3292, 10 PPM, Female, Adult	0.5	1	10400
E01-1256-28737	454-109-3294, 10 PPM, Female, Adult	0.5	1	7660
E01-1256-28739	454-109-3296, 10 PPM, Female, Adult	0.5	1	10800
E01-1256-28740	454-109-9583, 0 PPM, Female, Offspring	0.15	1	<100
E01-1256-28742	454-109-9589, 0 PPM, Female, Offspring	0.2	1	<100
E01-1256-28743	454-109-9592, 0 PPM, Female, Offspring	0.2	1	<100
E01-1256-28744	454-109-9598, 0 PPM, Female, Offspring	0.1	1	<100
E01-1256-28745	454-109-9603, 0 PPM, Female, Offspring	0.2	1	<100
E01-1256-28747	454-109-9610, 0 PPM, Female, Offspring	0.2	1	<100
E01-1256-28748	454-109-9573, 0 PPM, Female, Offspring	0.05	1	<100
E01-1256-28751	454-109-9629, 10 PPM, Female, Offspring	0.1	1	5130
E01-1256-28752	454-109-9633, 10 PPM, Female, Offspring	0.1	1	3420
E01-1256-28753	454-109-9634, 10 PPM, Female, Offspring	0.5	1	6760
E01-1256-28754	454-109-9645, 10 PPM, Female, Offspring	0.1	1	3750
E01-1256-28755	454-109-9648, 10 PPM, Female, Offspring	0.2	1	4900
E01-1256-28758	454-109-9654, 10 PPM, Female, Offspring	0.1	1	5450
E01-1256-28759	454-109-9659, 10 PPM, Female, Offspring	0.1	1	5350

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

Table 11. C₈F₁₇SO₃⁻ Data Summary, Male Mallard Sera (Adult and Offspring)

3M SAMPLE NUMBER	SAMPLE DESCRIPTION	AMOUNT OF SERA USED (mL)	ADDITIONAL DILUTION	CONC. OF C ₈ F ₁₇ SO ₃ ⁻ IN SAMPLE (NG/ML OF SERA)
E01-1256-28662	454-109-3217, 0 PPM, Male, Adult	0.5	1	107
E01-1256-28664	454-109-3219, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28666	454-109-3221, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28668	454-109-3223, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28671	454-109-3227, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28673	454-109-3229, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28675	454-109-3231, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28677	454-109-3233, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28680	454-109-3237, 0 PPM, Male, Adult	0.3	1	<100
E01-1256-28682	454-109-3239, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28684	454-109-3241, 0 PPM, Male, Adult	0.5	1	1400
E01-1256-28686	454-109-3243, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28688	454-109-3245, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28690	454-109-3247, 0 PPM, Male, Adult	0.5	1	162
E01-1256-28692	454-109-3249, 0 PPM, Male, Adult	0.5	1	1070
E01-1256-28694	454-109-3251, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28696	454-109-3253, 0 PPM, Male, Adult	0.1	1	<100
E01-1256-28698	454-109-3255, 0 PPM, Male, Adult	0.5	1	<100
E01-1256-28700	454-109-3257, 10 PPM, Male, Adult	0.5	10	78800
E01-1256-28702	454-109-3259, 10 PPM, Male, Adult	0.2	10	83300
E01-1256-28704	454-109-3261, 10 PPM, Male, Adult	0.5	10	68700
E01-1256-28706	454-109-3263, 10 PPM, Male, Adult	0.06	100	210000•
E01-1256-28708	454-109-3265, 10 PPM, Male, Adult	0.2	10	93800
E01-1256-28710	454-109-3267, 10 PPM, Male, Adult	0.5	10	75700
E01-1256-28712	454-109-3269, 10 PPM, Male, Adult	0.5	10	80600
E01-1256-28714	454-109-3271, 10 PPM, Male, Adult	0.5	10	11300•
E01-1256-28716	454-109-3273, 10 PPM, Male, Adult	0.5	10	106000
E01-1256-28718	454-109-3275, 10 PPM, Male, Adult	0.5	10	80500
E01-1256-28720	454-109-3277, 10 PPM, Male, Adult	0.5	10	80400
E01-1256-28722	454-109-3279, 10 PPM, Male, Adult	0.5	10	103000
E01-1256-28724	454-109-3281, 10 PPM, Male, Adult	0.5	10	74300
E01-1256-28726	454-109-3283, 10 PPM, Male, Adult	0.5	10	91800
E01-1256-28728	454-109-3285, 10 PPM, Male, Adult	0.5	10	108000
E01-1256-28730	454-109-3287, 10 PPM, Male, Adult	0.3	10	78700
E01-1256-28732	454-109-3289, 10 PPM, Male, Adult	0.3	10	97900
E01-1256-28734	454-109-3291, 10 PPM, Male, Adult	0.3	10	73500
E01-1256-28736	454-109-3293, 10 PPM, Male, Adult	0.5	10	96800
E01-1256-28738	454-109-3295, 10 PPM, Male, Adult	0.5	10	99700
E01-1256-28741	454-109-9591, 0 PPM, Male, Offspring	0.1	1	<100

3M SAMPLE NUMBER	SAMPLE DESCRIPTION	AMOUNT OF SERA USED (mL)	ADDITIONAL DILUTION	CONC. OF $\text{C}_8\text{F}_{17}\text{SO}_3^-$ IN SAMPLE (ng/mL OF SERA)
E01-1256-28746	454-109-9606, 0 PPM, Male, Offspring	0.2	1	103
E01-1256-28749	454-109-9578, 0 PPM, Male, Offspring	0.2	1	<100
E01-1256-28750	454-109-9625, 10 PPM, Male, Offspring	0.2	1	5450
E01-1256-28756	454-109-9636, 10 PPM, Male, Offspring	0.1	1	3670
E01-1256-28757	454-109-9642, 10 PPM, Male, Offspring	0.2	1	4110

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

Table 12. C₈F₁₇SO₃⁻ Data Summary, Female Mallard Liver (Adult and Offspring)

3M SAMPLE NUMBER	SAMPLE DESCRIPTION	AMOUNT OF LIVER WEIGHED (G)	ADDITIONAL DILUTION	CONC. OF C ₈ F ₁₇ SO ₃ ⁻ IN SAMPLE (NG/G OF LIVER)
E01-1326-29482	0 ppm a.i. 3218 F Liver, adult	1.0095	1	<50
E01-1326-29583	0 ppm a.i. 3220 F Liver, adult	1.0944	1	<50
E01-1326-29601	0 ppm a.i. 3222 F Liver, adult	1.0435	1	<50
E01-1326-29619	0 ppm a.i. 3224 F Liver, adult	1.0425	1	<50
E01-1326-29637	0 ppm a.i. 3226 F Liver, adult	1.0703	1	<50
E01-1326-29655	0 ppm a.i. 3228 F Liver, adult	1.0526	1	<50
E01-1326-29673	0 ppm a.i. 3230 F Liver, adult	1.0411	1	<50
E01-1326-29691	0 ppm a.i. 3232 F Liver, adult	1.1704	1	<50
E01-1326-29709	0 ppm a.i. 3234 F Liver, adult	1.1290	1	<50
E01-1326-29727	0 ppm a.i. 3236 F Liver, adult	1.0800	1	<50
E01-1326-29745	0 ppm a.i. 3238 F Liver, adult	1.1170	1	<50
E01-1326-29763	0 ppm a.i. 3240 F Liver, adult	1.0086	1	<50
E01-1326-29781	0 ppm a.i. 3242 F Liver, adult	1.0907	1	<50
E01-1326-29799	0 ppm a.i. 3244 F Liver, adult	1.0291	1	<50
E01-1326-29817	0 ppm a.i. 3246 F Liver, adult	1.1294	1	<50
E01-1326-29835	0 ppm a.i. 3248 F Liver, adult	1.1075	1	<50
E01-1326-29853	0 ppm a.i. 3250 F Liver, adult	1.1709	1	<50
E01-1326-29871	0 ppm a.i. 3252 F Liver, adult	1.0761	1	<50
E01-1326-29889	0 ppm a.i. 3254 F Liver, adult	1.1344	1	<50
E01-1326-29907	0 ppm a.i. 3256 F Liver, adult	1.0669	1	<50
E01-1326-29925	10 ppm a.i. 3258 F Liver, adult	1.0034	1	7080
E01-1326-29943	10 ppm a.i. 3260 F Liver, adult	1.1331	1	6780
E01-1326-29961	10 ppm a.i. 3262 F Liver, adult	1.1294	10	13600
E01-1326-29980	10 ppm a.i. 3264 F Liver, adult	1.1829	1	6080
E01-1326-29998	10 ppm a.i. 3266 F Liver, adult	1.0487	1	5380
E01-1326-30016	10 ppm a.i. 3268 F Liver, adult	1.1214	1	5730
E01-1326-30034	10 ppm a.i. 3270 F Liver, adult	1.0751	10	13200
E01-1326-30052	10 ppm a.i. 3272 F Liver, adult	1.0658	1	5800
E01-1326-30070	10 ppm a.i. 3274 F Liver, adult	1.1226	10	13800
E01-1326-30088	10 ppm a.i. 3276 F Liver, adult	1.0953	10	30700
E01-1326-30106	10 ppm a.i. 3278 F Liver, adult	1.1956	1	5960
E01-1326-30124	10 ppm a.i. 3280 F Liver, adult	1.0600	1	2480
E01-1326-30142	10 ppm a.i. 3282 F Liver, adult	1.0275	1	4560
E01-1326-30160	10 ppm a.i. 3284 F Liver, adult	1.0915	1	6330
E01-1326-30178	10 ppm a.i. 3286 F Liver, adult	1.0657	10	28200
E01-1326-30196	10 ppm a.i. 3288 F Liver, adult	1.0330	10	24100
E01-1326-30214	10 ppm a.i. 3290 F Liver, adult	1.0352	10	19800
E01-1326-30232	10 ppm a.i. 3292 F Liver, adult	1.0658	1	4600
E01-1326-30250	10 ppm a.i. 3294 F Liver, adult	1.0627	1	4760

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)
E01-1326-30268	10 ppm a.i. 3296 F Liver, adult	1.1238	1	6330
E01-1367-31295	0 ppm a.i. 9583 F Liver, offspring	1.1308	1	<50
E01-1367-31307	0 ppm a.i. 9589 F Liver, offspring	1.0005	1	<50
E01-1367-31313	0 ppm a.i. 9592 F Liver, offspring	1.0390	1	<50
E01-1367-31319	0 ppm a.i. 9598 F Liver, offspring	1.1204	1	<50
E01-1367-31325	0 ppm a.i. 9603 F Liver, offspring	1.1550	1	<50
E01-1367-31337	0 ppm a.i. 9610 F Liver, offspring	1.0217	1	<50
E01-1367-31343	0 ppm a.i. 9573 F Liver, offspring	1.0122	1	<50
E01-1367-31362	10 ppm a.i. 9629 F Liver, offspring	1.1676	1	2950
E01-1367-31368	10 ppm a.i. 9633 F Liver, offspring	1.0510	1	2240
E01-1367-31374	10 ppm a.i. 9634 F Liver, offspring	1.1638	1	4520
E01-1367-31380	10 ppm a.i. 9645 F Liver, offspring	1.1510	1	2580
E01-1367-31386	10 ppm a.i. 9648 F Liver, offspring	1.1125	1	3500
E01-1367-31404	10 ppm a.i. 9654 F Liver, offspring	1.0770	1	5930
E01-1367-31410	10 ppm a.i. 9659 F Liver, offspring	1.0581	1	3580

Table 13. C₈F₁₇SO₃⁻ Data Summary, Male Mallard Liver (Adult and Offspring)

3M SAMPLE NUMBER	SAMPLE DESCRIPTION	AMOUNT OF LIVER WEIGHED (G)	ADDITIONAL DILUTION	CONC. OF C ₈ F ₁₇ SO ₃ ⁻ IN SAMPLE (NG/G OF LIVER)
E01-1326-29473	0 ppm a.i. 3217 M Liver, adult	1.1169	1	<50
E01-1326-29574	0 ppm a.i. 3219 M Liver, adult	1.1847	1	<50
E01-1326-29592	0 ppm a.i. 3221 M Liver, adult	1.1181	1	<50
E01-1326-29610	0 ppm a.i. 3223 M Liver, adult	1.0788	1	<50
E01-1326-29628	0 ppm a.i. 3225 M Liver, adult	1.1043	1	<50
E01-1326-29646	0 ppm a.i. 3227 M Liver, adult	1.0486	1	<50
E01-1326-29664	0 ppm a.i. 3229 M Liver, adult	1.0336	1	<50
E01-1326-29682	0 ppm a.i. 3231 M Liver, adult	1.0165	1	<50
E01-1326-29700	0 ppm a.i. 3233 M Liver, adult	1.0395	1	<50
E01-1326-29718	0 ppm a.i. 3235 M Liver, adult	1.0373	1	<50
E01-1326-29736	0 ppm a.i. 3237 M Liver, adult	1.1679	1	<50
E01-1326-29754	0 ppm a.i. 3239 M Liver, adult	1.1493	1	<50
E01-1326-29772	0 ppm a.i. 3241 M Liver, adult	1.0619	1	<50
E01-1326-29790	0 ppm a.i. 3243 M Liver, adult	1.0263	1	<50
E01-1326-29808	0 ppm a.i. 3245 M Liver, adult	1.1541	1	<50
E01-1326-29826	0 ppm a.i. 3247 M Liver, adult	1.0813	1	<50
E01-1326-29844	0 ppm a.i. 3249 M Liver, adult	1.0263	1	<50
E01-1326-29862	0 ppm a.i. 3251 M Liver, adult	1.0088	1	<50
E01-1326-29880	0 ppm a.i. 3253 M Liver, adult	1.0294	1	<50
E01-1326-29898	0 ppm a.i. 3255 M Liver, adult	1.0926	1	<50
E01-1326-29916	10 ppm a.i. 3257 M Liver, adult	1.0654	10	72100
E01-1326-29934	10 ppm a.i. 3259 M Liver, adult	1.1316	10	49200
E01-1326-29952	10 ppm a.i. 3261 M Liver, adult	1.1330	10	70100
E01-1326-29970	10 ppm a.i. 3263 M Liver, adult	1.1404	100	125000
E01-1326-29989	10 ppm a.i. 3265 M Liver, adult	1.1480	10	61700
E01-1326-30007	10 ppm a.i. 3267 M Liver, adult	1.0620	10	49900
E01-1326-30025	10 ppm a.i. 3269 M Liver, adult	1.1687	10	57900
E01-1326-30043	10 ppm a.i. 3271 M Liver, adult	1.0313	10	63000
E01-1326-30061	10 ppm a.i. 3273 M Liver, adult	1.1399	10	85000
E01-1326-30079	10 ppm a.i. 3275 M Liver, adult	1.1143	10	54900
E01-1326-30097	10 ppm a.i. 3277 M Liver, adult	1.0062	10	53900
E01-1326-30115	10 ppm a.i. 3279 M Liver, adult	1.0380	10	69000
E01-1326-30133	10 ppm a.i. 3281 M Liver, adult	1.0669	10	49900
E01-1326-30151	10 ppm a.i. 3283 M Liver, adult	1.0955	10	67100
E01-1326-30169	10 ppm a.i. 3285 M Liver, adult	1.1981	10	55500
E01-1326-30187	10 ppm a.i. 3287 M Liver, adult	1.0500	10	52600
E01-1326-30205	10 ppm a.i. 3289 M Liver, adult	1.0251	10	59300
E01-1326-30223	10 ppm a.i. 3291 M Liver, adult	1.0441	10	46500
E01-1326-30241	10 ppm a.i. 3293 M Liver, adult	1.0979	10	24200
E01-1326-30259	10 ppm a.i. 3295 M Liver, adult	1.0815	10	50400

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)
E01-1367-31301	0 ppm a.i. 9591 M Liver, offspring	1.0411	1	<50
E01-1367-31331	0 ppm a.i. 9606 M Liver, offspring	1.1314	1	<50
E01-1367-31349	0 ppm a.i. 9578 M Liver, offspring	1.0413	1	<50
E01-1367-31356	10 ppm a.i. 9625 M Liver, offspring	1.0166	1	4640
E01-1367-31392	10 ppm a.i. 9636 M Liver, offspring	1.1367	1	2200
E01-1367-31398	10 ppm a.i. 9642 M Liver, offspring	1.1252	1	2680

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 14 of 18 mallards (<100ng/mL) and quantifiable concentrations of $C_8F_{17}SO_3^-$ in 4 of 18 (range 107-1400ng/mL, Table 14). The sera results from the female control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 15 of 17 birds (<100ng/mL) and quantifiable concentrations of $C_8F_{17}SO_3^-$ in 2 of 17 (range 166-172ng/mL, Table 15). Analytical results for eighteen sera samples from the 10ppm dosed male group showed an average of 87300ng/mL $C_8F_{17}SO_3^-$ (range 68700-108000ng/mL, Table 15). Analytical results for nineteen sera samples from the 10ppm dosed female group showed an average of 16600ng/mL $C_8F_{17}SO_3^-$ (range 6350-42800ng/mL, Table 15).

Sera results from the male offspring control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 2 of 3 birds (<100ng/mL) and a quantifiable concentration in 1 of 3 (103 ng/mL, Table 14). The sera results from the female offspring control group portion of this study showed no detectable levels of $C_8F_{17}SO_3^-$ in 7 of 7 birds (<100ng/mL, Table 14). Analytical results for three sera samples from the 10ppm dosed male offspring group showed an average of 4410ng/mL (range 3670-5450ng/mL, Table 14). Analytical results for seven sera samples from the 10ppm dosed female offspring group showed an average of 4960ng/mL (range 3420-6760ng/mL, Table 14).

Liver results from the male control group showed no detectable levels of $C_8F_{17}SO_3^-$ in 20 of 20 birds (<50ng/g, Table 14). The liver results from the female control group also showed no detectable levels of $C_8F_{17}SO_3^-$ in 20 of 20 birds (<50ng/g, Table 14). Analytical results for twenty liver samples from the 10ppm dosed male group showed an average of 60900ng/g $C_8F_{17}SO_3^-$ (range 24200-125000ng/g, Table 14). Analytical results for twenty liver samples from the 10ppm dosed female group showed an average of 10800ng/g $C_8F_{17}SO_3^-$ (range 2480-30700ng/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 3 of 3 birds (<50ng/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 7 of 7 birds (<50ng/g, Table 14). Analytical results for three liver samples from the 10ppm dosed male offspring group showed an average of 3170ng/g (range 2200-4640ng/g, Table 14). Analytical results for seven liver samples from the 10ppm dosed female offspring group showed an average of 3620ng/g (range 2240-5930ng/g, Table 14).

Table 14. Summary Table, C₈F₁₇SO₃⁻ Concentration in Sera and Liver

SAMPLING EVENT	NUMBER OF SERA SAMPLES	AVERAGE C ₈ F ₁₇ SO ₃ ⁻ CONC. SERA	Range of Results	NUMBER OF LIVER SAMPLES	AVERAGE C ₈ F ₁₇ SO ₃ ⁻ CONC. LIVER	Range of Results
Male Control Group	18	NA	<100 ng/mL (14 of 18) 107-1400 ng/mL range (4 of 18)	20	<50 ng/g	NA
Female Control Group	20	NA	<100 ng/mL (15 of 20) 166-2680 ng/mL range (5 of 20)	20	<50 ng/g	NA
Male 10ppm Group	20	89700 ng/mL	11300-210000 ng/mL	20	60900 ng/g	24200-125000 ng/g
Female 10ppm Group	20	20300 ng/mL	6350-91400 ng/mL	20	10800 ng/g	2480-30700 ng/g
Male Offspring Control Group	3	NA	<100 ng/mL (2 of 3) 103 ng/mL (1 of 3)	3	<50 ng/g	NA
Female Offspring Control Group	7	NA	<100 ng/mL (7 of 7)	7	<50 ng/g	NA
Male Offspring 10ppm Group	3	4410 ng/mL	3670 - 5450 ng/mL	3	3170 ng/g	2200-4640 ng/g
Female Offspring 10ppm Group	7	4960 ng/mL	3420 - 6760 ng/mL	7	3620 ng/g	2240-5930 ng/g

For individual samples values see Tables 10 to 13 and Attachment B.

Table 15. Summary Table, C₈F₁₇SO₃⁻ Concentration in Sera and Liver with Outliers Removed

SAMPLING EVENT	NUMBER OF SERA SAMPLES	AVERAGE C ₈ F ₁₇ SO ₃ ⁻ CONC. SERA	Range of Results	NUMBER OF LIVER SAMPLES	AVERAGE C ₈ F ₁₇ SO ₃ ⁻ CONC. LIVER	Range of Results
Male Control Group	18*	•	•	20*	*	*
Female Control Group	17	NA	<100 ng/mL (15 of 17) 166-172 ng/mL range (2 of 17)	20*	*	*
Male 10ppm Group	18	87300 ng/mL	68700-108000 ng/mL	20*	*	*
Female 10ppm Group	19	16600 ng/mL	6350-42800 ng/mL	20*	•	•
Male Offspring Control Group	3*	*	*	3*	•	•
Female Offspring Control Group	7*	*	*	7*	*	*
Male Offspring 10ppm Group	3*	*	*	3*	*	*
Female Offspring 10ppm Group	7*	*	*	7*	*	*

* No outliers removed from this data set. Refer to Table 14 for results. For individual samples values see Tables 10 to 13 and Attachment B.

STATISTICAL METHODS

Statistical methods included calculating means, standard deviations, % coefficient of variation, linear regressions, percent recoveries, 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 that 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 only measurement is the quality control and matrix spike results. Accuracy (the observed amount in the QC and MS samples, divided by the amount spiked, expressed as a percent) was found to range from 79.7% to 172%; while precision, expressed as the standard deviation of the recoveries, was found to range from 0.717% to 15.7%.

REFERENCES

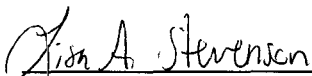
1. Wildlife International, Ltd. Protocol No. 454-109 "PFOS: A Reproduction Study with the Mallard".
2. Wildlife International, Ltd. Protocol No. 454/120700/MR/SUB454 (3M Environmental Laboratory Project Number U2723) "PFOS: A Reproduction Study with the Mallard".
3. Centre Analytical Laboratories, Inc. Study 023-044, "Validation of Analytical Methods "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC Electrospray/Mass Spectrometry" and "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC Electrospray/Mass Spectrometry in Quail Serum and Liver."
4. 3M Environmental Laboratory Standard Operating Procedure ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds form Biological Matrices".

LIST OF ATTACHMENTS

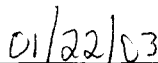
- Attachment A: Extraction and Analytical Method
- Attachment B: Data Summary Tables
- Attachment C: Sample Chromatograms, Calibration Information, and Instrument Parameters
- Attachment D: Sample Preparation Sheets
- Attachment E: Study Protocol, Protocol Amendments, and Deviations
- Attachment F: Example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

SIGNATURE PAGE

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



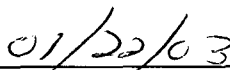
Lisa A. Stevenson
Principal Analytical Investigator



Date



William K. Reagen
Laboratory Management



Date

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

3M Environmental Laboratory

Method

Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices

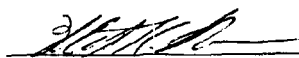
Method Number: ETS-8-231.1

Adoption Date: 11/13/01

Revision Date: 2/18/02

Effective Date: 2/18/02

Approved By:



William K. Reagen
Laboratory Manager

02/18/02

Date

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

Page 1 of 19

1 Scope and Application

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

2 Method Summary

An amount of biological material, determined by the analyst, is prepared (fluids diluted and tissues homogenized) at a 1/6 dilution, or other dilution as determined by the analyst using reagent grade water. An aliquot of the dilution/homogenate is spiked with the appropriate surrogate or analyte mixture. Acetonitrile (ACN) is added as an extraction solvent and also serves to precipitate the proteins. The sample is capped, mixed, and put on the centrifuge to clarify the supernatant. The supernatant is transferred to a clean tube, diluted with water, and passed through a pre-conditioned C₁₈ SPE cartridge. Finally, the analytes of interest are eluted from the SPE cartridge and analyzed by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ES/MS/MS).

3 Definitions

3.1 Dilution

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

3.2 SPE cartridge

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

3.3 Reagent grade water

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

3.4 Quality control sample

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

4 Warnings and Cautions

4.1 Health and Safety Warnings

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

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

4.2 Cautions

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

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

Do not run solvent pumps to dryness.

5 Interferences

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

6 Instrumentation, Supplies, and Materials

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

6.1 Instrumentation

Vortex mixer, VWR, Vortex Genie 2

Ultra-Turrax T25 tissue homogenizer

Vacuum Pump

SPE Extraction Manifold

Centrifuge, Mistral 1000 or IEC

Shaker, Eberbach or VWR

Balance (+/- 0.1000 g)

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

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

6.2 Supplies and Materials

Eppendorf or disposable pipettes, plastic or glass

Dissecting scalpels

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

Volumetric flasks, glass, type A

40 mL glass vials (ICHEM)

Plastic 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 C₁₈ (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

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

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

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

Allow samples to thaw to room temperature prior to extraction.

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

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

9 Quality Control

9.1 Blanks

9.1.1 Solvent Blank

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

9.1.2 Method Blank

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

9.1.3 Matrix Blank

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

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

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

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

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

9.2 Sample Replicate

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

9.3 Surrogate standard

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

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

9.4 Internal standard

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

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

9.5 Lab Control Sample

Lab control samples are not a component of this method.

9.6 Quality Control (QC) Sample

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

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

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

Low level: 3X to 5X the LLOQ,

Mid-level: equivalent to a point near the middle of the calibration curve,

High level: 80% of the ULOQ

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

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

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

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

9.7 Sample Dilution

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

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

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

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

10 Calibration and Standardization

10.1 Instrument Calibration

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

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

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

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

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

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

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

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

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

TABLE 1 APPROXIMATE SPIKING AMOUNTS FOR STANDARDS AND SPIKES USING 1.0 mL OF MATRIX			
Working standard (approximate concentration)	μL	Approximate final concentration of analyte in Matrix diluted 1:5	Approximate final concentration of analyte in Final 2.0 mL volume
-	-	Blank	Blank
0.500 $\mu\text{g/mL}$	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 (± 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 ± 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 μ L injection

Inject/sample = 1

Cycle time = 10.0 minutes

Flow rate = 300 μ 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 μ 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:

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

Calculate relative standard deviation using the following equation:

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

Calculate percent deviation using the following equation:

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

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

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

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

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

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 < 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

Sample waste is disposed of in noninfectious biohazard waste containers.

Flammable solvent waste is disposed of in high BTU containers.

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

15 Records

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

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

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

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

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

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

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

16 Attachments

Attachment A: Extraction Worksheet

Attachment B: Sample Weight/Volume Worksheet

Attachment C: Calibration Standard Concentration Worksheet

Attachment D: Dilutions Summary Worksheet

17 References

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

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

18 Affected Documents

None

19 Revisions

<u>Revision Number</u>	<u>Revision Description</u>	<u>Revision 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 _____ μ L 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.

ETS-8-231.1

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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: _____

Attachment C: Calibration Standard Concentration Worksheet

Prep date(s):

Standard number:

Analyte(s):

Equipment number:

Sample matrix:

Final solvent and TN:

Method/revision:

Blank Tissue or Fluid/identifier:

Analyte mix std approx. 0.500 ug/mL:

Analyte mix std approx. 5.00 ug/mL:

Analyte mix std approx. 50.0 ug/mL:

Surrogate std approx. 100 ug/mL:

Actual concentrations of standards in the analyte mix

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

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

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

ETS-8-231.1

Solid Phase Extraction and Analysis of Fluorochemical
Compounds from Biological Matrices

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Attachment D: Dilutions Summary Worksheet

Study:
Dilution Date/Analyst:
Box Number:

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

Sample Number 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: _____

ATTACHMENT B: DATA SUMMARY TABLES

Study number E01-1256 - MALLARD SERA DATA SUMMARY, BLANKS AND SPIKES

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL)	Conc. of PFOS in Sample (ng/mL)	log for control samples
E01-1256-36231	02/25/2002	Blank H2O-day 1	d020225037	0.05	40	1	1.00	40.0	* 40.0
E01-1256-36259	02/26/2002	Blank H2O-day 2	d020226025	0.05	40	1	2.51	100.4	* 100.4
E01-1256-36263	02/27/2002	Blank H2O-day 3	d020228024	0.05	40	1	2.51	100.4	* 100.4
E01-1256-36263	02/27/2002	Blank H2O-day 3	d020301099	0.05	40	1	1.00	40.0	* 40.0
E01-1256-36267	02/28/2002	Blank H2O-day 4	d020228107	0.05	40	1	1.00	40.0	* 40.0
E01-1256-36231	02/25/2002	Blank H2O-day 1	d020308036	0.05	40	1	2.51	100.4	* 100.4
E01-1256-36232	02/25/2002	Blank mallard sera-day 1	d020225038	0.05	40	1	1.00	40.0	* 40.0
E01-1256-36260	02/26/2002	Blank mallard sera-day 2	d020226026	0.05	40	1	2.51	100.4	* 100.4
E01-1256-36264	02/27/2002	Blank mallard sera-day 3	d020228025	0.05	40	1	2.51	100.4	* 100.4
E01-1256-36264	02/27/2002	Blank mallard sera-day 3	d020301100	0.05	40	1	1.00	40.0	* 40.0
E01-1256-36268	02/28/2002	Blank mallard sera-day 4	d020228108	0.05	40	1	1.00	40.0	* 40.0
E01-1256-36232	02/25/2002	Blank mallard sera-day 1	d020308037	0.05	40	1	2.51	100.4	* 100.4

3M Sample Number	Sampled Date	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL)	Conc. of PFOS in Sample (ng/mL)	% Rec
E01-1256-36233	02/25/2002	Mallard Sera MS-day 1	d020225039	0.05	40	1	27.79	1112	110.7
E01-1256-36261	02/26/2002	Mallard Sera MS-day 2	d020226027	0.05	40	1	29.50	1180	117.5
E01-1256-36265	02/27/2002	Mallard Sera MS-day 3	d020228026	0.05	40	1	28.85	1154	114.9
E01-1256-36265	02/27/2002	Mallard Sera MS-day 3	d020301101	0.05	40	1	27.76	1110	110.6
E01-1256-36269	02/28/2002	Mallard Sera MS-day 4	d020228109	0.05	40	1	28.01	1120	111.6
E01-1256-36233	02/25/2002	Mallard Sera MS-day 1	d020308038	0.05	40	1	29.03	1161	115.7
E01-1256-36234	02/25/2002	Mallard Sera MSD-day 1	d020225040	0.05	40	1	30.44	1218	121.3
E01-1256-36262	02/26/2002	Mallard Sera MSD-day 2	d020226028	0.05	40	1	29.80	1192	118.7
E01-1256-36266	02/27/2002	Mallard Sera MSD-day 3	d020228027	0.05	40	1	24.76	990	98.65
E01-1256-36266	02/27/2002	Mallard Sera MSD-day 3	d020301102	0.05	40	1	24.01	960	95.66
E01-1256-36270	02/28/2002	Mallard Sera MSD-day 4	d020228110	0.05	40	1	28.23	1129	112.5
E01-1256-36234	02/25/2002	Mallard Sera MSD-day 1	d020308039	0.05	40	1	31.34	1254	124.9

* Concentration <LOQ

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 (mL) = amount of sera used/total amount of sera + water (1/20 dilution). = 1/20 = 0.05 mL

for both ms and msd

avg %rec	112.7
std dev of %rec	8.487
avg conc	1132
std dev of conc	85.21

Study number E01-1256 - MALLARD SERA

Absolute Recoveries

versus unextracted curve

3M Sample Number	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	Conc. of PFOS in Sample (ng/mL of Sera)	% Rec	
e01-1256-36250	QC- 25ppb -1 (1004 ng/mL diluted sera)	d020405036	0.05	40	1	48.29	1932	192.4	avg 177.3
e01-1256-36251	QC- 25ppb -2 (1004 ng/mL diluted sera)	d020405037	0.05	40	1	39.92	1597	159.0	std dev 16.90
e01-1256-36252	QC- 25ppb -3 (1004 ng/mL diluted sera)	d020405038	0.05	40	1	45.30	1812	180.5	%RSD 9.531
e01-1256-36253	QC- 250ppb -1 (10040 ng/mL diluted sera)	d020405039	0.05	40	1	288.00	11520	114.7	avg 116.2
e01-1256-36254	QC- 250ppb -2 (10040 ng/mL diluted sera)	d020405040	0.05	40	1	294.03	11761	117.1	std dev 1.293
e01-1256-36255	QC- 250ppb -3 (10040 ng/mL diluted sera)	d020405041	0.05	40	1	293.10	11724	116.8	%RSD 1.113
e01-1256-36256	QC- 4.0 ppm -1 (160640 ng/mL diluted sera)	d020405042	0.05	40	10	523.44	209376	130.3	avg 113.8
e01-1256-36257	QC- 4.0 ppm -2 (160640 ng/mL diluted sera)	d020405043	0.05	40	10	432.18	172872	107.6	std dev 14.51
e01-1256-36258	QC- 4.0 ppm -3 (160640 ng/mL diluted sera)	d020405044	0.05	40	10	415.07	166028	103.4	%RSD 12.75

A = Not within +/-25% criteria

for all QC samples (absolute recovery)

avg %rec	135.8
std dev of %rec	33.11

Study number E01-1256 - MALLARD SERA

versus extracted curve

original

QC Sample Recoveries

3M Sample Number	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	Conc. of PFOS in Sample (ng/mL of Sera)	% Rec	
e01-1256-36250	QC- 25ppb -1 (1004 ng/mL diluted sera)	d020225071	0.05	40	1	39.15	1566	156.0	avg 145.3
e01-1256-36251	QC- 25ppb -2 (1004 ng/mL diluted sera)	d020225072	0.05	40	1	32.41	1296	129.1	std dev 14.24
e01-1256-36252	QC- 25ppb -3 (1004 ng/mL diluted sera)	d020225073	0.05	40	1	37.84	1514	150.8	%RSD 9.800
e01-1256-36253	QC- 250ppb -1 (10040 ng/mL diluted sera)	d020225074	0.05	40	1	246.03	9841	98.02	avg 97.93
e01-1256-36254	QC- 250ppb -2 (10040 ng/mL diluted sera)	d020225075	0.05	40	1	252.86	10114	100.7	std dev 2.860
e01-1256-36255	QC- 250ppb -3 (10040 ng/mL diluted sera)	d020225076	0.05	40	1	238.51	9540	95.02	%RSD 2.920
e01-1256-36256	QC- 4.0 ppm -1 (160640 ng/mL diluted sera)	d020308045	0.05	40	10	320.09	128036	79.70	avg 84.67
e01-1256-36257	QC- 4.0 ppm -2 (160640 ng/mL diluted sera)	d020308046	0.05	40	10	364.90	145960	90.86	std dev 5.680
e01-1256-36258	QC- 4.0 ppm -3 (160640 ng/mL diluted sera)	d020308047	0.05	40	10	335.08	134032	83.44	%RSD 6.708

A = Not within +/-25% criteria

for all QC samples original analysis (recovery)

avg %rec	109.3
std dev of %rec	28.68

versus extracted curve

rerun

3M Sample Number	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	Conc. of PFOS in Sample (ng/mL of Sera)	% Rec	
e01-1256-36250	QC- 25ppb -1 (1004 ng/mL diluted sera)	d020405036	0.05	40	1	43.07	1723	171.6	avg 157.6
e01-1256-36251	QC- 25ppb -2 (1004 ng/mL diluted sera)	d020405037	0.05	40	1	35.30	1412	140.6	std dev 15.69
e01-1256-36252	QC- 25ppb -3 (1004 ng/mL diluted sera)	d020405038	0.05	40	1	40.31	1612	160.6	%RSD 9.957
e01-1256-36253	QC- 250ppb -1 (10040 ng/mL diluted sera)	d020405039	0.05	40	1	264.06	10562	105.2	avg 106.6
e01-1256-36254	QC- 250ppb -2 (10040 ng/mL diluted sera)	d020405040	0.05	40	1	269.60	10784	107.4	std dev 1.189
e01-1256-36255	QC- 250ppb -3 (10040 ng/mL diluted sera)	d020405041	0.05	40	1	268.75	10750	107.1	%RSD 1.115
e01-1256-36256	QC- 4.0 ppm -1 (160640 ng/mL diluted sera)	d020405042	0.05	40	10	479.65	191860	119.4	avg 104.3
e01-1256-36257	QC- 4.0 ppm -2 (160640 ng/mL diluted sera)	d020405043	0.05	40	10	395.97	158388	98.60	std dev 13.29
e01-1256-36258	QC- 4.0 ppm -3 (160640 ng/mL diluted sera)	d020405044	0.05	40	10	380.39	152156	94.72	%RSD 12.75

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 (mL) = amount of sera used/total amount of sera + water (1/20 dilution). = 1/20 = 0.05 mL

for all QC samples rerun analysis (recovery)

avg %rec	122.8
std dev of %rec	28.08

Blanks QCs and MSs

Report E01-1256
Study number E01-1256 - MALLARD SERA

PFOS: A Reproduction Study with the Mallard

3M Sample Number	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	Conc. of PFOS in Sample (ng/mL of Sera)	log for control samples	
E01-1256-28663	454-109-3218, 0 PPM, Female, Adult	d020225047	0.5	40	1	1.90	76.0	40.0	
E01-1256-28665	454-109-3220, 0 PPM, Female, Adult	d020225049	0.5	40	1	1.00	40.0	* 40.0	
E01-1256-28667	454-109-3222, 0 PPM, Female, Adult	d020225051	0.5	40	1	2.21	88.4	40.0	
E01-1256-28669	454-109-3224, 0 PPM, Female, Adult	d020225053	0.5	40	1	1.00	40.0	* 40.0	
E01-1256-28670	454-109-3226, 0 PPM, Female, Adult	d020225054	0.5	40	1	1.53	61.2	40.0	
E01-1256-28672	454-109-3228, 0 PPM, Female, Adult	d020225061	0.5	40	1	66.94	2678	40.0	rejected
E01-1256-28674	454-109-3230, 0 PPM, Female, Adult	d020225063	0.5	40	1	1.00	40.0	* 40.0	
E01-1256-28676	454-109-3232, 0 PPM, Female, Adult	d020225065	0.5	40	1	4.30	172	40.0	
E01-1256-28678	454-109-3234, 0 PPM, Female, Adult	d020226050	0.5	40	1	2.51	100.4	* 100.4	
E01-1256-28679	454-109-3236, 0 PPM, Female, Adult	d020226051	0.5	40	1	2.51	100.4	* 100.4	
E01-1256-28681	454-109-3238, 0 PPM, Female, Adult	d020226052	0.5	40	1	4.16	166	100.4	
E01-1256-28683	454-109-3240, 0 PPM, Female, Adult	d020226053	0.5	40	1	2.51	100.4	* 100.4	
E01-1256-28685	454-109-3242, 0 PPM, Female, Adult	d020226054	0.5	40	1	2.51	100.4	* 100.4	With outliers
E01-1256-28687	454-109-3244, 0 PPM, Female, Adult	d020226055	0.5	40	1	2.51	100.4	* 100.4	avg 271
E01-1256-28689	454-109-3246, 0 PPM, Female, Adult	d020226056	0.5	40	1	2.51	100.4	* 100.4	std dev 589
E01-1256-28691	454-109-3248, 0 PPM, Female, Adult	d020226057	0.5	40	1	9.96	398	100.4	%RSD 218
E01-1256-28693	454-109-3250, 0 PPM, Female, Adult	d020226058	0.5	40	1	2.51	100.4	* 100.4	rejected
E01-1256-28695	454-109-3252, 0 PPM, Female, Adult	d020226064	0.5	40	1	18.92	757	100.4	Without outliers
E01-1256-28697	454-109-3254, 0 PPM, Female, Adult	d020226065	0.5	40	1	2.51	100.4	* 100.4	avg 93.4
E01-1256-28699	454-109-3256, 0 PPM, Female, Adult	d020226066	0.5	40	1	2.51	100.4	* 100.4	std dev 37.0
E01-1256-28701	454-109-3258, 10 PPM, Female, Adult	d020226067	0.5	40	1	340.30	13612		%RSD 39.6
E01-1256-28703	454-109-3260, 10 PPM, Female, Adult	d020226068	0.5	40	1	235.54	9422		
E01-1256-28705	454-109-3262, 10 PPM, Female, Adult	d020226069	0.5	40	1	425.04	17002		
E01-1256-28707	454-109-3264, 10 PPM, Female, Adult	d020226070	0.5	40	1	158.72	6349		
E01-1256-28709	454-109-3266, 10 PPM, Female, Adult	d020226071	0.5	40	1	197.38	7895		
E01-1256-28711	454-109-3268, 10 PPM, Female, Adult	d020228031	0.5	40	1	168.16	6726		
E01-1256-28713	454-109-3270, 10 PPM, Female, Adult	d020228032	0.5	40	10	228.48	91392		rejected
E01-1256-28715	454-109-3272, 10 PPM, Female, Adult	d020228033	0.5	40	1	192.81	7712		
E01-1256-28717	454-109-3274, 10 PPM, Female, Adult	d020405045	0.5	40	10	73.64	29456		
E01-1256-28719	454-109-3276, 10 PPM, Female, Adult	d020228035	0.5	40	10	101.64	40656		
E01-1256-28721	454-109-3278, 10 PPM, Female, Adult	d020228036	0.5	40	1	245.98	9839		
E01-1256-28723	454-109-3280, 10 PPM, Female, Adult	d020308048	0.2	40	10	80.62	32248		With outliers
E01-1256-28725	454-109-3282, 10 PPM, Female, Adult	d020228038	0.5	40	1	268.84	10754		avg 20312
E01-1256-28727	454-109-3284, 10 PPM, Female, Adult	d020228039	0.5	40	1	164.14	6566		std dev 20385
E01-1256-28729	454-109-3286, 10 PPM, Female, Adult	d020308049	0.5	40	10	72.23	28892		%RSD 100
E01-1256-28731	454-109-3288, 10 PPM, Female, Adult	d020228044	0.5	40	10	106.99	42796		
E01-1256-28733	454-109-3290, 10 PPM, Female, Adult	d020228045	0.5	40	1	400.80	16032		Without outliers
E01-1256-28735	454-109-3292, 10 PPM, Female, Adult	d020228046	0.5	40	1	260.05	10402		avg 16571
E01-1256-28737	454-109-3294, 10 PPM, Female, Adult	d020228047	0.5	40	1	191.39	7656		std dev 11965
E01-1256-28739	454-109-3296, 10 PPM, Female, Adult	d020228048	0.5	40	1	270.66	10826		%RSD 72.2
E01-1256-28740	454-109-9583, 0 PPM, Female, Offspring	d020228114	0.15	40	1	2.51	100.4	* 100.4	
E01-1256-28742	454-109-9589, 0 PPM, Female, Offspring	d020228115	0.2	40	1	2.51	100.4	* 100.4	
E01-1256-28743	454-109-9592, 0 PPM, Female, Offspring	d020228116	0.2	40	1	2.51	100.4	* 100.4	
E01-1256-28744	454-109-9598, 0 PPM, Female, Offspring	d020228117	0.1	40	1	2.51	100.4	* 100.4	
E01-1256-28745	454-109-9603, 0 PPM, Female, Offspring	d020228118	0.2	40	1	2.51	100.4	* 100.4	avg 100.4
E01-1256-28747	454-109-9610, 0 PPM, Female, Offspring	d020228119	0.2	40	1	2.51	100.4	* 100.4	std dev 0
E01-1256-28748	454-109-9573, 0 PPM, Female, Offspring	d020228120	0.05	40	1	2.51	100.4	* 100.4	%RSD -
E01-1256-28751	454-109-9629, 10 PPM, Female, Offspring	d020228121	0.1	40	1	128.28	5131		
E01-1256-28752	454-109-9633, 10 PPM, Female, Offspring	d020228122	0.1	40	1	85.41	3416		
E01-1256-28753	454-109-9634, 10 PPM, Female, Offspring	d020228123	0.5	40	1	168.96	6758		
E01-1256-28754	454-109-9645, 10 PPM, Female, Offspring	d020228127	0.1	40	1	93.74	3750		
E01-1256-28755	454-109-9648, 10 PPM, Female, Offspring	d020228128	0.2	40	1	122.59	4904		avg 4965
E01-1256-28758	454-109-9654, 10 PPM, Female, Offspring	d020228129	0.1	40	1	136.20	5448		std dev 1118
E01-1256-28759	454-109-9659, 10 PPM, Female, Offspring	d020228130	0.1	40	1	133.73	5349		%RSD 22.5

* Concentration <LOQ

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 (mL) = amount of sera used/total amount of sera + water (1/20 dilution). = 1/20 = 0.05 mL

3M Sample Number	Sample Description	Inst. File Name	amount of sera used (mL)	dilution (diluted sera)	additional dilution	Inst Conc. (ng/mL of Methanol)	Conc. of PFOS in Sample (ng/mL of Sera)	log for control samples	
E01-1256-28662	454-109-3217, 0 PPM, Male, Adult	d020225046	0.5	40	1	2.68	107	40.0	
E01-1256-28664	454-109-3219, 0 PPM, Male, Adult	d020225048	0.5	40	1	1.49	59.6	40.0	
E01-1256-28666	454-109-3221, 0 PPM, Male, Adult	d020225050	0.5	40	1	1.00	40.0	40.0	*
E01-1256-28668	454-109-3223, 0 PPM, Male, Adult	d020225052	0.5	40	1	1.07	42.8	40.0	
E01-1256-28671	454-109-3227, 0 PPM, Male, Adult	d020225060	0.5	40	1	1.99	79.6	40.0	
E01-1256-28673	454-109-3229, 0 PPM, Male, Adult	d020225062	0.5	40	1	1.00	40.0	40.0	*
E01-1256-28675	454-109-3231, 0 PPM, Male, Adult	d020225064	0.5	40	1	1.20	48.0	40.0	
E01-1256-28677	454-109-3233, 0 PPM, Male, Adult	d020226034	0.5	40	1	2.51	100.4	100.4	*
E01-1256-28680	454-109-3237, 0 PPM, Male, Adult	d020226035	0.3	40	1	2.51	100.4	100.4	*
E01-1256-28682	454-109-3239, 0 PPM, Male, Adult	d020226036	0.5	40	1	2.51	100.4	100.4	*
E01-1256-28684	454-109-3241, 0 PPM, Male, Adult	d020226037	0.5	40	1	34.88	1395	100.4	
E01-1256-28686	454-109-3243, 0 PPM, Male, Adult	d020226038	0.5	40	1	2.51	100.4	100.4	*
E01-1256-28688	454-109-3245, 0 PPM, Male, Adult	d020226039	0.5	40	1	2.51	100.4	100.4	*
E01-1256-28690	454-109-3247, 0 PPM, Male, Adult	d020226040	0.5	40	1	4.05	162	100.4	
E01-1256-28692	454-109-3249, 0 PPM, Male, Adult	d020226041	0.5	40	1	26.82	1073	100.4	
E01-1256-28694	454-109-3251, 0 PPM, Male, Adult	d020226042	0.5	40	1	2.51	100.4	100.4	*
E01-1256-28696	454-109-3253, 0 PPM, Male, Adult	d020226043	0.1	40	1	2.51	100.4	100.4	*
E01-1256-28698	454-109-3255, 0 PPM, Male, Adult	d020226049	0.5	40	1	2.51	100.4	100.4	*
									avg 214
									std dev 376
									%RSD 176
E01-1256-28700	454-109-3257, 10 PPM, Male, Adult	d020301106	0.5	40	10	196.96	78784		
E01-1256-28702	454-109-3259, 10 PPM, Male, Adult	d020301107	0.2	40	10	208.27	83308		
E01-1256-28704	454-109-3261, 10 PPM, Male, Adult	d020301108	0.5	40	10	171.66	68664		
E01-1256-28706	454-109-3263, 10 PPM, Male, Adult	d020411024	0.06	40	100	52.51	210040		rejected
E01-1256-28708	454-109-3265, 10 PPM, Male, Adult	d020301110	0.2	40	10	234.38	93752		
E01-1256-28710	454-109-3267, 10 PPM, Male, Adult	d020228049	0.5	40	10	189.30	75720		
E01-1256-28712	454-109-3269, 10 PPM, Male, Adult	d020228050	0.5	40	10	201.59	80636		
E01-1256-28714	454-109-3271, 10 PPM, Male, Adult	d020228051	0.5	40	10	28.18	11272		rejected
E01-1256-28716	454-109-3273, 10 PPM, Male, Adult	d020228052	0.5	40	10	265.08	106032		
E01-1256-28718	454-109-3275, 10 PPM, Male, Adult	d020228053	0.5	40	10	201.27	80508		
E01-1256-28720	454-109-3277, 10 PPM, Male, Adult	d020228057	0.5	40	10	201.00	80400		
E01-1256-28722	454-109-3279, 10 PPM, Male, Adult	d020228058	0.5	40	10	258.46	103384		With outliers
E01-1256-28724	454-109-3281, 10 PPM, Male, Adult	d020228059	0.5	40	10	185.77	74308		avg 89652
E01-1256-28726	454-109-3283, 10 PPM, Male, Adult	d020228060	0.5	40	10	229.40	91760		std dev 35035
E01-1256-28728	454-109-3285, 10 PPM, Male, Adult	d020228061	0.5	40	10	269.40	107760		%RSD 39.1
E01-1256-28730	454-109-3287, 10 PPM, Male, Adult	d020228062	0.3	40	10	196.75	78700		
E01-1256-28732	454-109-3289, 10 PPM, Male, Adult	d020228063	0.3	40	10	244.83	97932		Without outliers
E01-1256-28734	454-109-3291, 10 PPM, Male, Adult	d020228064	0.3	40	10	183.85	73540		avg 87318
E01-1256-28736	454-109-3293, 10 PPM, Male, Adult	d020228065	0.5	40	10	242.13	96852		std dev 12337
E01-1256-28738	454-109-3295, 10 PPM, Male, Adult	d020228066	0.5	40	10	249.20	99680		%RSD 14.1
E01-1256-28741	454-109-9591, 0 PPM, Male, Offspring	d020228131	0.1	40	1	2.51	100.4	100.4	*
E01-1256-28746	454-109-9606, 0 PPM, Male, Offspring	d020228132	0.2	40	1	2.57	103	100.4	std dev 1.39
E01-1256-28749	454-109-9578, 0 PPM, Male, Offspring	d020228133	0.2	40	1	2.51	100.4	100.4	%RSD 1.37
E01-1256-28750	454-109-9625, 10 PPM, Male, Offspring	d020228134	0.2	40	1	136.31	5452		avg 4408
E01-1256-28756	454-109-9636, 10 PPM, Male, Offspring	d020228135	0.1	40	1	91.65	3666		std dev 931
E01-1256-28757	454-109-9642, 10 PPM, Male, Offspring	d020228136	0.2	40	1	102.66	4106		%RSD 21.1

* Concentration <LOQ

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 (mL) = amount of sera used/total amount of sera + water (1/20 dilution). = 1/20 = 0.05 mL

Study number E01-1256 - MALLARD LIVER DATA SUMMARY, BLANKS AND SPIKES

3M Sample Number	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)	Conc. of PFOS in Sample (ng/g of Liver)	log for control samples
E01-1326-36778	Blank H2O-day 1	d020304037	1.00	9.00	2.00	0.100000	1	2.51	50.2	* 50.2
E01-1326-36778	Blank H2O-day 1	d020305036	1.00	9.00	2.00	0.100000	1	1.00	20.0	* 20.0
E01-1326-36807	Blank H2O-day 2	d020306024	1.00	9.00	2.00	0.100000	1	2.51	50.2	* 50.2
E01-1326-36812	Blank H2O-day 3	d020306104	1.00	9.00	2.00	0.100000	1	2.51	50.2	* 50.2
E01-1326-36817	Blank H2O-day 4	d020307024	1.00	9.00	2.00	0.100000	1	2.51	50.2	* 50.2
E01-1326-36780	Blank mallard liver-day 1	d020304038	5.0597	45.0	2.00	0.101073	1	2.51	50.2	* 50.2
E01-1326-36780	Blank mallard liver-day 1	d020305037	5.0597	45.0	2.00	0.101073	1	1.00	20.0	* 20.0
E01-1326-36809	Blank mallard liver-day 2	d020306025	5.0597	45.0	2.00	0.101073	1	2.51	50.2	* 50.2
E01-1326-36814	Blank mallard liver-day 3	d020306105	5.0597	45.0	2.00	0.101073	1	2.51	50.2	* 50.2
E01-1326-36819	Blank mallard liver-day 4	d020307025	5.0597	45.0	2.00	0.101073	1	2.51	50.2	* 50.2

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)	Conc. of PFOS in Sample (ng/g of Liver)	% Rec
E01-1326-36781	Mallard Liver MS-day 1	d020304039	5.0597	45.0	2.00	0.101073	1	28.50	564	113.5
E01-1326-36781	Mallard Liver MS-day 1	d020305038	5.0597	45.0	2.00	0.101073	1	26.91	532	107.1
E01-1326-36810	Mallard Liver MS-day 2	d020306026	5.0597	45.0	2.00	0.101073	1	27.95	553	111.3
E01-1326-36815	Mallard Liver MS-day 3	d020306106	5.0597	45.0	2.00	0.101073	1	27.72	549	110.4
E01-1326-36820	Mallard Liver MS-day 4	d020307026	5.0597	45.0	2.00	0.101073	1	27.84	551	110.8
E01-1326-36782	Mallard Liver MSD-day 1	d020304040	5.0597	45.0	2.00	0.101073	1	26.86	531	106.9
E01-1326-36782	Mallard Liver MSD-day 1	d020305039	5.0597	45.0	2.00	0.101073	1	26.18	518	104.2
E01-1326-36811	Mallard Liver MSD-day 2	d020306027	5.0597	45.0	2.00	0.101073	1	26.20	518	104.3
E01-1326-36816	Mallard Liver MSD-day 3	d020306107	5.0597	45.0	2.00	0.101073	1	27.50	544	109.5
E01-1326-36821	Mallard Liver MSD-day 4	d020307027	5.0597	45.0	2.00	0.101073	1	27.68	548	110.2

* Concentration <LOQ

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

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

for both ms and msd

avg %rec	108.8
std dev of %rec	3.059
avg conc	540.9
std dev of conc	15.20

Study number E01-1256 - MALLARD LIVER DATA SUMMARY**Absolute Recoveries**

versus unextracted curve

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)	Conc. of PFOS in Sample (ng/g of Liver)	% Rec.	
E01-1326-36798	QC- 25ppb -1 (497 ng/g homogenate)	d020305045	5.0597	45.0	2.00	0.101073	1	28.87	571	114.9	avg 124.5
E01-1326-36799	QC- 25ppb -2 (497 ng/g homogenate)	d020305046	5.0597	45.0	2.00	0.101073	1	29.22	578	116.3	std dev 15.41
E01-1326-36800	QC- 25ppb -3 (497 ng/g homogenate)	d020305047	5.0597	45.0	2.00	0.101073	1	35.74	707	A 142.3	%RSD 12.37
E01-1326-36801	QC- 250ppb -1 (4967 ng/g homogenate)	d020305048	5.0597	45.0	2.00	0.101073	1	242.46	4798	96.59	avg 102.3
E01-1326-36802	QC- 250ppb -2 (4967 ng/g homogenate)	d020305049	5.0597	45.0	2.00	0.101073	1	249.01	4927	99.20	std dev 7.662
E01-1326-36803	QC- 250ppb -3 (4967 ng/g homogenate)	d020305050	5.0597	45.0	2.00	0.101073	1	278.56	5512	111.0	%RSD 7.493
E01-1326-36804	QC- 4.0 ppm -1 (79467 ng/g homogenate)	d020405065	5.0597	45.0	2.00	0.101073	10	440.20	87105	109.6	avg 109.6
E01-1326-36805	QC- 4.0 ppm -2 (79467 ng/g homogenate)	d020405066	5.0597	45.0	2.00	0.101073	10	443.16	87691	110.3	std dev 0.7785
E01-1326-36806	QC- 4.0 ppm -3 (79467 ng/g homogenate)	d020405067	5.0597	45.0	2.00	0.101073	10	436.91	86454	108.8	%RSD 0.7104

A = Not within +/-25% criteria

for all QC samples (absolute recovery)

avg %rec	112.1
std dev of %rec	13.07

Study number E01-1256 - MALLARD LIVER DATA SUMMARY**QC Sample Recoveries**

versus extracted curve

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)	Conc. of PFOS in Sample (ng/g)	% Rec.	
E01-1326-36798	QC- 25ppb -1 (497 ng/g homogenate)	d020305045	5.0597	45.0	2.00	0.101073	1	25.41	503	101.2	avg 109.3
E01-1326-36799	QC- 25ppb -2 (497 ng/g homogenate)	d020305046	5.0597	45.0	2.00	0.101073	1	25.71	509	102.4	std dev 13.07
E01-1326-36800	QC- 25ppb -3 (497 ng/g homogenate)	d020305047	5.0597	45.0	2.00	0.101073	1	31.24	618	124.4	%RSD 11.96
E01-1326-36801	QC- 250ppb -1 (4967 ng/g homogenate)	d020305048	5.0597	45.0	2.00	0.101073	1	221.45	4382	88.22	avg 94.30
E01-1326-36802	QC- 250ppb -2 (4967 ng/g homogenate)	d020305049	5.0597	45.0	2.00	0.101073	1	228.29	4517	90.95	std dev 8.274
E01-1326-36803	QC- 250ppb -3 (4967 ng/g homogenate)	d020305050	5.0597	45.0	2.00	0.101073	1	260.35	5152	103.7	%RSD 8.774
E01-1326-36804	QC- 4.0 ppm -1 (79467 ng/g homogenate)	d020405065	5.0597	45.0	2.00	0.101073	10	415.43	82204	103.4	avg 103.4
E01-1326-36805	QC- 4.0 ppm -2 (79467 ng/g homogenate)	d020405066	5.0597	45.0	2.00	0.101073	10	418.16	82744	104.1	std dev 0.7175
E01-1326-36806	QC- 4.0 ppm -3 (79467 ng/g homogenate)	d020405067	5.0597	45.0	2.00	0.101073	10	412.40	81604	102.7	%RSD 0.6937

A = Not within +/-25% criteria

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 all QC samples (recovery)

avg %rec	102.3
std dev of %rec	10.14

Study number E01-1256 - MALLARD LIVER DATA SUMMARY

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)	Conc. of PFOS in Sample (ng/g of Liver)	log for control samples
E01-1326-29482	0 ppm a.i. 3218 F Liver	d020305059	1.0095	9.00	2.00	0.100854	1	1.00	20.0	*
E01-1326-29583	0 ppm a.i. 3220 F Liver	d020305060	1.0944	9.00	2.00	0.108417	1	1.00	20.0	*
E01-1326-29601	0 ppm a.i. 3222 F Liver	d020305061	1.0435	9.00	2.00	0.103898	1	1.00	20.0	*
E01-1326-29619	0 ppm a.i. 3224 F Liver	d020305062	1.0425	9.00	2.00	0.103809	1	1.00	20.0	*
E01-1326-29637	0 ppm a.i. 3226 F Liver	d020305063	1.0703	9.00	2.00	0.106283	1	1.00	20.0	*
E01-1326-29655	0 ppm a.i. 3228 F Liver	d020305064	1.0526	9.00	2.00	0.104709	1	1.00	20.0	*
E01-1326-29673	0 ppm a.i. 3230 F Liver	d020305065	1.0411	9.00	2.00	0.103684	1	1.00	20.0	*
E01-1326-29691	0 ppm a.i. 3232 F Liver	d020306031	1.1704	9.00	2.00	0.115079	1	2.51	50.2	*
E01-1326-29709	0 ppm a.i. 3234 F Liver	d020306032	1.1290	9.00	2.00	0.111462	1	2.51	50.2	*
E01-1326-29727	0 ppm a.i. 3236 F Liver	d020306033	1.0800	9.00	2.00	0.107143	1	2.51	50.2	*
E01-1326-29745	0 ppm a.i. 3238 F Liver	d020306034	1.1170	9.00	2.00	0.110408	1	2.51	50.2	*
E01-1326-29763	0 ppm a.i. 3240 F Liver	d020306035	1.0086	9.00	2.00	0.100773	1	2.51	50.2	*
E01-1326-29781	0 ppm a.i. 3242 F Liver	d020306036	1.0907	9.00	2.00	0.108090	1	2.51	50.2	*
E01-1326-29799	0 ppm a.i. 3244 F Liver	d020306037	1.0291	9.00	2.00	0.102611	1	2.51	50.2	*
E01-1326-29817	0 ppm a.i. 3246 F Liver	d020306038	1.1294	9.00	2.00	0.111497	1	2.51	50.2	*
E01-1326-29835	0 ppm a.i. 3248 F Liver	d020306039	1.1075	9.00	2.00	0.109572	1	2.51	50.2	*
E01-1326-29853	0 ppm a.i. 3250 F Liver	d020306040	1.1709	9.00	2.00	0.115123	1	2.51	50.2	*
E01-1326-29871	0 ppm a.i. 3252 F Liver	d020306044	1.0761	9.00	2.00	0.106797	1	2.51	50.2	*
E01-1326-29889	0 ppm a.i. 3254 F Liver	d020306045	1.1344	9.00	2.00	0.111936	1	2.51	50.2	*
E01-1326-29907	0 ppm a.i. 3256 F Liver	d020306046	1.0669	9.00	2.00	0.105981	1	2.51	50.2	*
E01-1326-29925	10 ppm a.i. 3258 F Liver	d020306066	1.0034	9.00	2.00	0.100306	1	355.07	7080	
E01-1326-29943	10 ppm a.i. 3260 F Liver	d020306067	1.1331	9.00	2.00	0.111822	1	379.32	6784	
E01-1326-29961	10 ppm a.i. 3262 F Liver	d020307057	1.1294	9.00	2.00	0.111497	10	76.07	13645	
E01-1326-29980	10 ppm a.i. 3264 F Liver	d020306111	1.1829	9.00	2.00	0.116165	1	352.91	6076	
E01-1326-29998	10 ppm a.i. 3266 F Liver	d020306112	1.0487	9.00	2.00	0.104362	1	280.63	5378	
E01-1326-30016	10 ppm a.i. 3268 F Liver	d020306113	1.1214	9.00	2.00	0.110795	1	317.25	5727	
E01-1326-30034	10 ppm a.i. 3270 F Liver	d020307059	1.0751	9.00	2.00	0.106709	10	70.37	13189	
E01-1326-30052	10 ppm a.i. 3272 F Liver	d020306115	1.0658	9.00	2.00	0.105883	1	307.09	5801	
E01-1326-30070	10 ppm a.i. 3274 F Liver	d020307060	1.1226	9.00	2.00	0.110900	10	76.85	13859	
E01-1326-30088	10 ppm a.i. 3276 F Liver	d020307061	1.0953	9.00	2.00	0.108496	10	166.74	30737	
E01-1326-30106	10 ppm a.i. 3278 F Liver	d020306118	1.1956	9.00	2.00	0.117266	1	349.71	5964	
E01-1326-30124	10 ppm a.i. 3280 F Liver	d020307062	1.0600	9.00	2.00	0.105368	1	130.88	2484	
E01-1326-30142	10 ppm a.i. 3282 F Liver	d020306120	1.0275	9.00	2.00	0.102468	1	233.84	4564	
E01-1326-30160	10 ppm a.i. 3284 F Liver	d020306124	1.0915	9.00	2.00	0.108160	1	342.26	6329	
E01-1326-30178	10 ppm a.i. 3286 F Liver	d020307063	1.0657	9.00	2.00	0.105874	10	149.38	28218	
E01-1326-30196	10 ppm a.i. 3288 F Liver	d020307064	1.0330	9.00	2.00	0.102960	10	124.22	24130	
E01-1326-30214	10 ppm a.i. 3290 F Liver	d020307065	1.0352	9.00	2.00	0.103157	10	101.98	19772	
E01-1326-30232	10 ppm a.i. 3292 F Liver	d020306128	1.0658	9.00	2.00	0.105883	1	243.62	4602	
E01-1326-30250	10 ppm a.i. 3294 F Liver	d020306129	1.0627	9.00	2.00	0.105608	1	251.58	4764	
E01-1326-30268	10 ppm a.i. 3296 F Liver	d020306130	1.1238	9.00	2.00	0.111006	1	351.37	6331	
E01-1367-31295	0 ppm a.i. 9583 F Liver	d020307031	1.1308	9.00	2.00	0.111620	1	2.51	50.2	*
E01-1367-31307	0 ppm a.i. 9589 F Liver	d020307032	1.0005	9.00	2.00	0.100045	1	2.51	50.2	*
E01-1367-31313	0 ppm a.i. 9592 F Liver	d020307033	1.0390	9.00	2.00	0.103496	1	2.51	50.2	*
E01-1367-31319	0 ppm a.i. 9598 F Liver	d020307034	1.1204	9.00	2.00	0.110707	1	2.51	50.2	*
E01-1367-31325	0 ppm a.i. 9603 F Liver	d020307035	1.1550	9.00	2.00	0.113737	1	2.51	50.2	*
E01-1367-31337	0 ppm a.i. 9610 F Liver	d020307036	1.0217	9.00	2.00	0.101949	1	2.51	50.2	*
E01-1367-31343	0 ppm a.i. 9573 F Liver	d020307037	1.0122	9.00	2.00	0.101097	1	2.51	50.2	*
E01-1367-31362	10 ppm a.i. 9629 F Liver	d020307044	1.1676	9.00	2.00	0.114835	1	169.63	2954	
E01-1367-31368	10 ppm a.i. 9633 F Liver	d020307045	1.0510	9.00	2.00	0.104567	1	117.36	2245	
E01-1367-31374	10 ppm a.i. 9634 F Liver	d020307046	1.1638	9.00	2.00	0.114504	1	258.53	4516	
E01-1367-31380	10 ppm a.i. 9645 F Liver	d020307047	1.1510	9.00	2.00	0.113388	1	146.41	2582	
E01-1367-31386	10 ppm a.i. 9648 F Liver	d020307048	1.1125	9.00	2.00	0.110012	1	192.68	3503	
E01-1367-31404	10 ppm a.i. 9654 F Liver	d020307049	1.0770	9.00	2.00	0.106877	1	316.88	5930	
E01-1367-31410	10 ppm a.i. 9659 F Liver	d020307050	1.0581	9.00	2.00	0.105199	1	188.42	3582	

avg 39.6
std dev 14.8
%RSD 37.3

avg 10772
std dev 8463
%RSD 78.6

avg 50.2
std dev 0
%RSD -

avg 3616
std dev 1263
%RSD 34.9

* 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 E01-1256 - MALLARD LIVER DATA SUMMARY

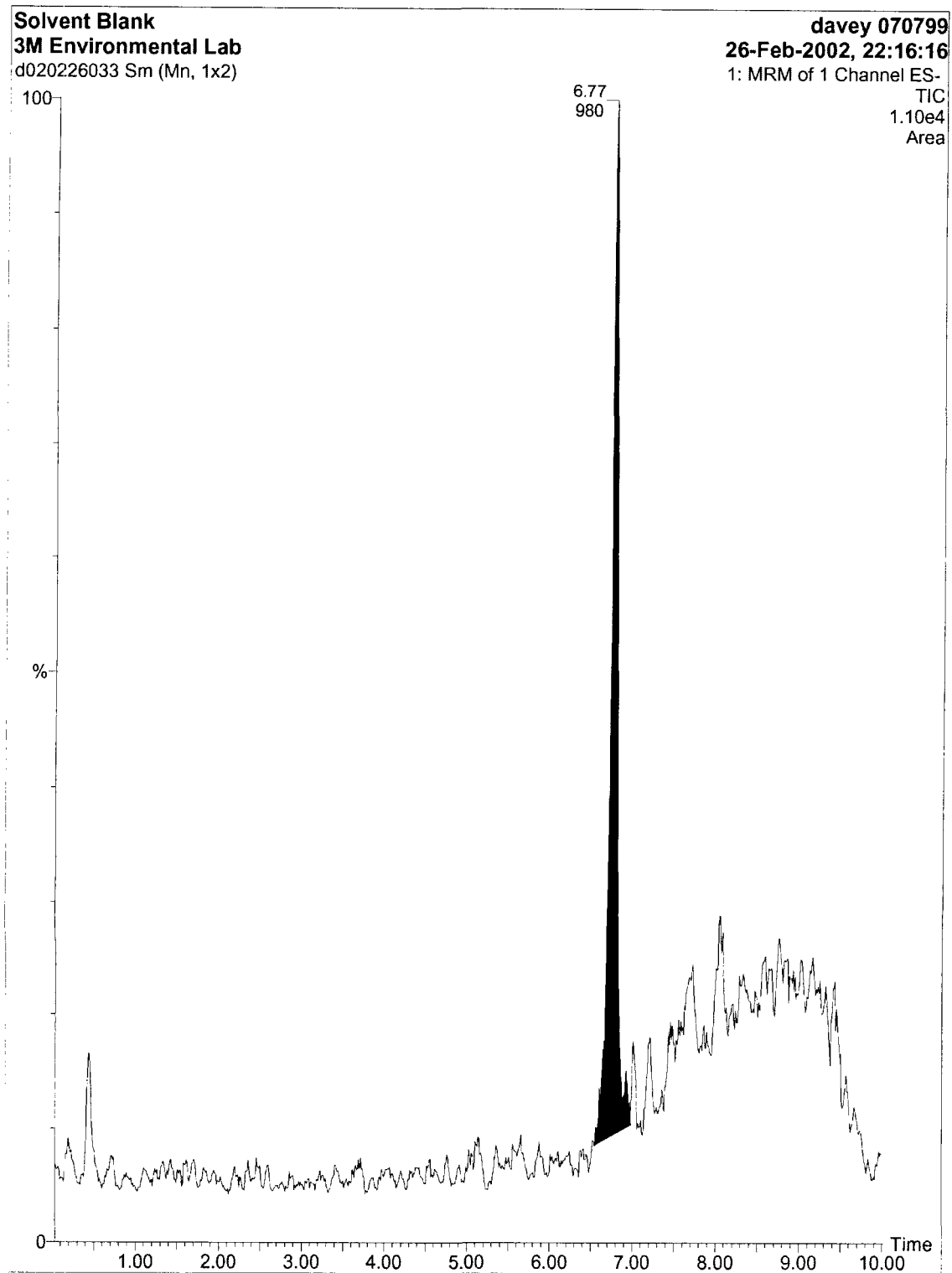
3M Sample Number	Sample Description	Inst. File Name	amount of liver weighed (g)	Water Added for Homogenizing (mL)	Final Volume (mL)	Amount of liver in diluted sample (g)	additional dilution	Inst Conc. (ng/mL of Methanol)	Conc. of PFOS in Sample (ng/g of Liver)	log for control samples	
E01-1326-29473	0 ppm a.i. 3217 M Liver	d020305071	1.1169	9.00	2.00	0.110399	1	1.00	20.0	*	20.0
E01-1326-29574	0 ppm a.i. 3219 M Liver	d020305072	1.1847	9.00	2.00	0.116322	1	1.00	20.0	*	20.0
E01-1326-29592	0 ppm a.i. 3221 M Liver	d020305073	1.1181	9.00	2.00	0.110505	1	1.00	20.0	*	20.0
E01-1326-29610	0 ppm a.i. 3223 M Liver	d020305074	1.0788	9.00	2.00	0.107037	1	1.00	20.0	*	20.0
E01-1326-29628	0 ppm a.i. 3225 M Liver	d020305075	1.1043	9.00	2.00	0.109290	1	1.00	20.0	*	20.0
E01-1326-29646	0 ppm a.i. 3227 M Liver	d020305076	1.0486	9.00	2.00	0.104353	1	1.00	20.0	*	20.0
E01-1326-29664	0 ppm a.i. 3229 M Liver	d020305077	1.0336	9.00	2.00	0.103014	1	1.00	20.0	*	20.0
E01-1326-29682	0 ppm a.i. 3231 M Liver	d020306047	1.0165	9.00	2.00	0.101483	1	2.51	50.2	*	50.2
E01-1326-29700	0 ppm a.i. 3233 M Liver	d020306048	1.0395	9.00	2.00	0.103541	1	2.51	50.2	*	50.2
E01-1326-29718	0 ppm a.i. 3235 M Liver	d020306049	1.0373	9.00	2.00	0.103345	1	2.51	50.2	*	50.2
E01-1326-29736	0 ppm a.i. 3237 M Liver	d020306050	1.1679	9.00	2.00	0.114861	1	2.51	50.2	*	50.2
E01-1326-29754	0 ppm a.i. 3239 M Liver	d020306051	1.1493	9.00	2.00	0.113239	1	2.51	50.2	*	50.2
E01-1326-29772	0 ppm a.i. 3241 M Liver	d020306052	1.0619	9.00	2.00	0.105537	1	2.51	50.2	*	50.2
E01-1326-29790	0 ppm a.i. 3243 M Liver	d020306053	1.0263	9.00	2.00	0.102361	1	2.51	50.2	*	50.2
E01-1326-29808	0 ppm a.i. 3245 M Liver	d020306057	1.1541	9.00	2.00	0.113659	1	2.51	50.2	*	50.2
E01-1326-29826	0 ppm a.i. 3247 M Liver	d020306058	1.0813	9.00	2.00	0.107258	1	2.51	50.2	*	50.2
E01-1326-29844	0 ppm a.i. 3249 M Liver	d020306059	1.0263	9.00	2.00	0.102361	1	2.51	50.2	*	50.2
E01-1326-29862	0 ppm a.i. 3251 M Liver	d020306060	1.0088	9.00	2.00	0.100791	1	2.51	50.2	*	50.2
E01-1326-29880	0 ppm a.i. 3253 M Liver	d020306061	1.0294	9.00	2.00	0.102638	1	2.51	50.2	*	50.2
E01-1326-29898	0 ppm a.i. 3255 M Liver	d020306062	1.0926	9.00	2.00	0.108258	1	2.51	50.2	*	50.2
E01-1326-29916	10 ppm a.i. 3257 M Liver	d020306069	1.0654	9.00	2.00	0.105848	10	381.46	72077		
E01-1326-29934	10 ppm a.i. 3259 M Liver	d020306070	1.1316	9.00	2.00	0.111690	10	274.77	49202		
E01-1326-29952	10 ppm a.i. 3261 M Liver	d020306071	1.1330	9.00	2.00	0.111813	10	392.11	70137		
E01-1326-29970	10 ppm a.i. 3263 M Liver	d020307058	1.1404	9.00	2.00	0.112461	100	70.46	125306		
E01-1326-29989	10 ppm a.i. 3265 M Liver	d020306131	1.1480	9.00	2.00	0.113126	10	349.14	61726		
E01-1326-30007	10 ppm a.i. 3267 M Liver	d020306132	1.0620	9.00	2.00	0.105546	10	263.55	49940		
E01-1326-30025	10 ppm a.i. 3269 M Liver	d020306133	1.1687	9.00	2.00	0.114931	10	332.69	57894		
E01-1326-30043	10 ppm a.i. 3271 M Liver	d020306137	1.0313	9.00	2.00	0.102808	10	323.76	62983		
E01-1326-30061	10 ppm a.i. 3273 M Liver	d020306138	1.1399	9.00	2.00	0.112417	10	477.80	85005		
E01-1326-30079	10 ppm a.i. 3275 M Liver	d020306139	1.1143	9.00	2.00	0.110171	10	302.45	54906		
E01-1326-30097	10 ppm a.i. 3277 M Liver	d020306140	1.0062	9.00	2.00	0.100558	10	271.03	53905		
E01-1326-30115	10 ppm a.i. 3279 M Liver	d020306141	1.0380	9.00	2.00	0.103407	10	356.95	69038		
E01-1326-30133	10 ppm a.i. 3281 M Liver	d020306142	1.0669	9.00	2.00	0.105981	10	264.50	49915		
E01-1326-30151	10 ppm a.i. 3283 M Liver	d020306143	1.0955	9.00	2.00	0.108514	10	363.85	67061		
E01-1326-30169	10 ppm a.i. 3285 M Liver	d020306144	1.1981	9.00	2.00	0.117483	10	325.94	55487		
E01-1326-30187	10 ppm a.i. 3287 M Liver	d020306145	1.0500	9.00	2.00	0.104478	10	274.59	52564		
E01-1326-30205	10 ppm a.i. 3289 M Liver	d020306146	1.0251	9.00	2.00	0.102253	10	303.06	59276		
E01-1326-30223	10 ppm a.i. 3291 M Liver	d020306150	1.0441	9.00	2.00	0.103952	10	241.84	46529		
E01-1326-30241	10 ppm a.i. 3293 M Liver	d020306151	1.0979	9.00	2.00	0.108726	10	131.53	24195		
E01-1326-30259	10 ppm a.i. 3295 M Liver	d020306152	1.0815	9.00	2.00	0.107276	10	270.60	50449		
E01-1367-31301	0 ppm a.i. 9591 M Liver	d020307038	1.0411	9.00	2.00	0.103684	1	2.51	50.2	*	50.2
E01-1367-31331	0 ppm a.i. 9606 M Liver	d020307039	1.1314	9.00	2.00	0.111673	1	2.51	50.2	*	50.2
E01-1367-31349	0 ppm a.i. 9578 M Liver	d020307040	1.0413	9.00	2.00	0.103702	1	2.51	50.2	*	50.2
E01-1367-31356	10 ppm a.i. 9625 M Liver	d020307051	1.0166	9.00	2.00	0.101492	1	235.62	4643		
E01-1367-31392	10 ppm a.i. 9636 M Liver	d020307052	1.1367	9.00	2.00	0.112137	1	123.08	2195		
E01-1367-31398	10 ppm a.i. 9642 M Liver	d020307053	1.1252	9.00	2.00	0.111129	1	149.00	2682		

* 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)

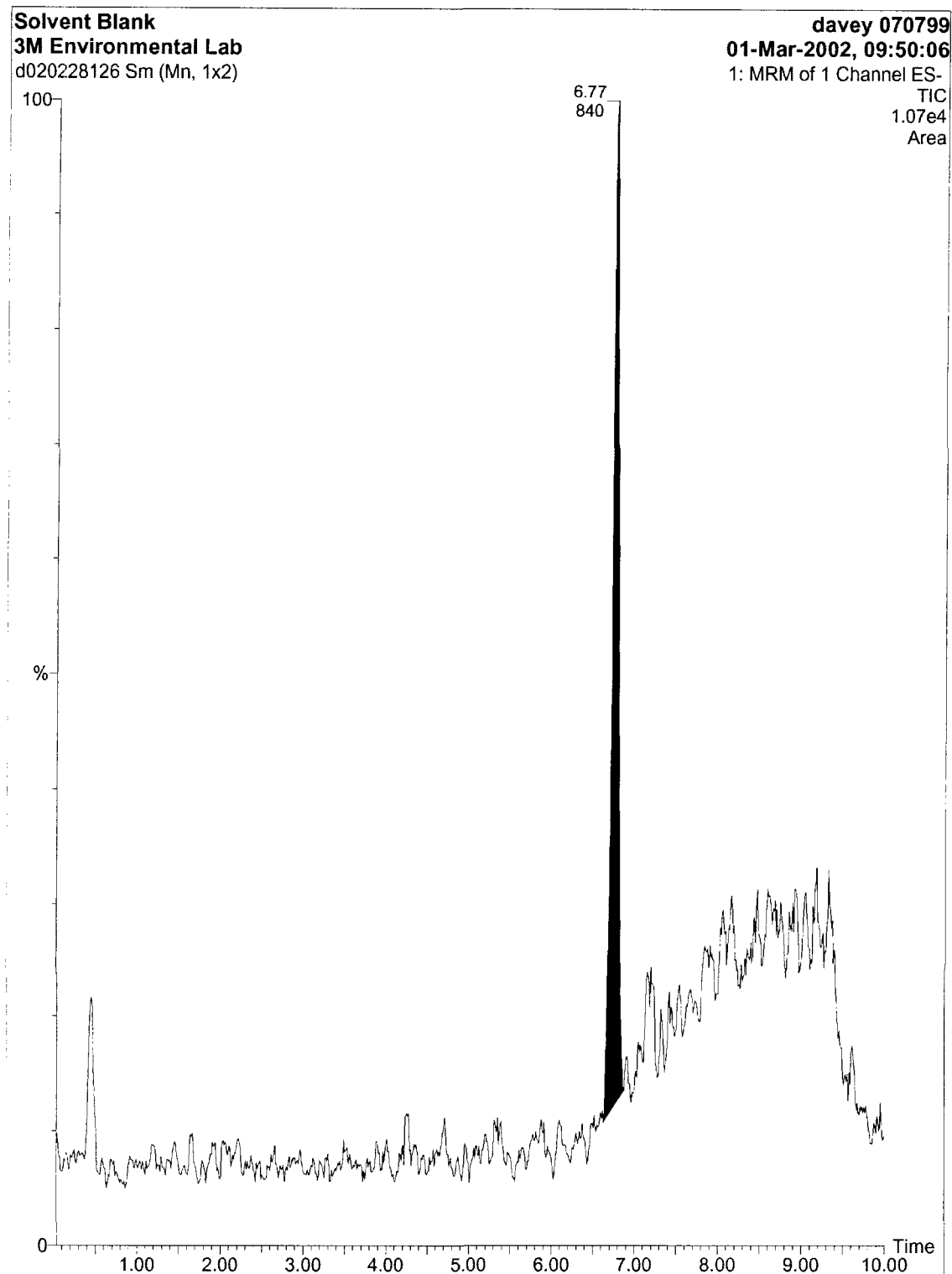
amount of liver in diluted sample (g) = amount of liver weighed (g)/ (water added for homogenizing (mL=g)) + amount of liver weighed(g)

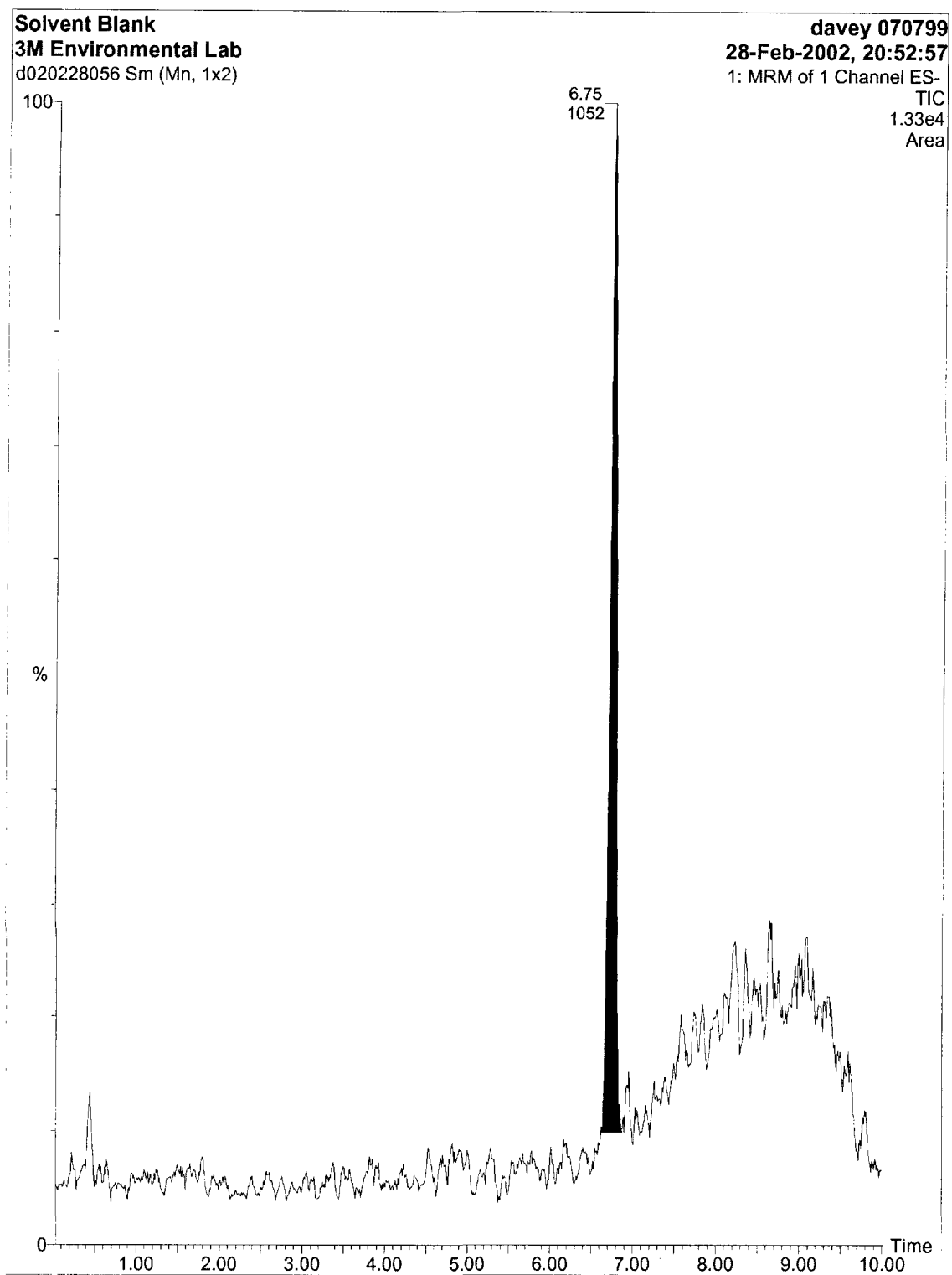
**ATTACHMENT C: SAMPLE CHROMATOGRAMS, CALIBRATION INFORMATION,
AND INSTRUMENT INFORMATION**

Methanol Solvent Blanks

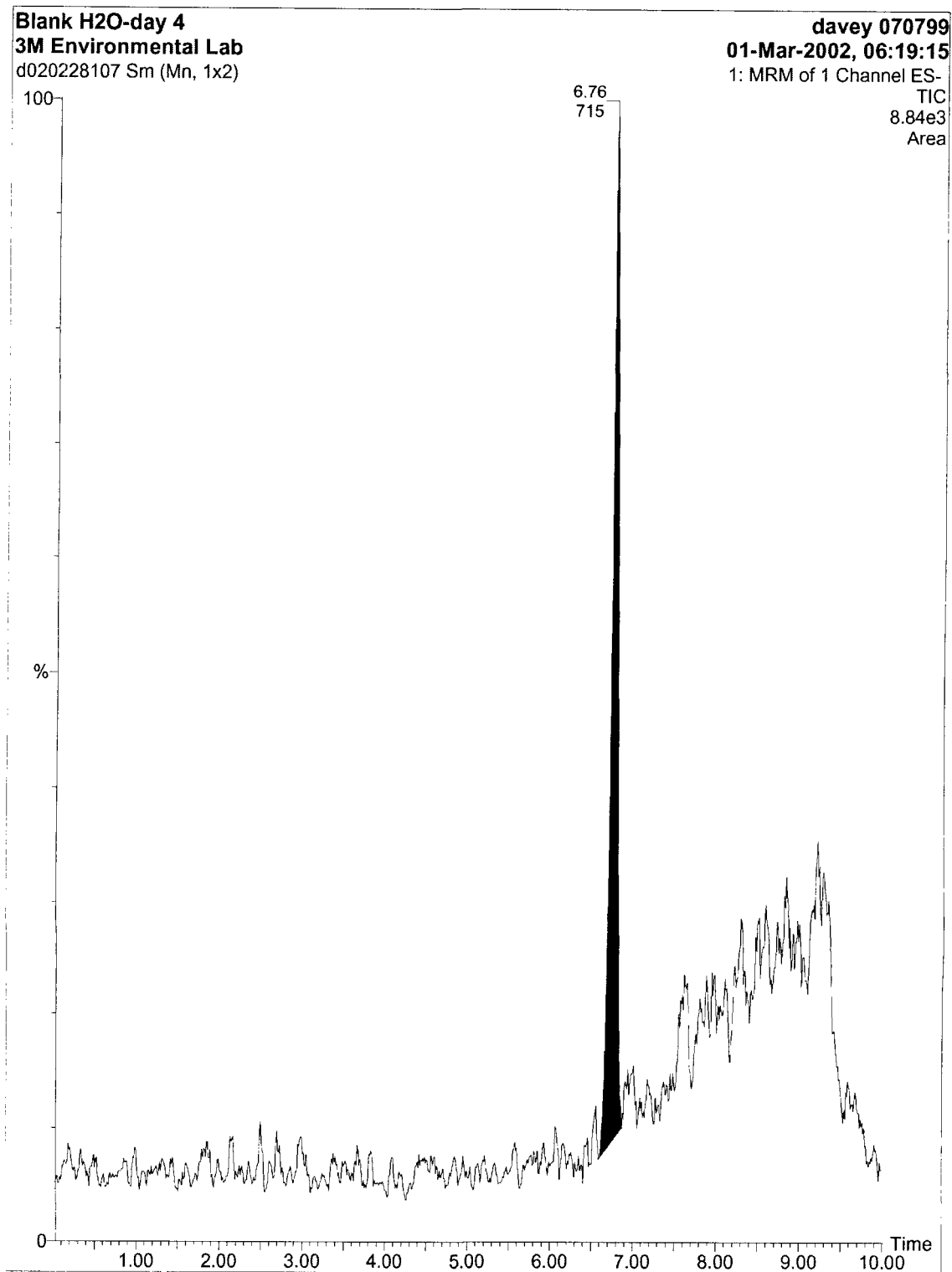
Report E01-1256

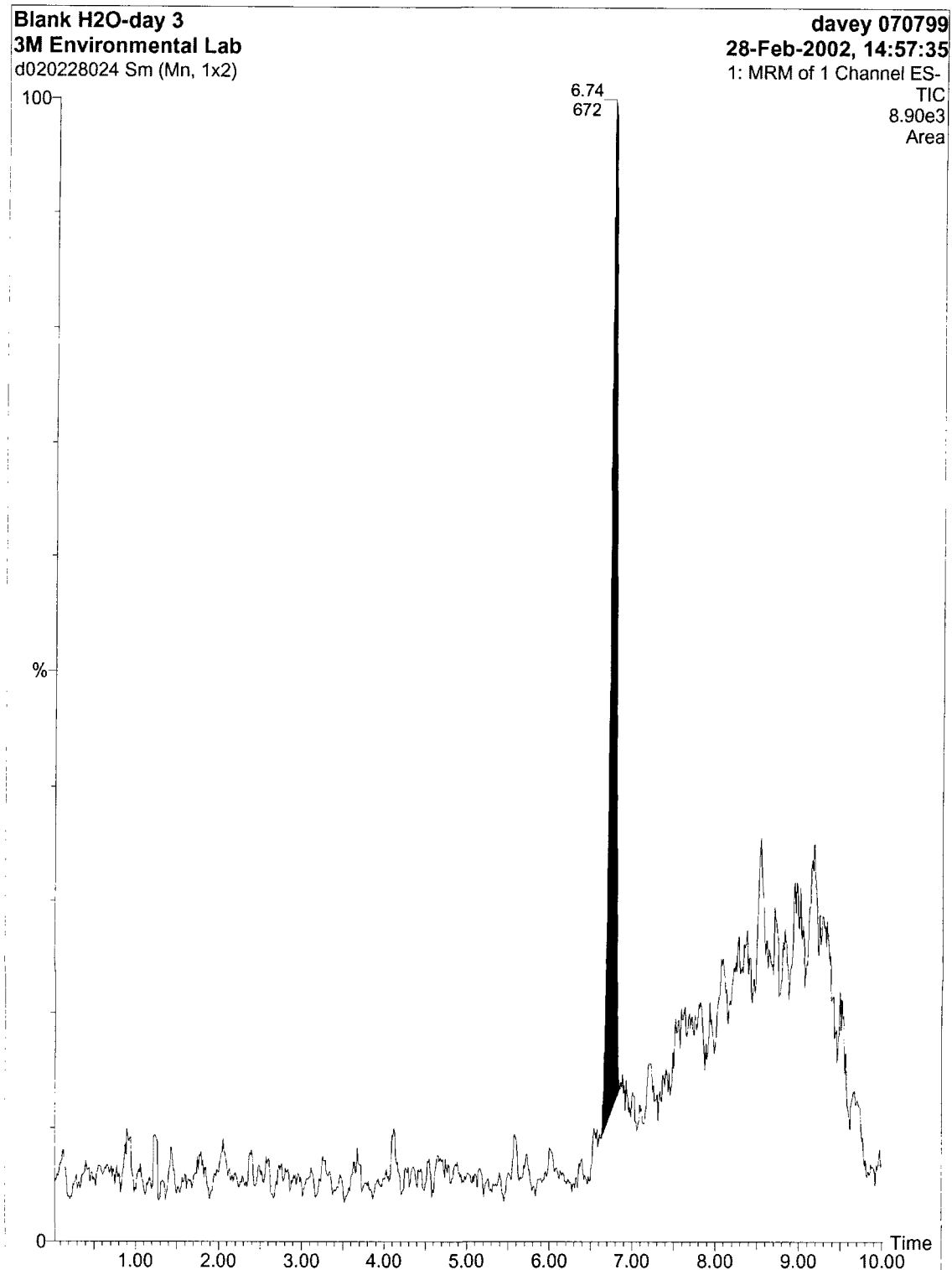
PFOS: A Reproduction Study with the Mallard

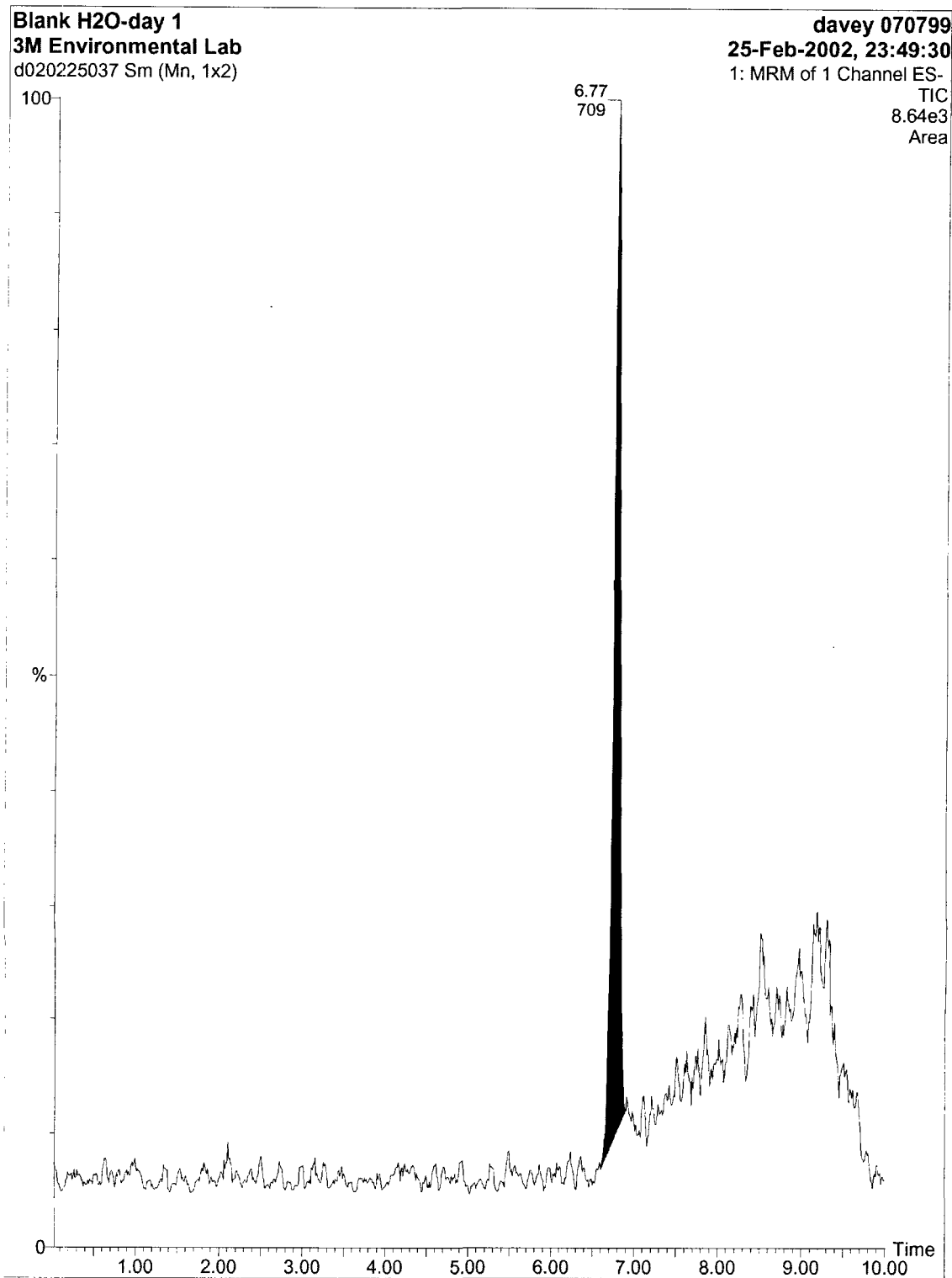




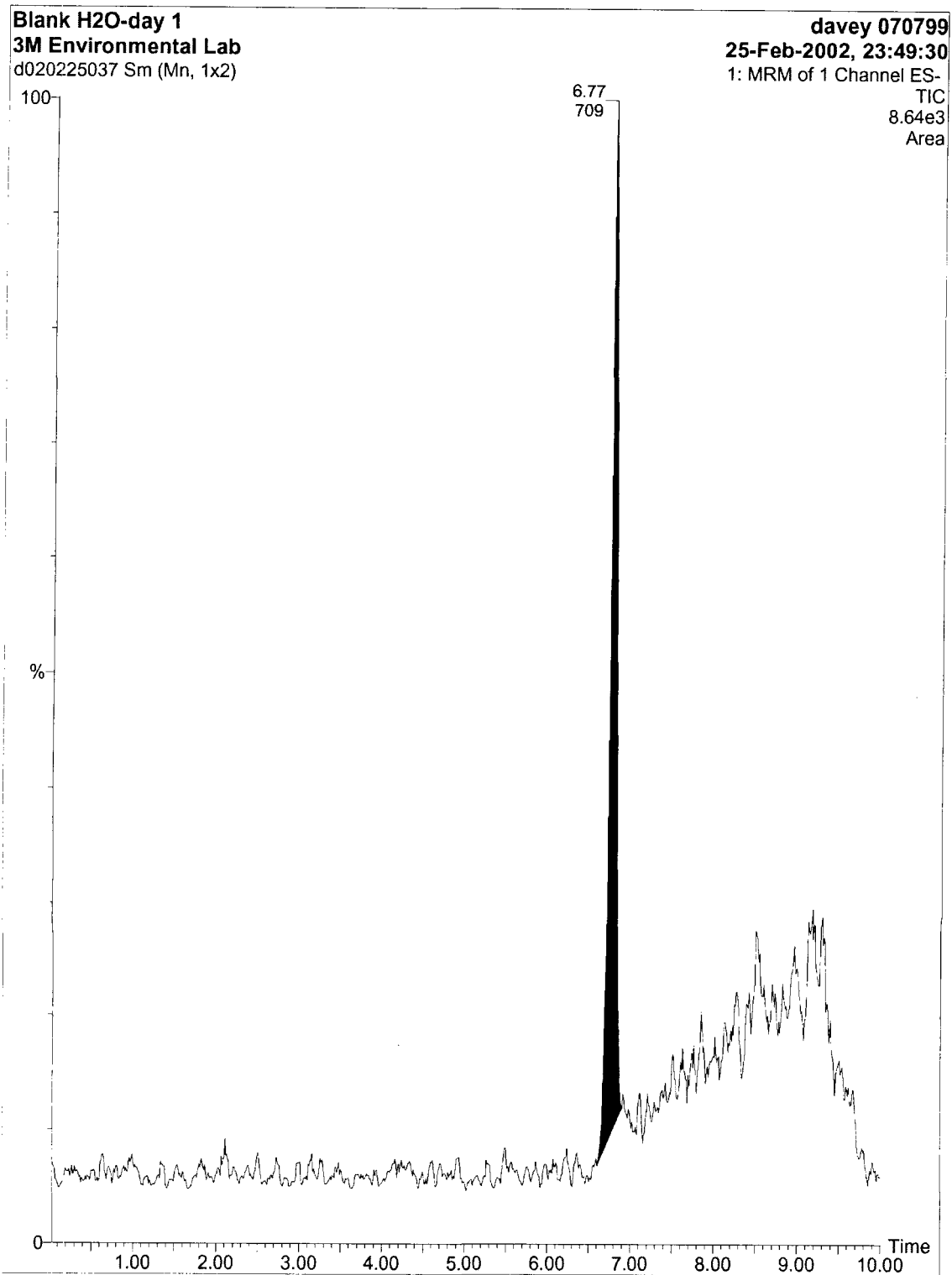
Water Blank Samples

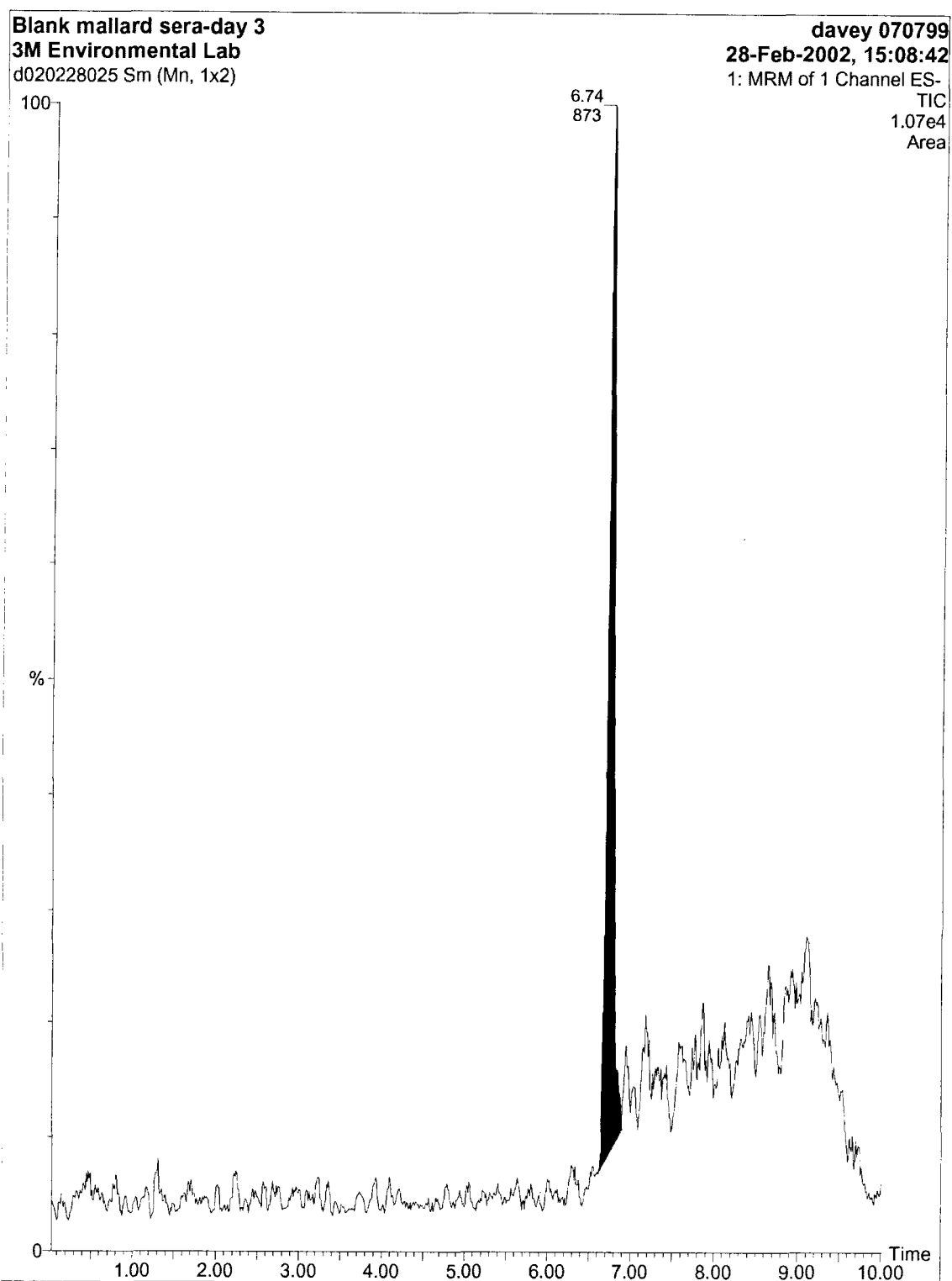


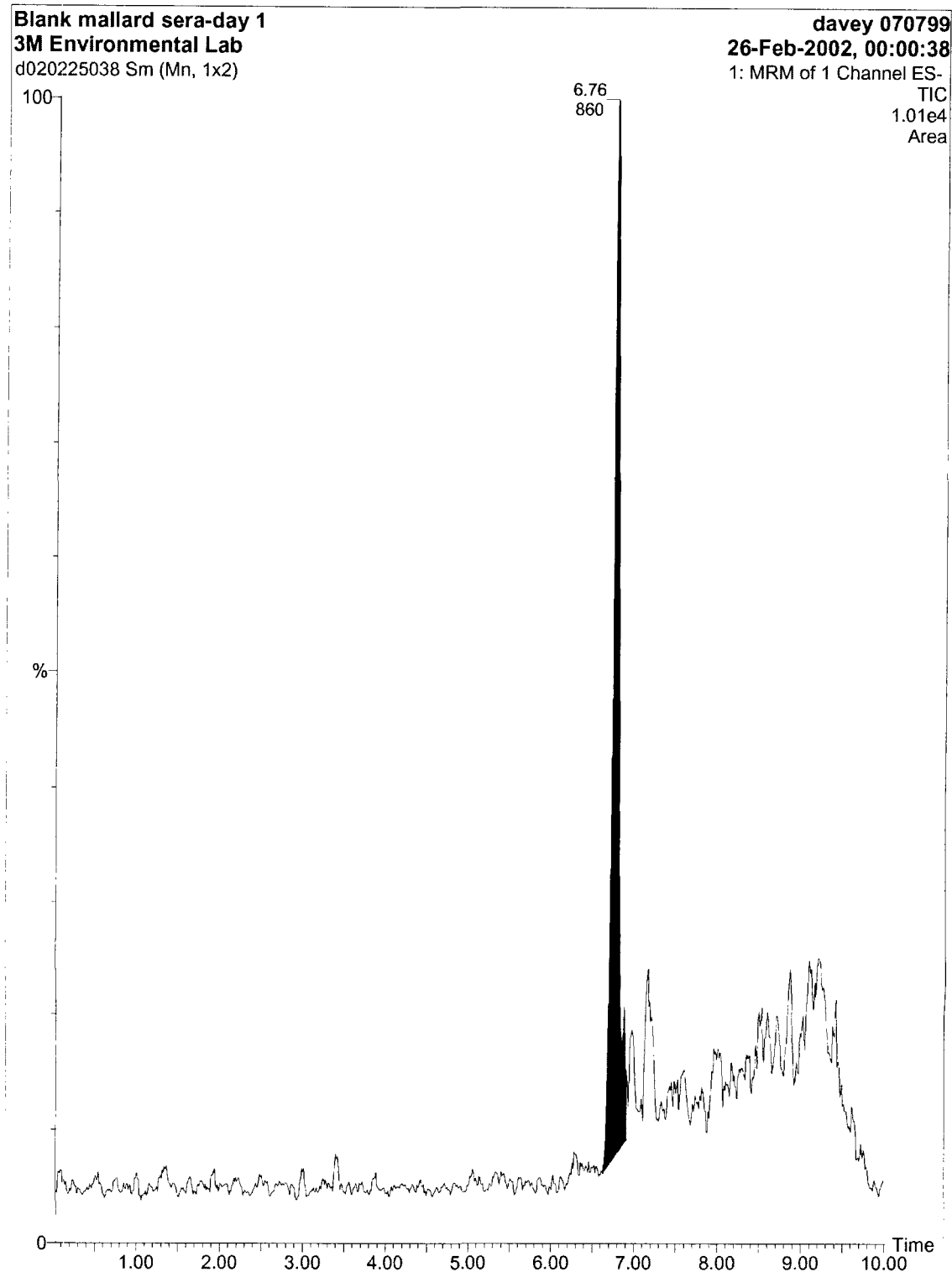




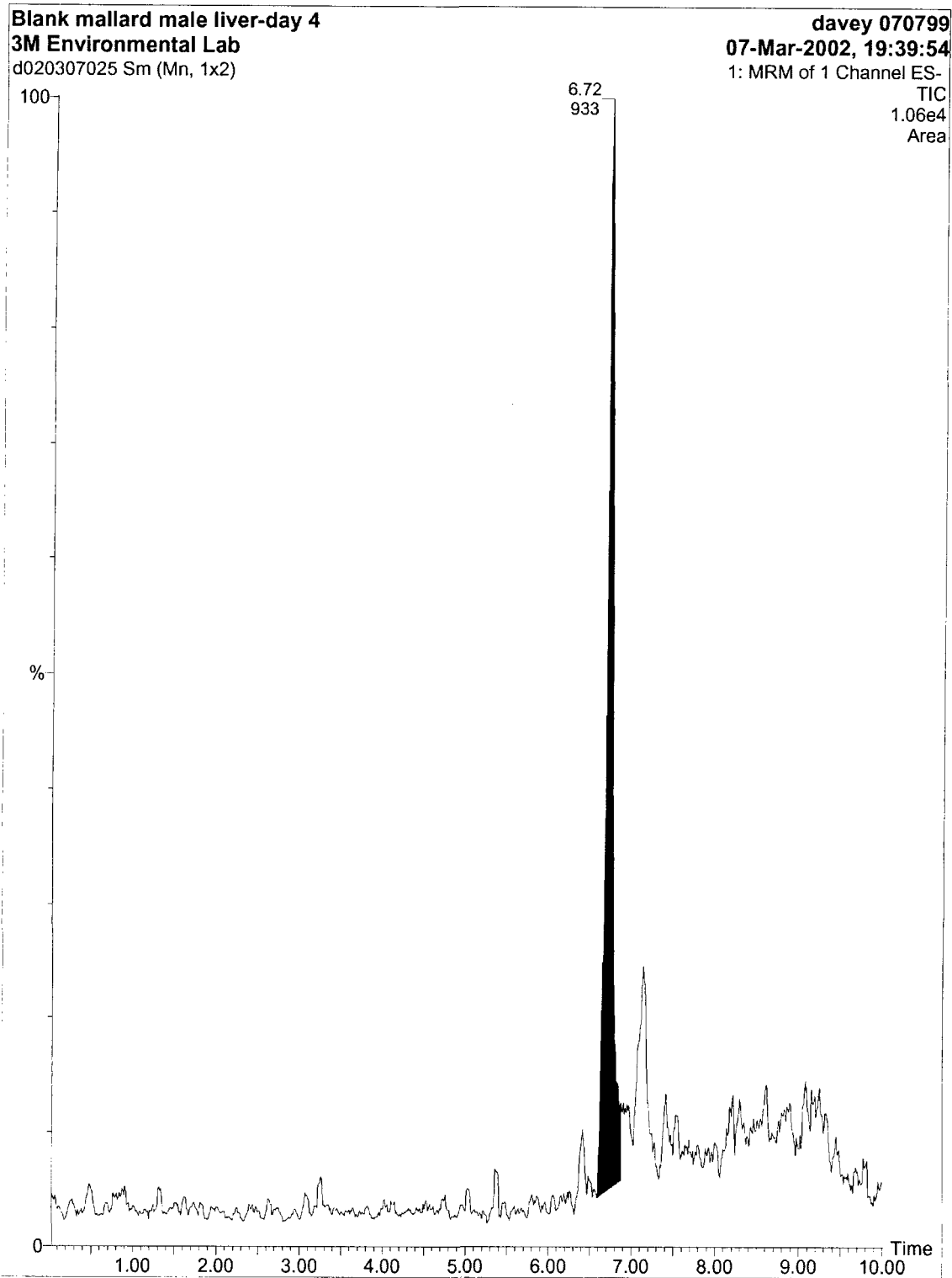
Sera Blank Samples

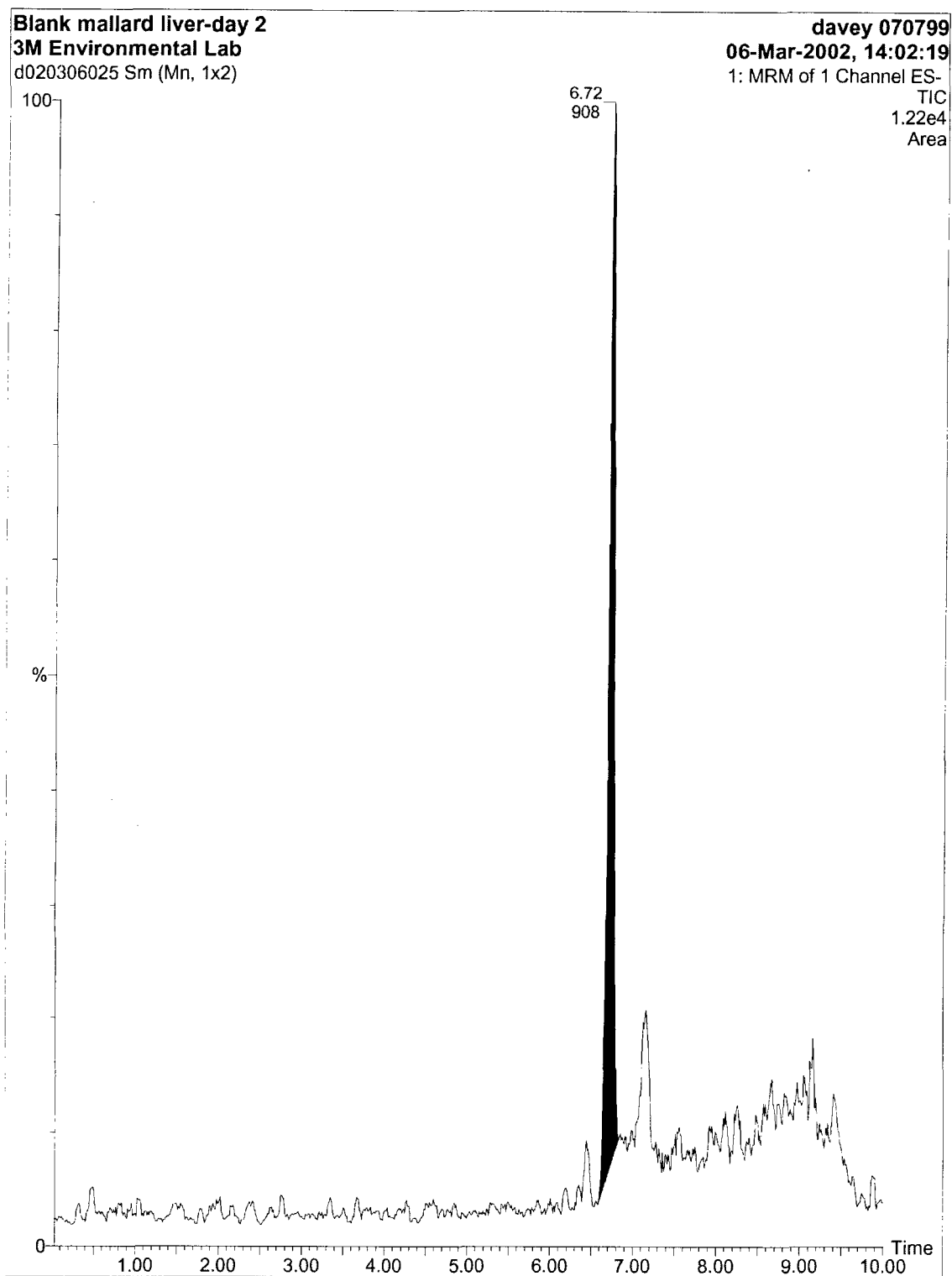


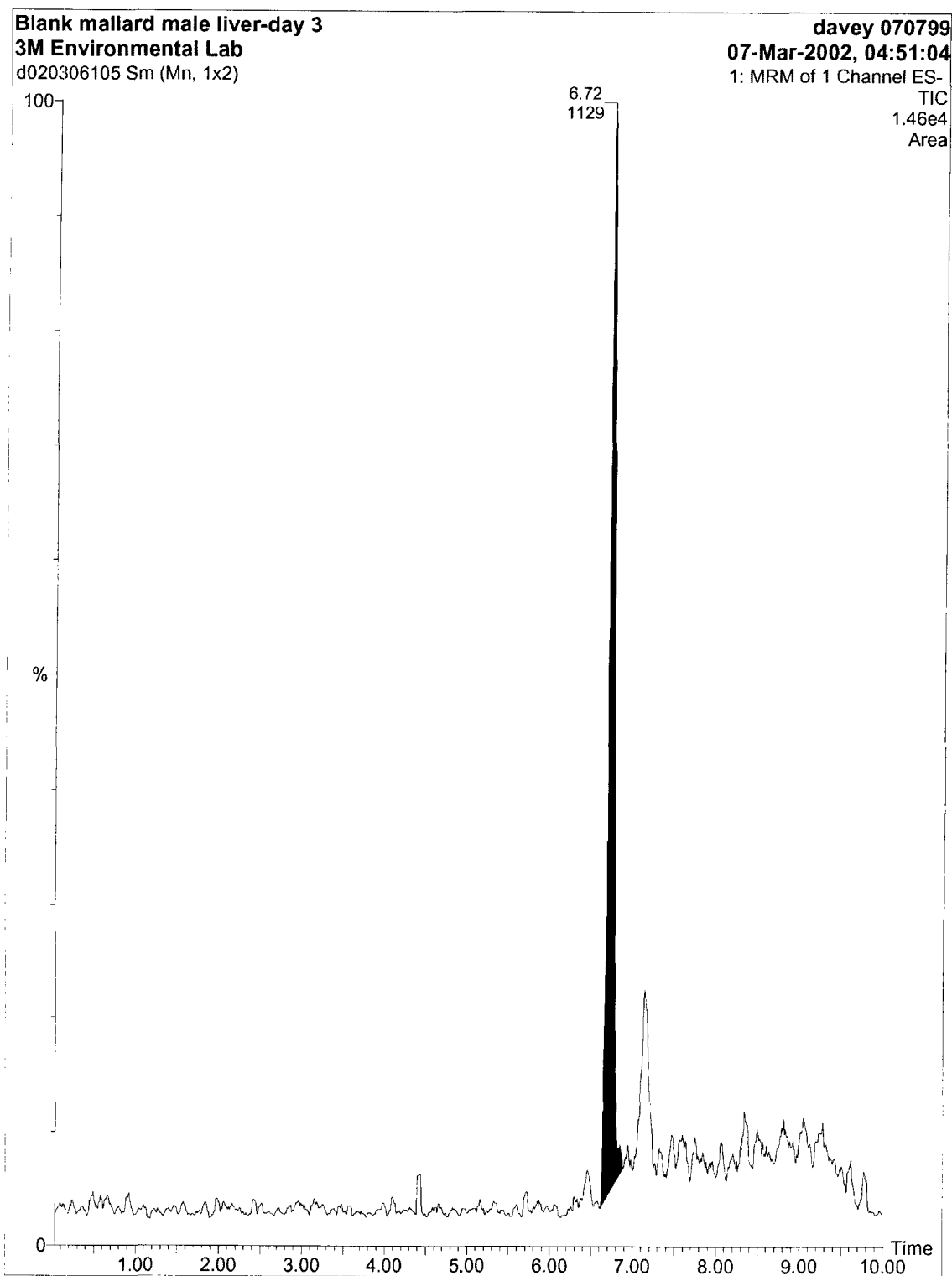


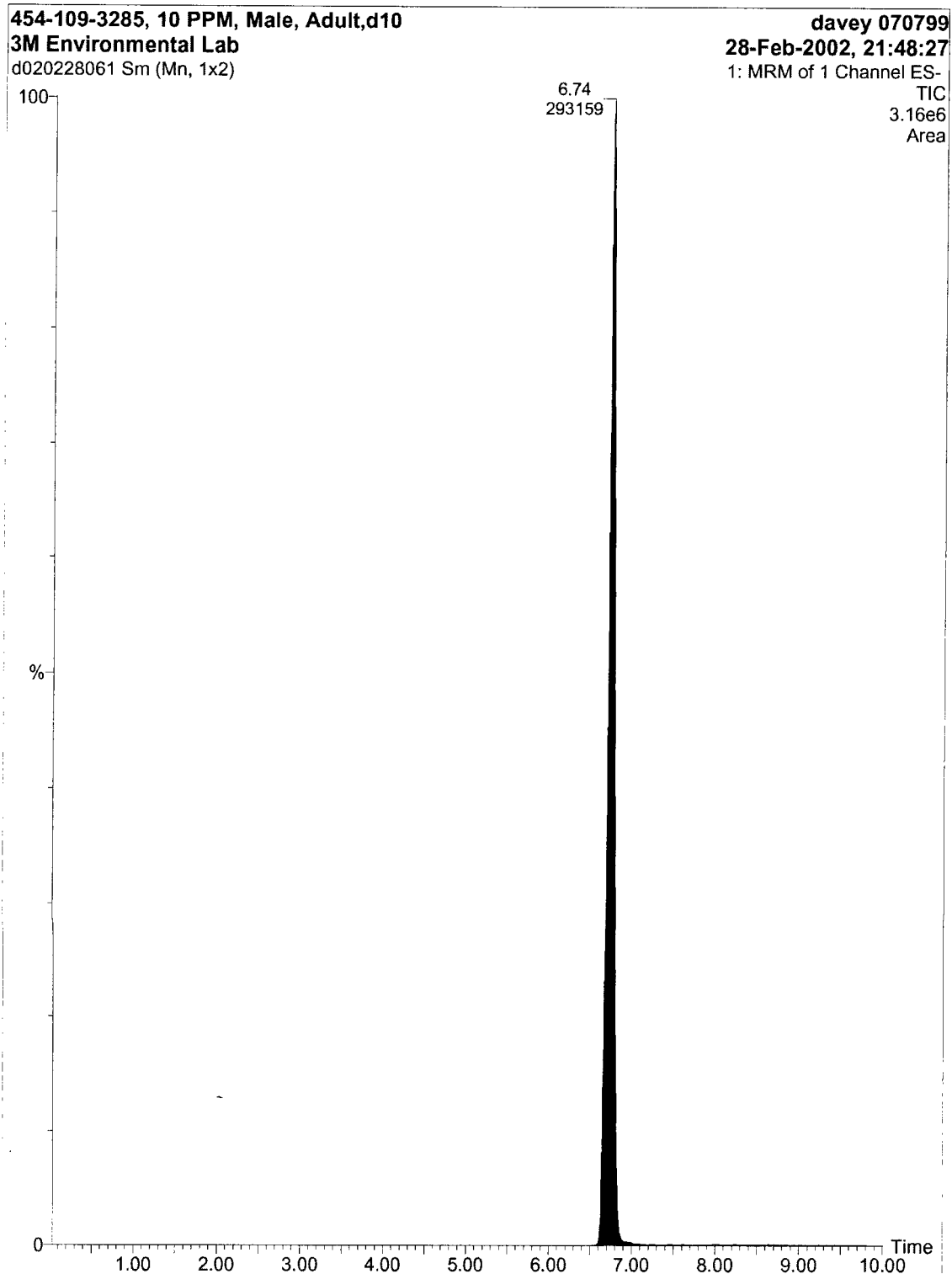


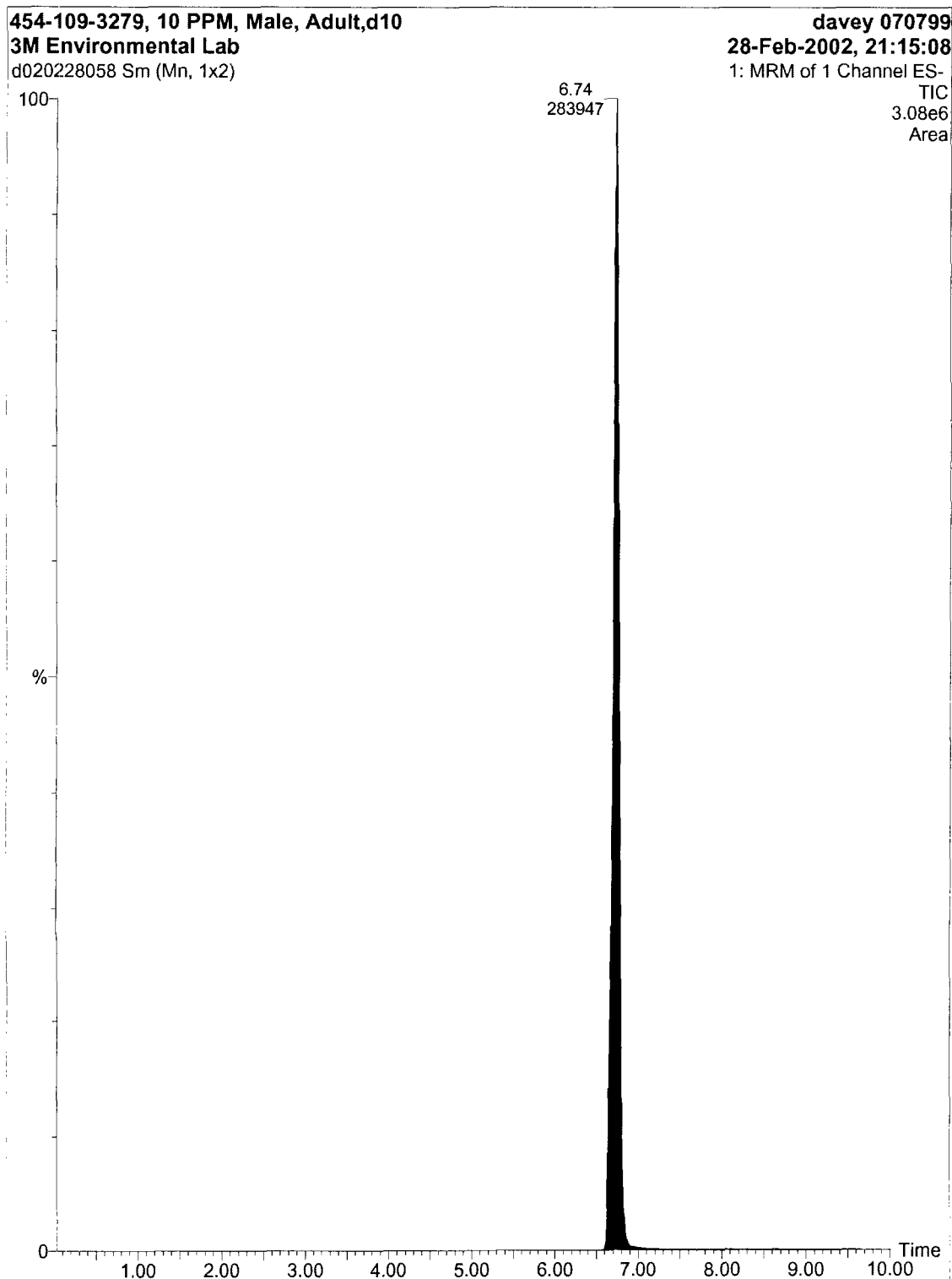
Liver Blank Samples

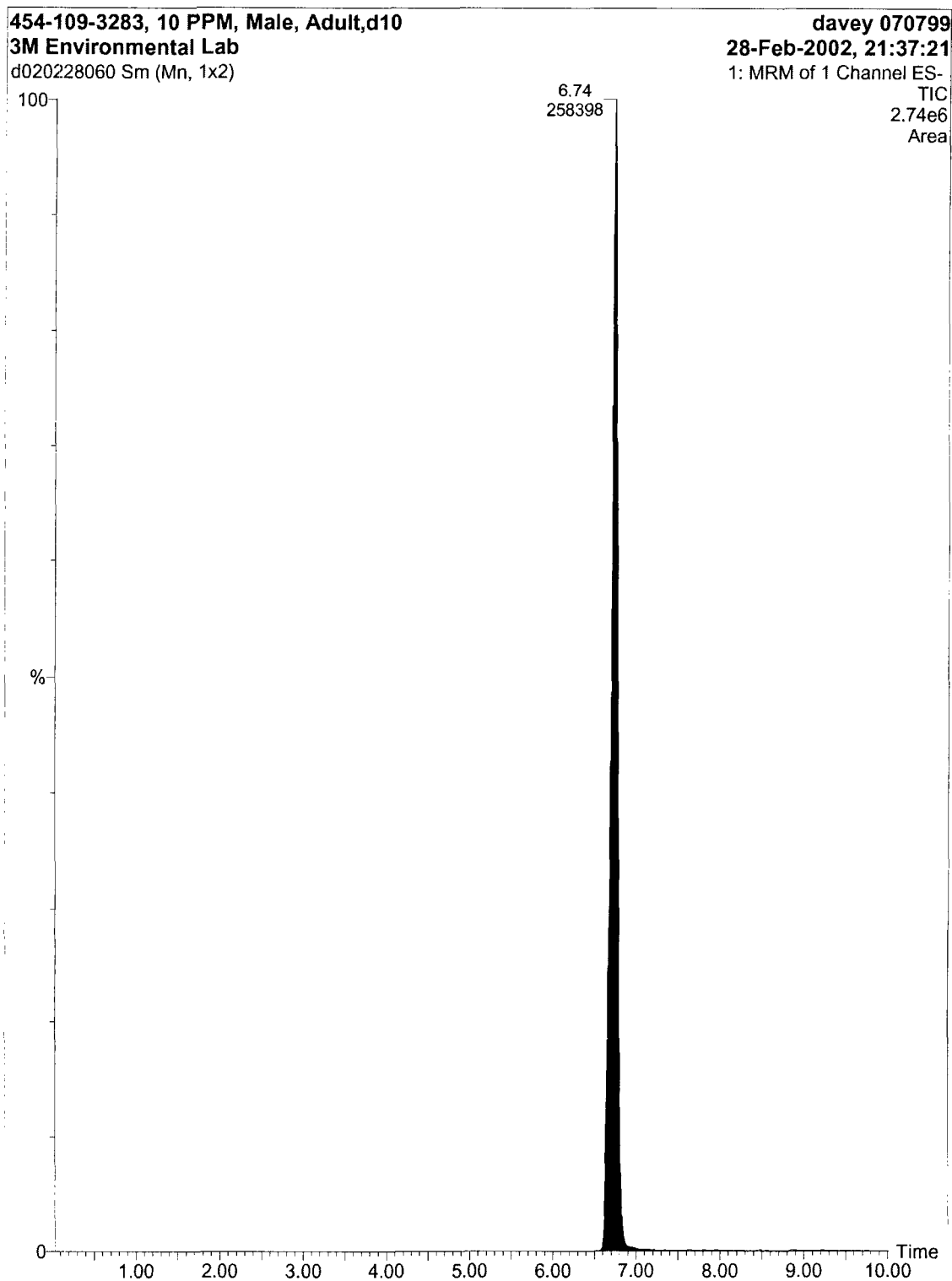






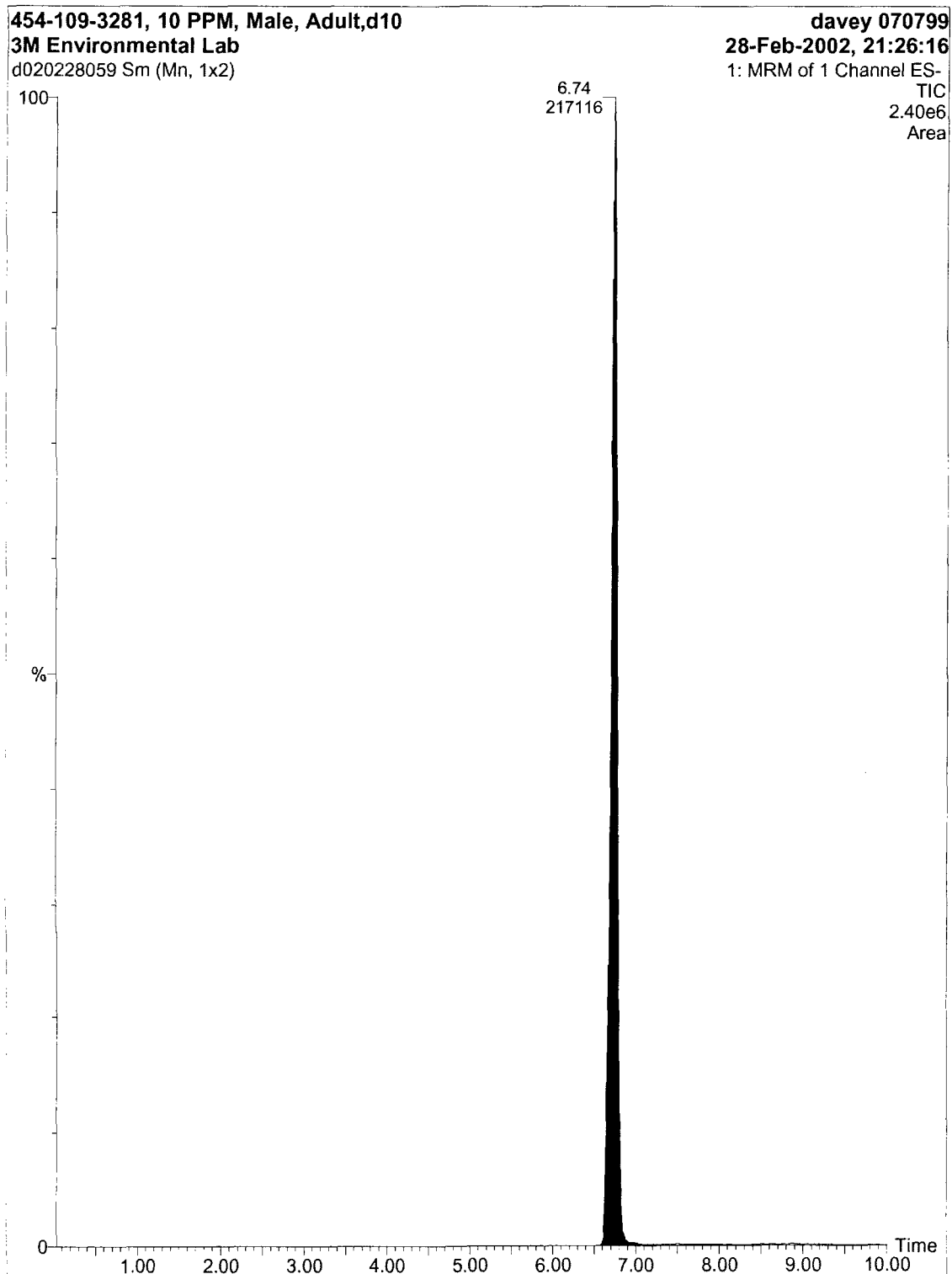
Dosed Group Adult Male Sera Samples, Diluted 1:10

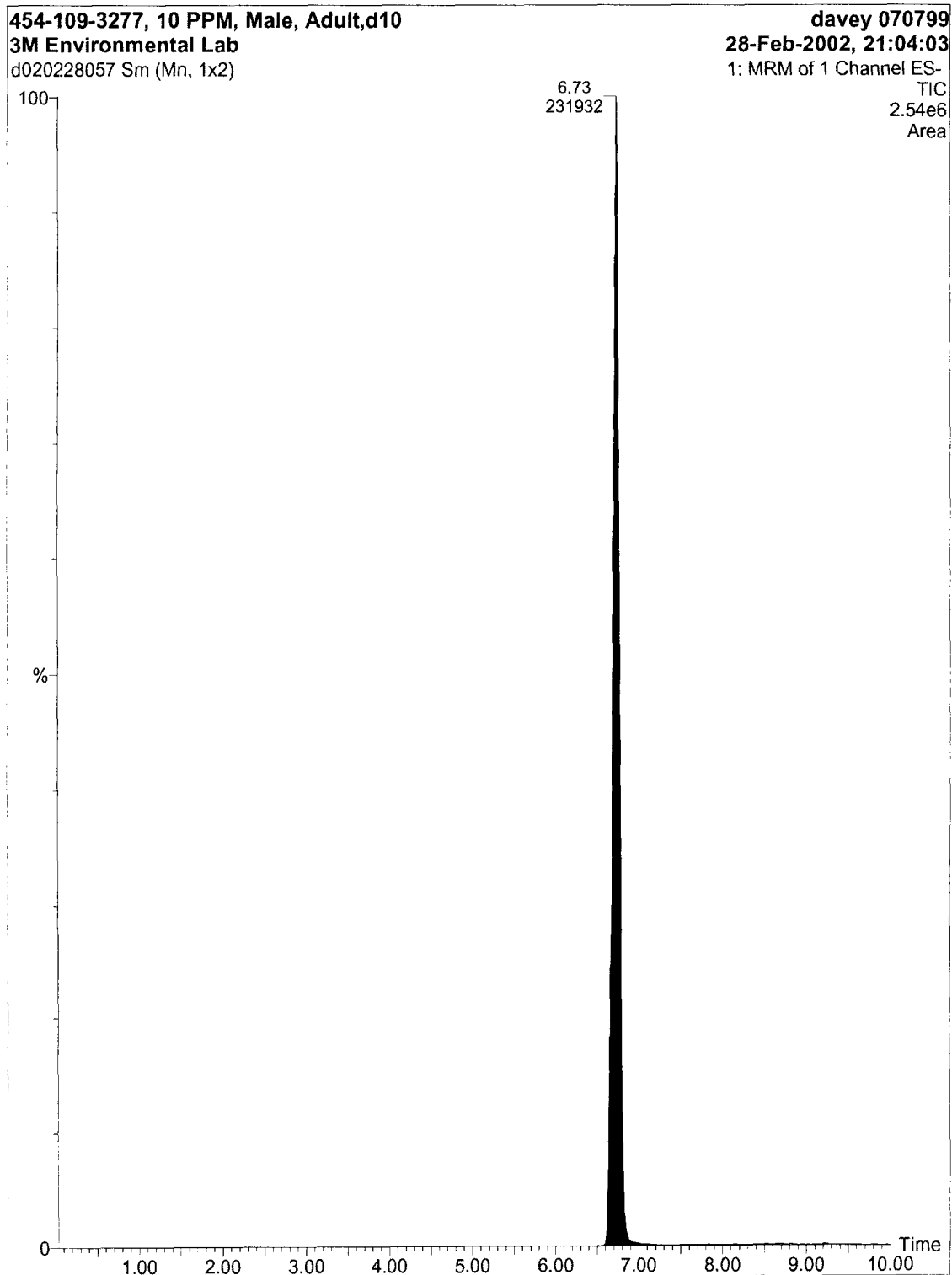


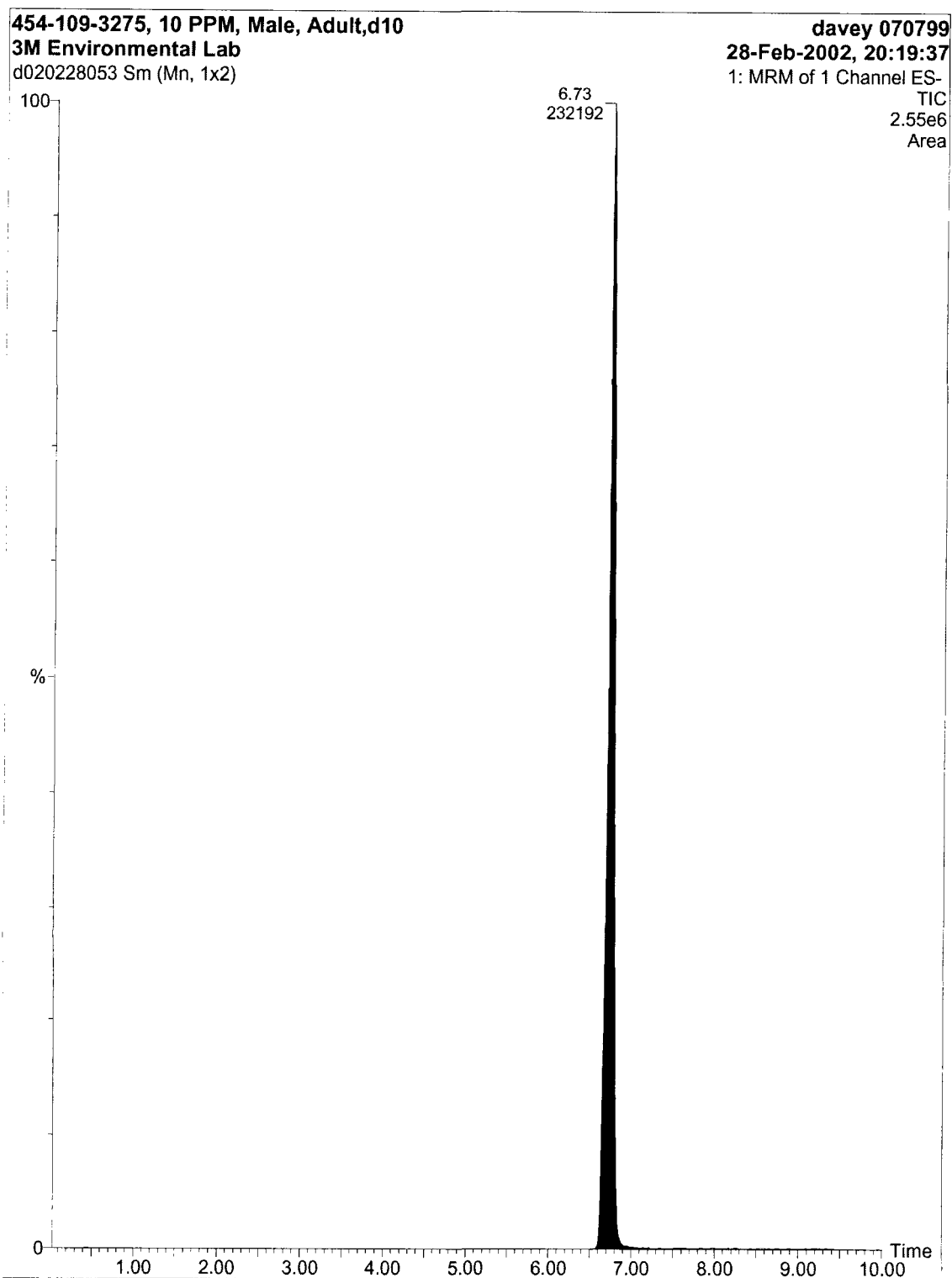


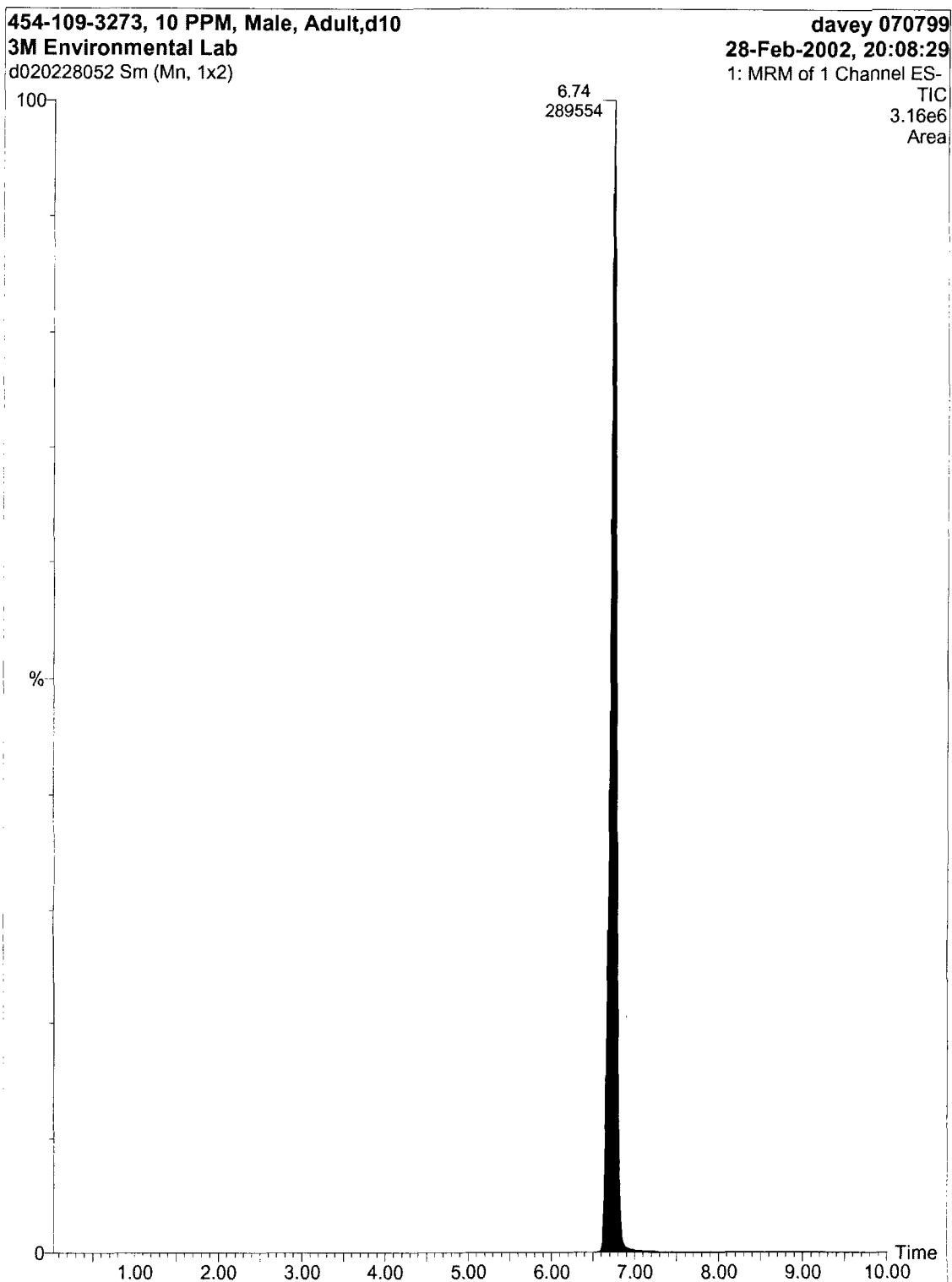
Report E01-1256

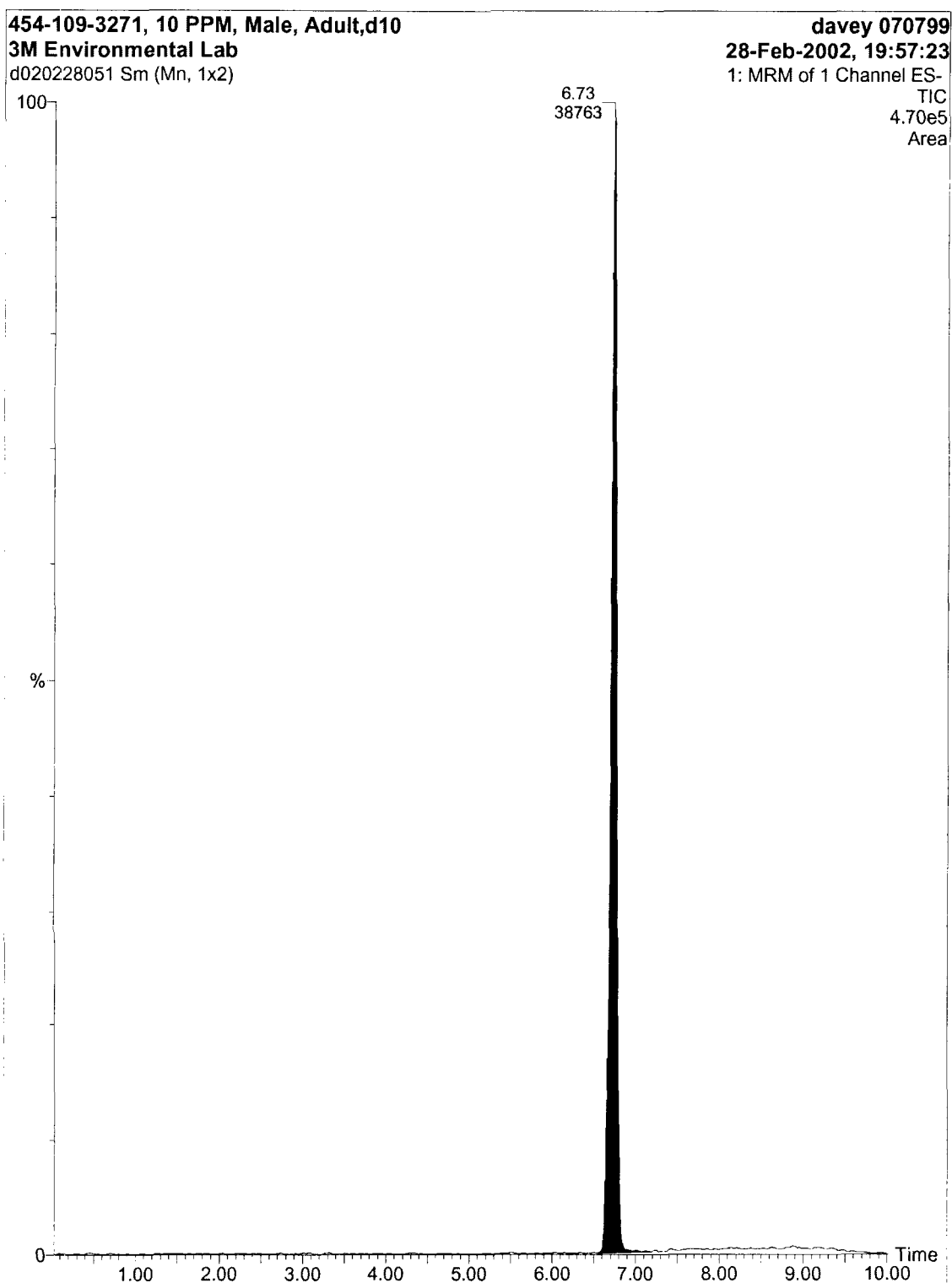
PFOS: A Reproduction Study with the Mallard

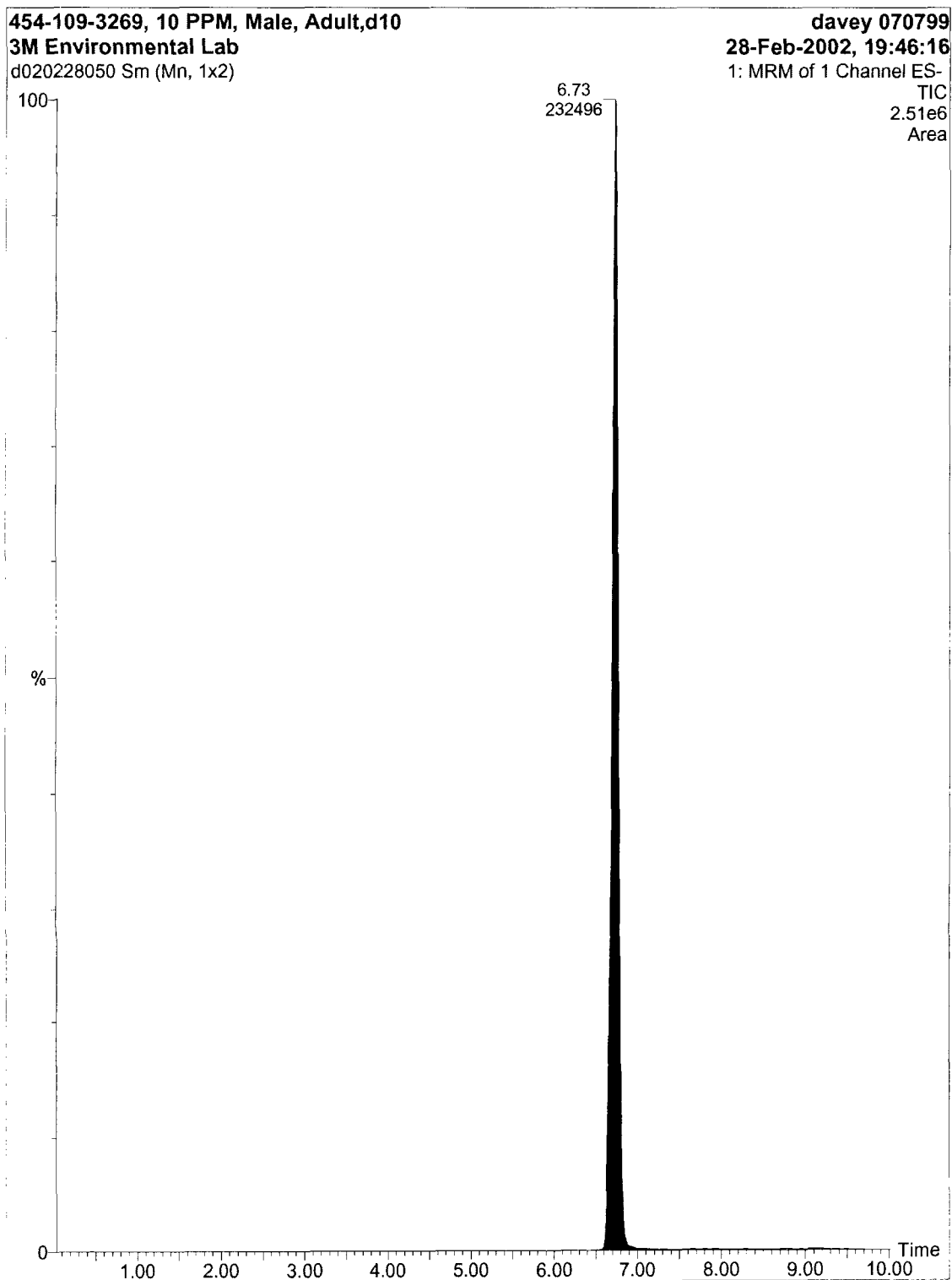


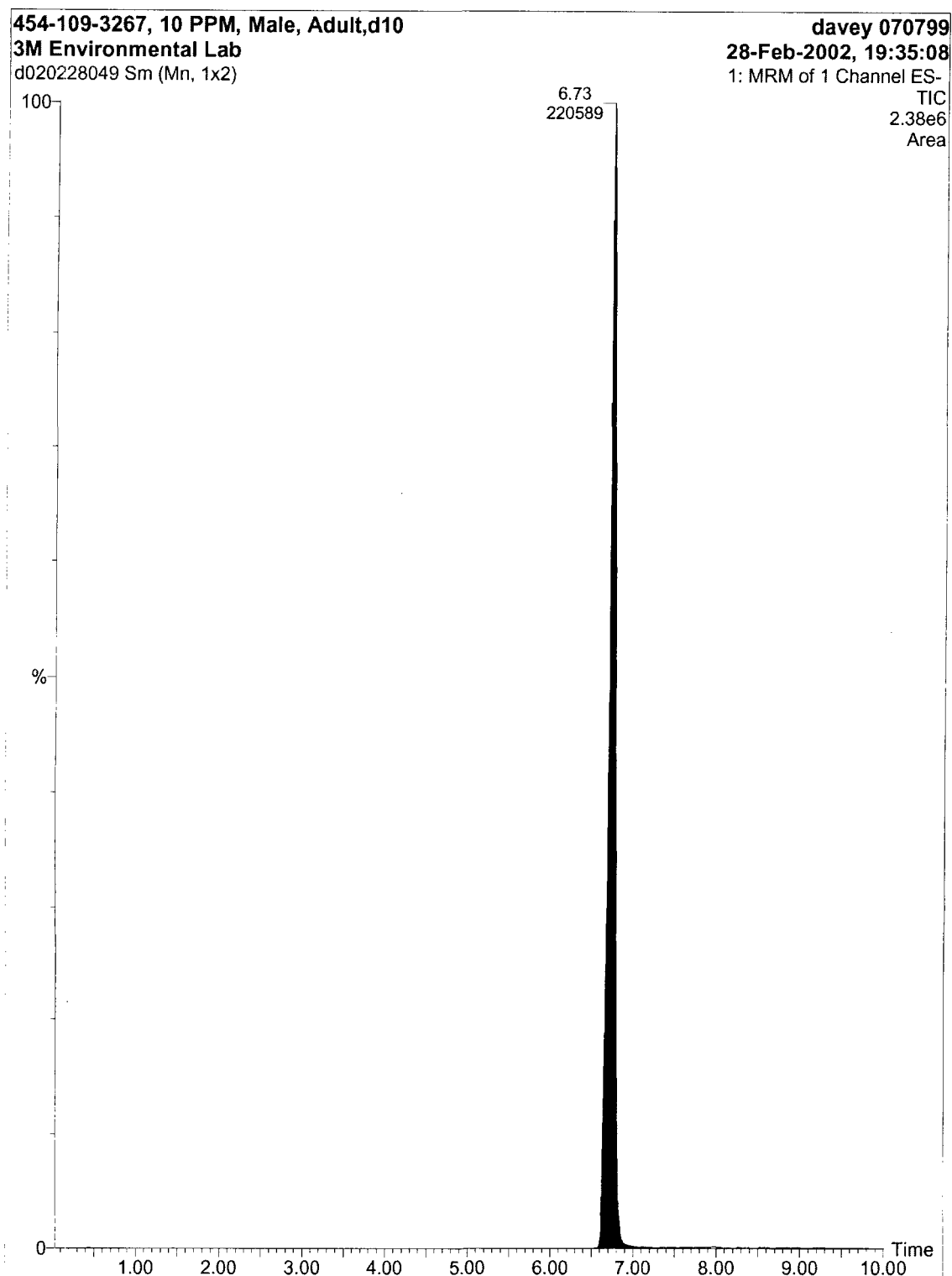






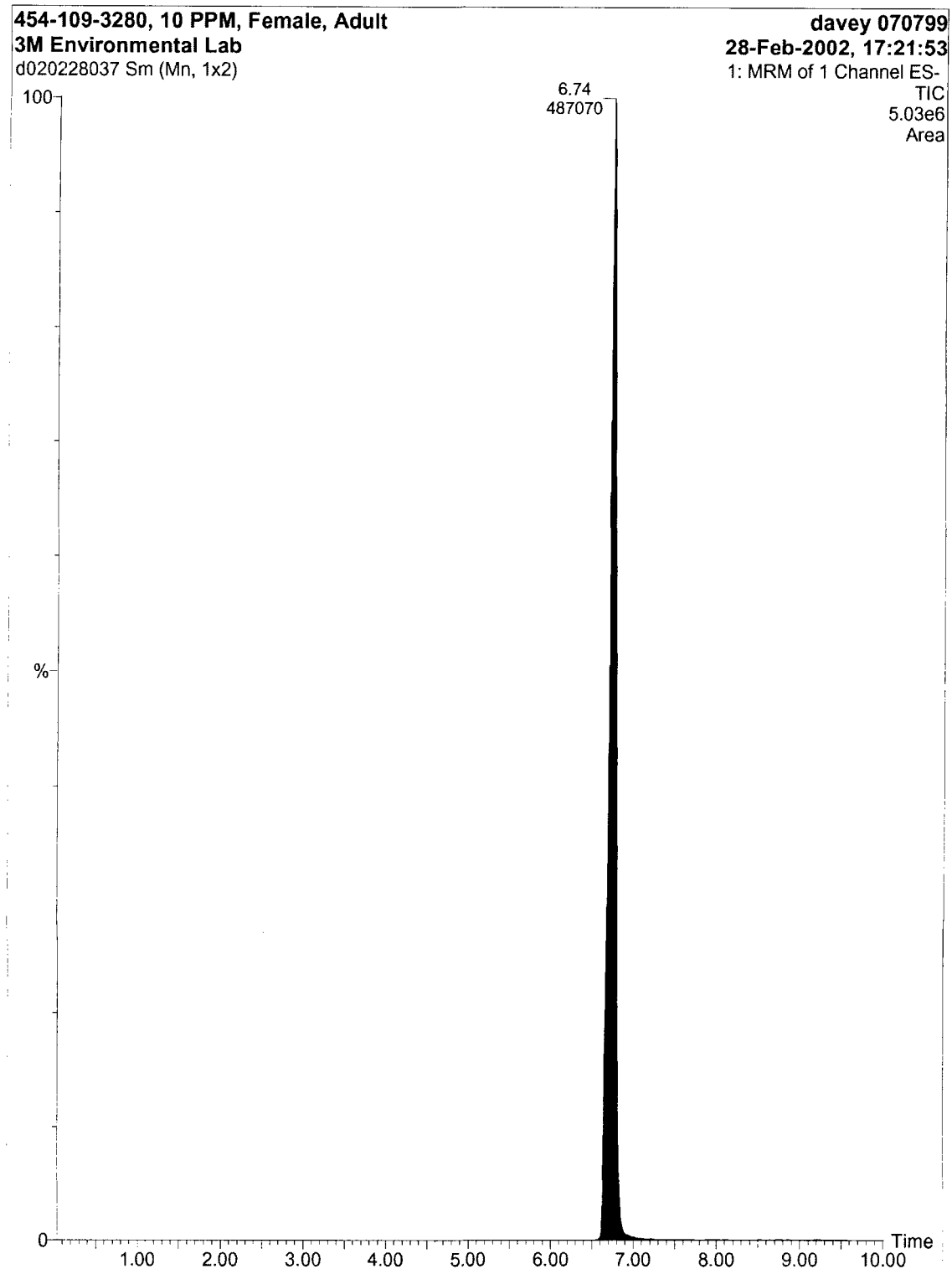


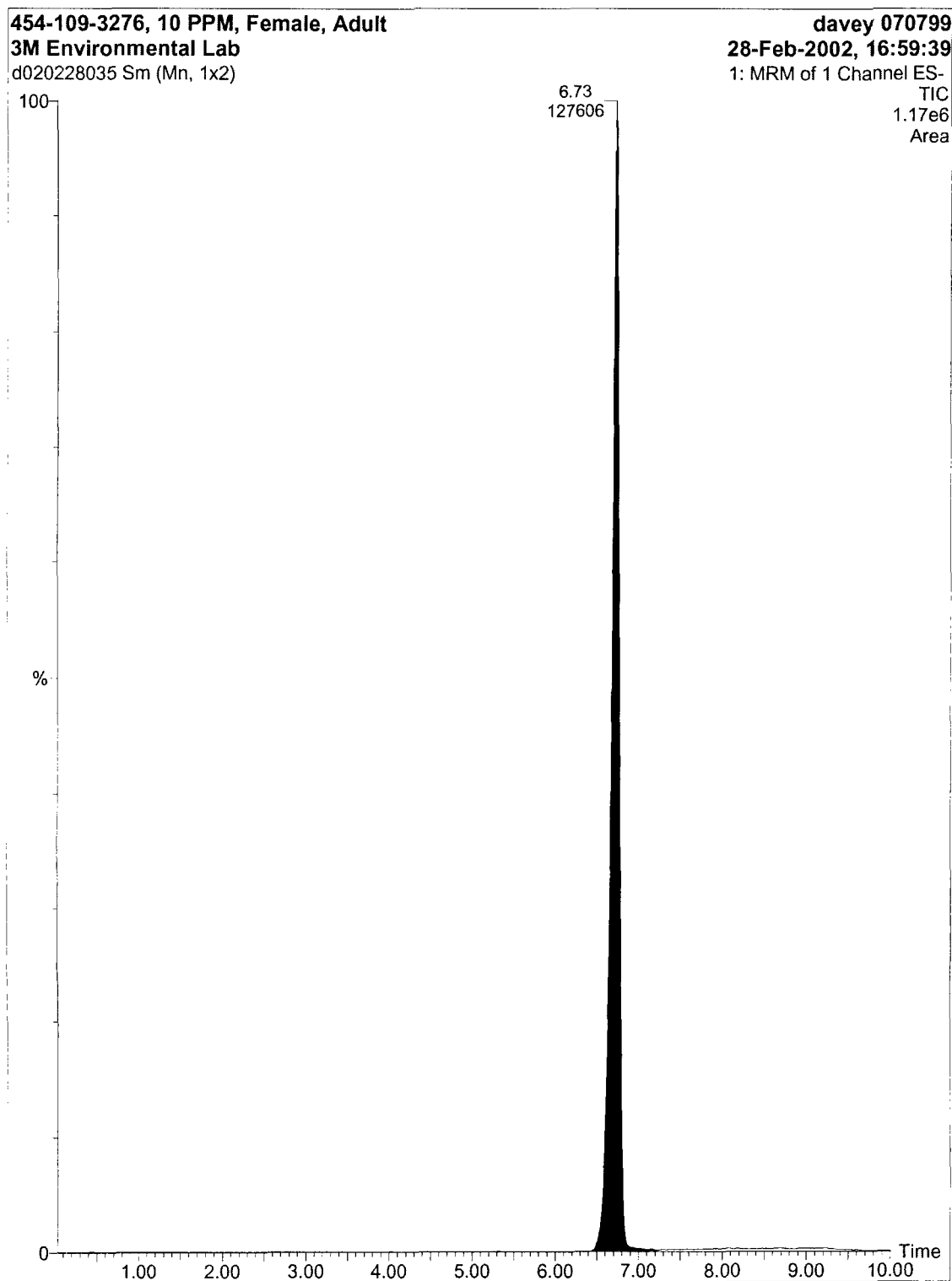


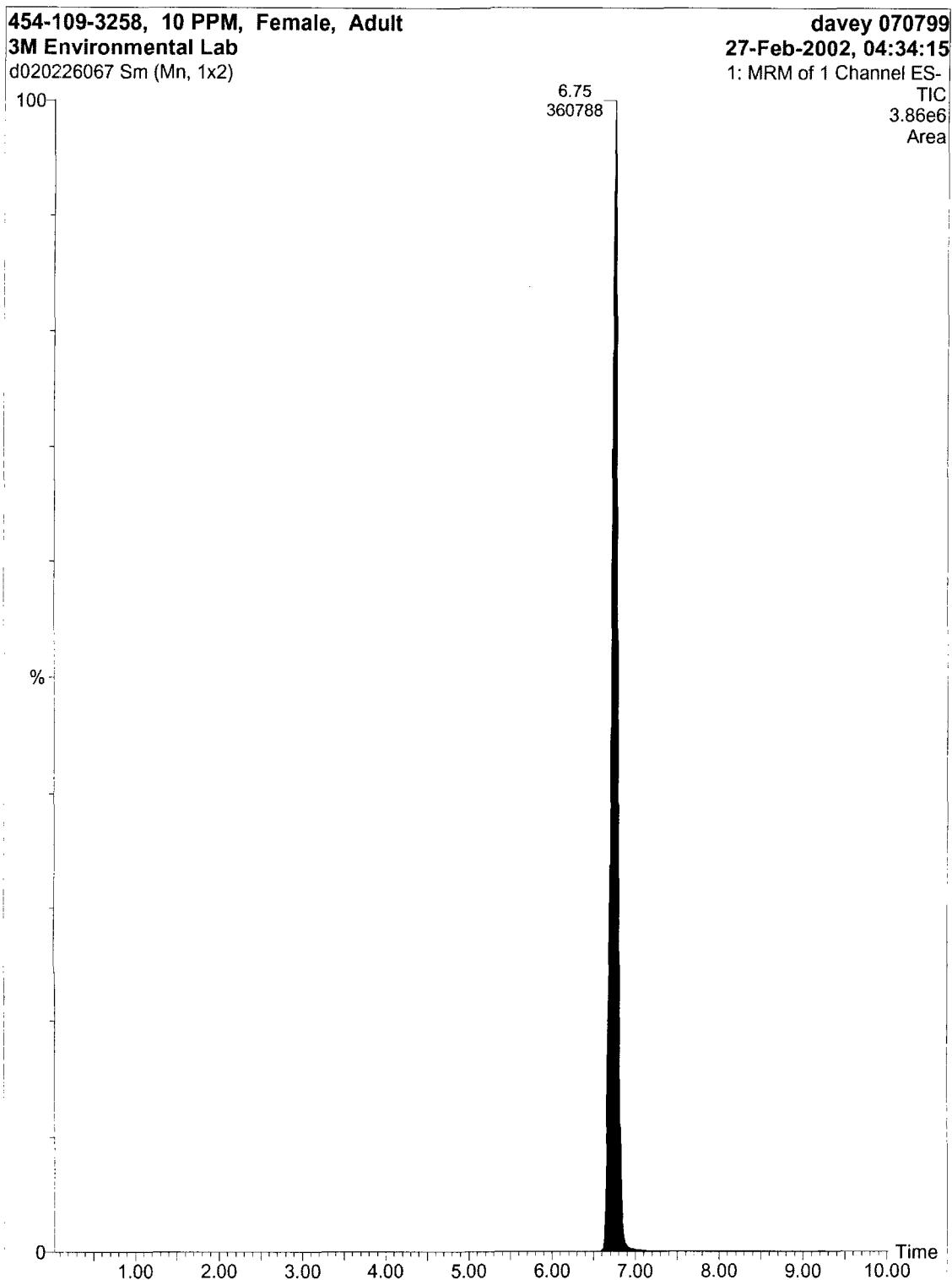


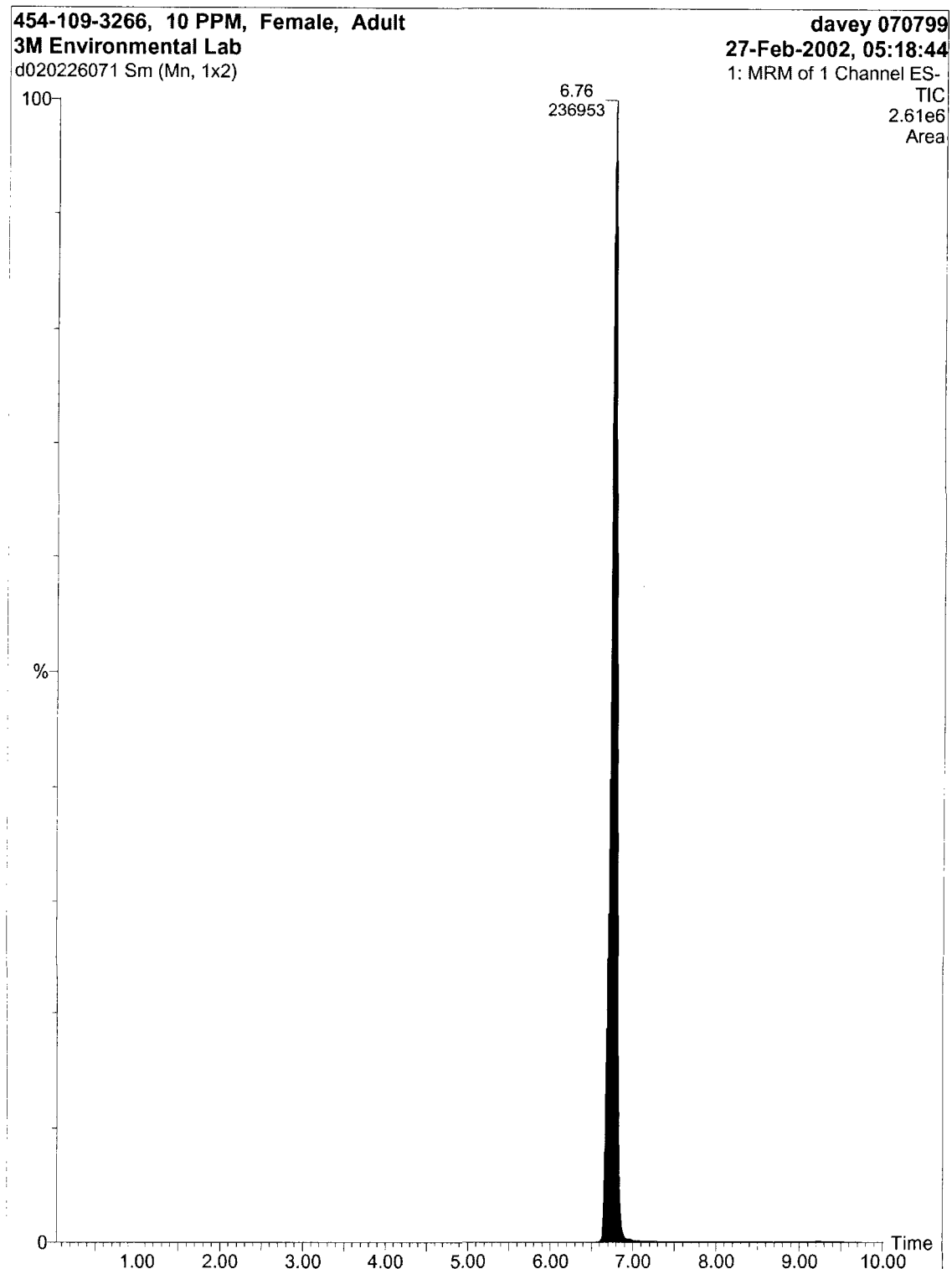
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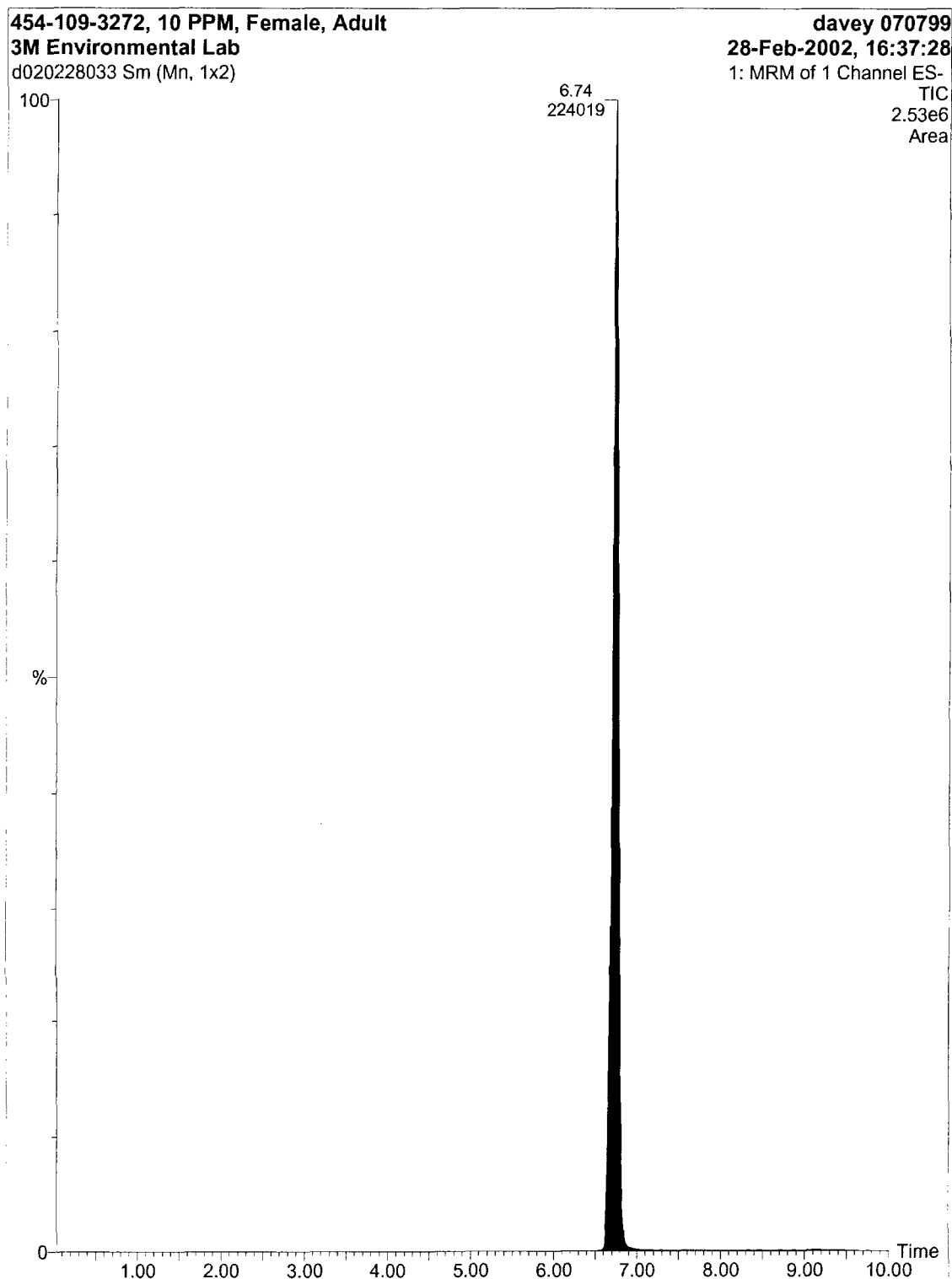
PFOS: A Reproduction Study with the Mallard

Dosed Group Adult Female Sera Samples



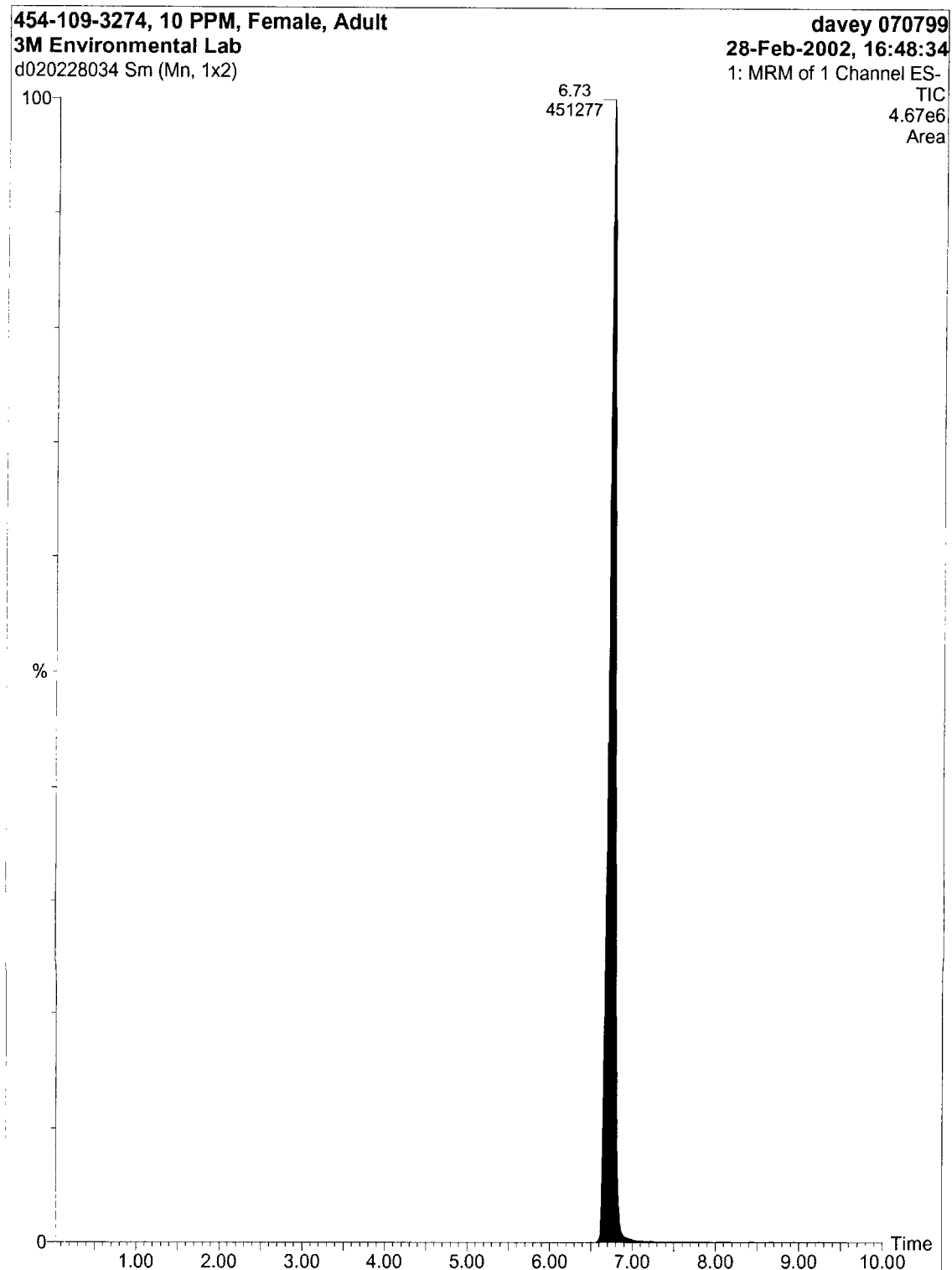






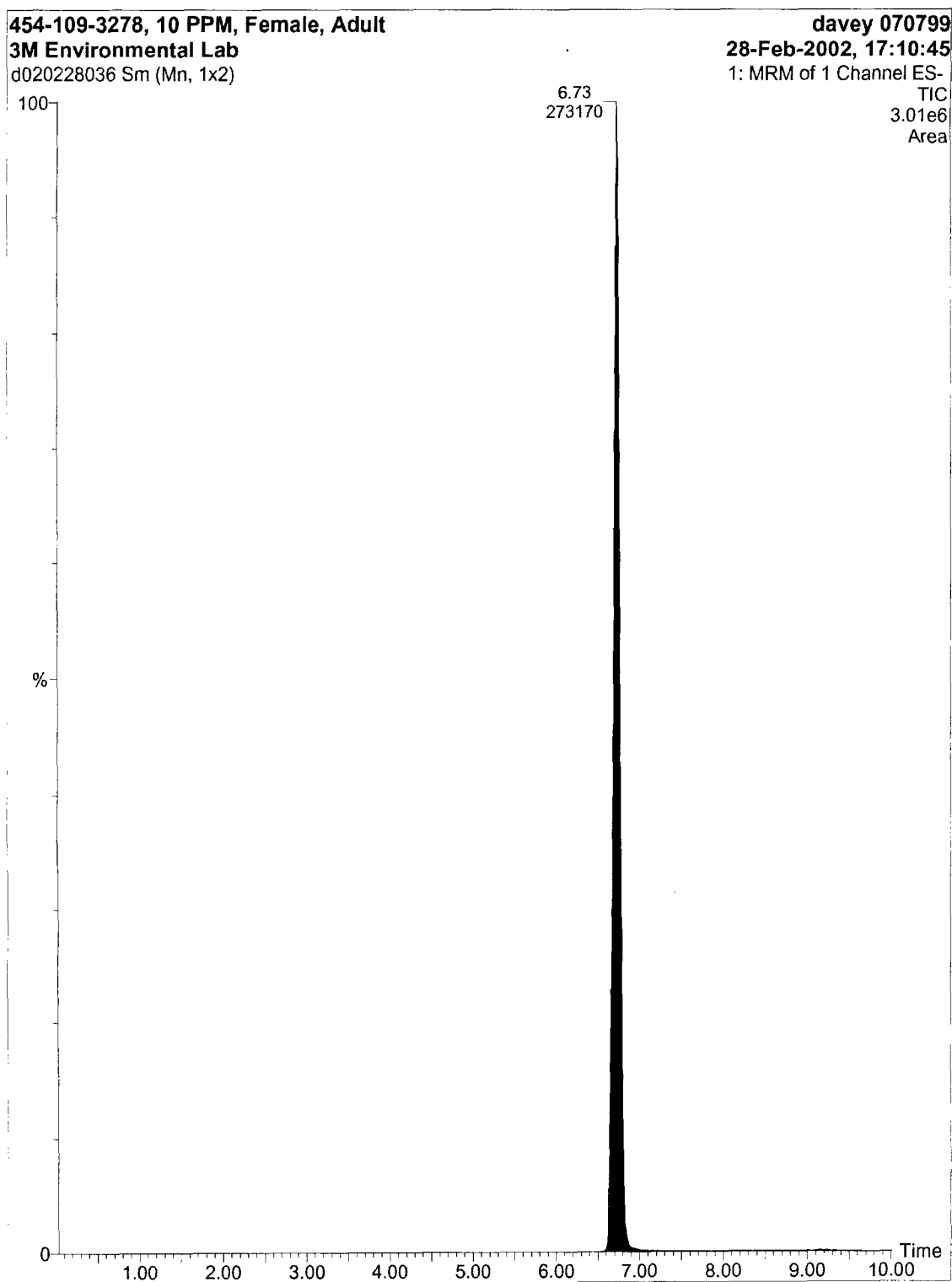
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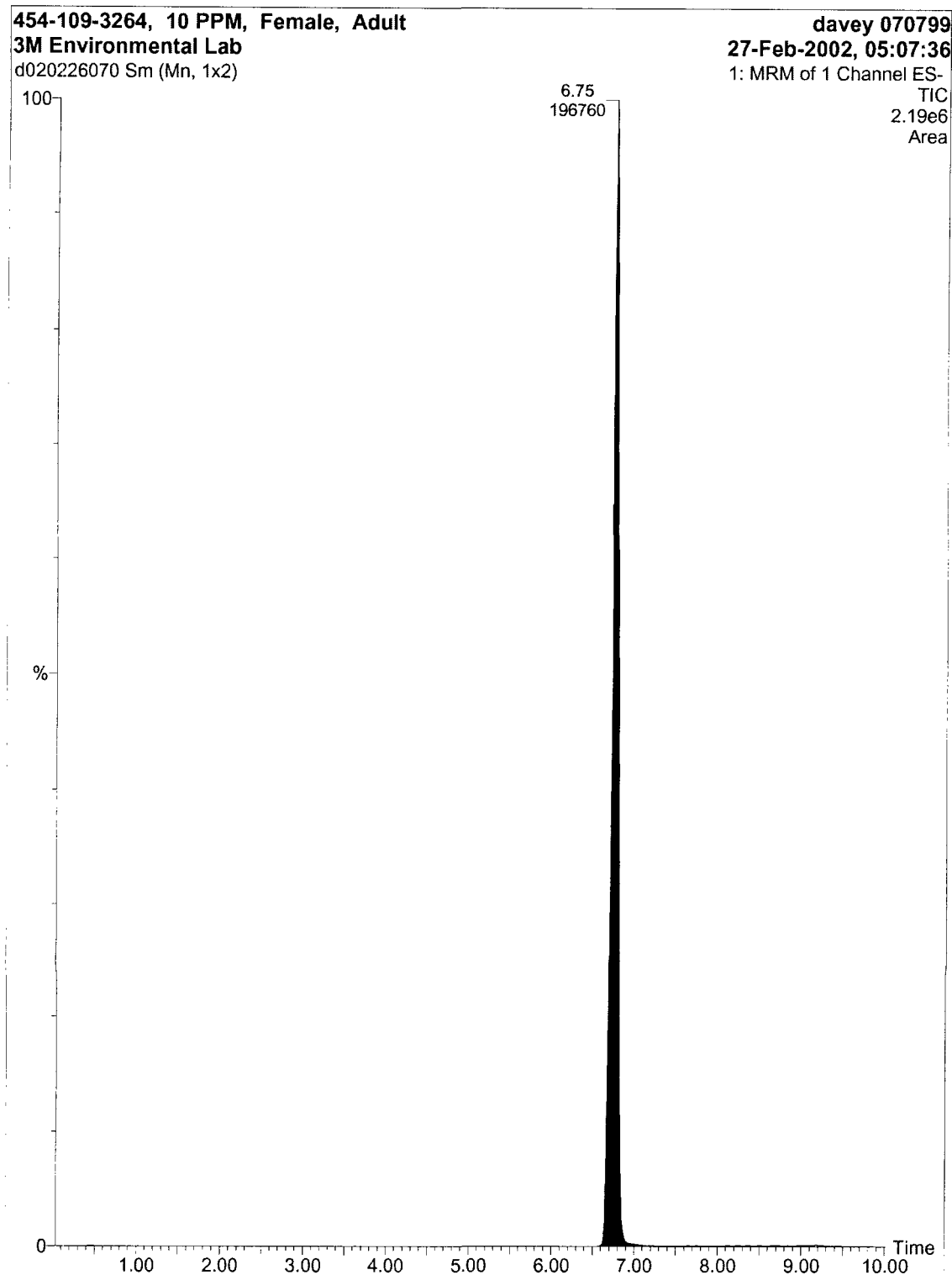
PFOS: A Reproduction Study with the Mallard

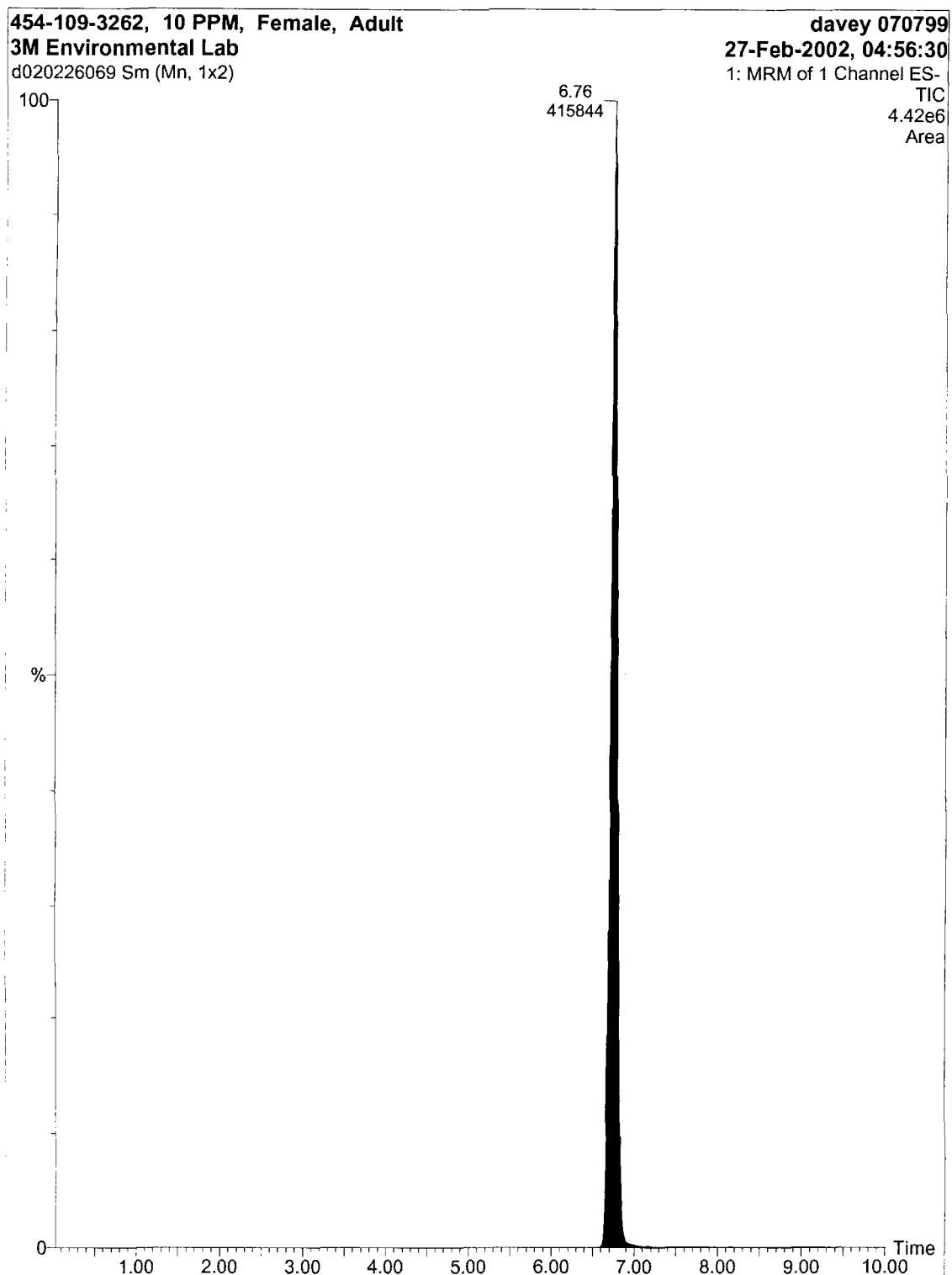


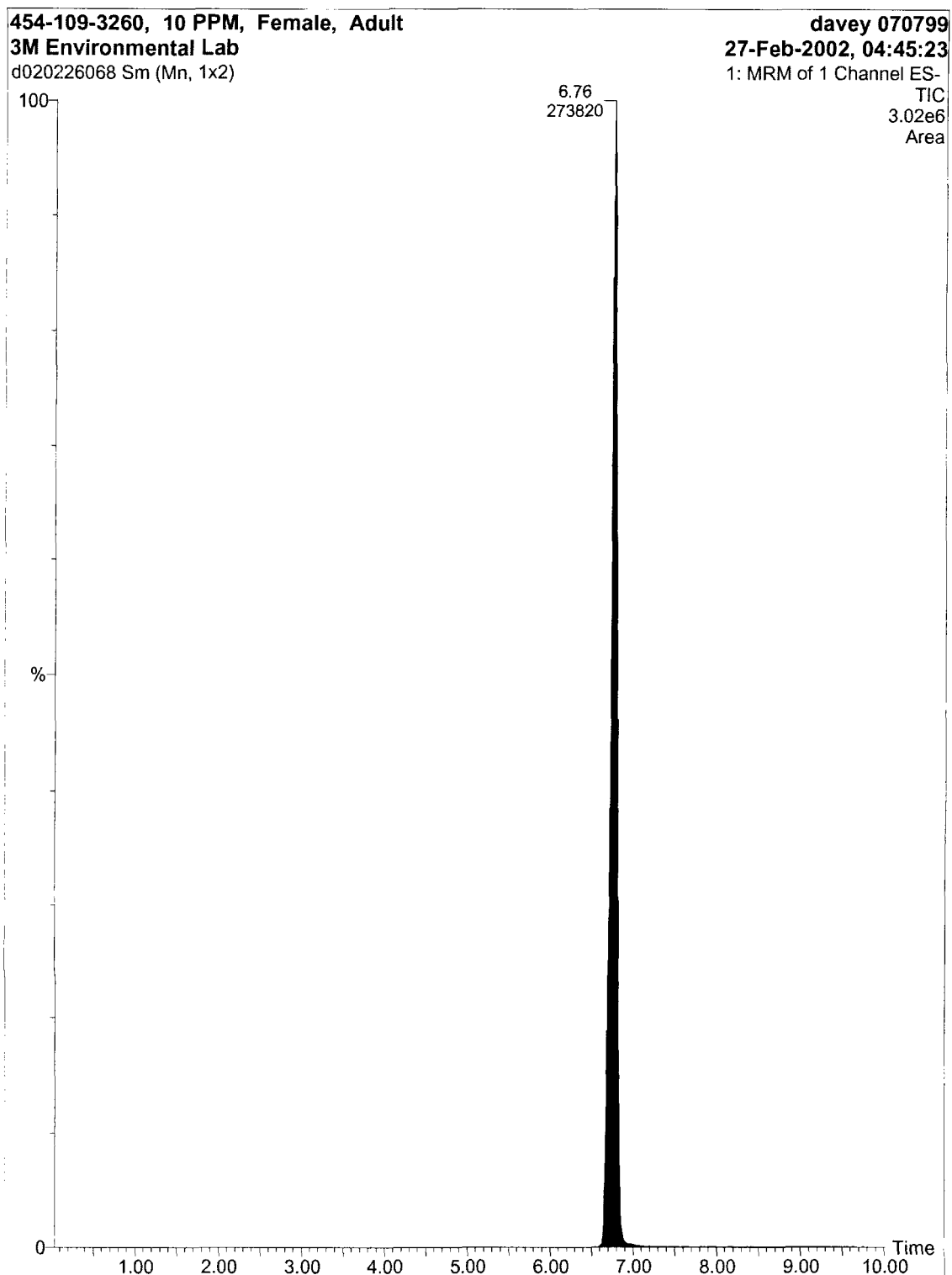
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PFOS: A Reproduction Study with the Mallard

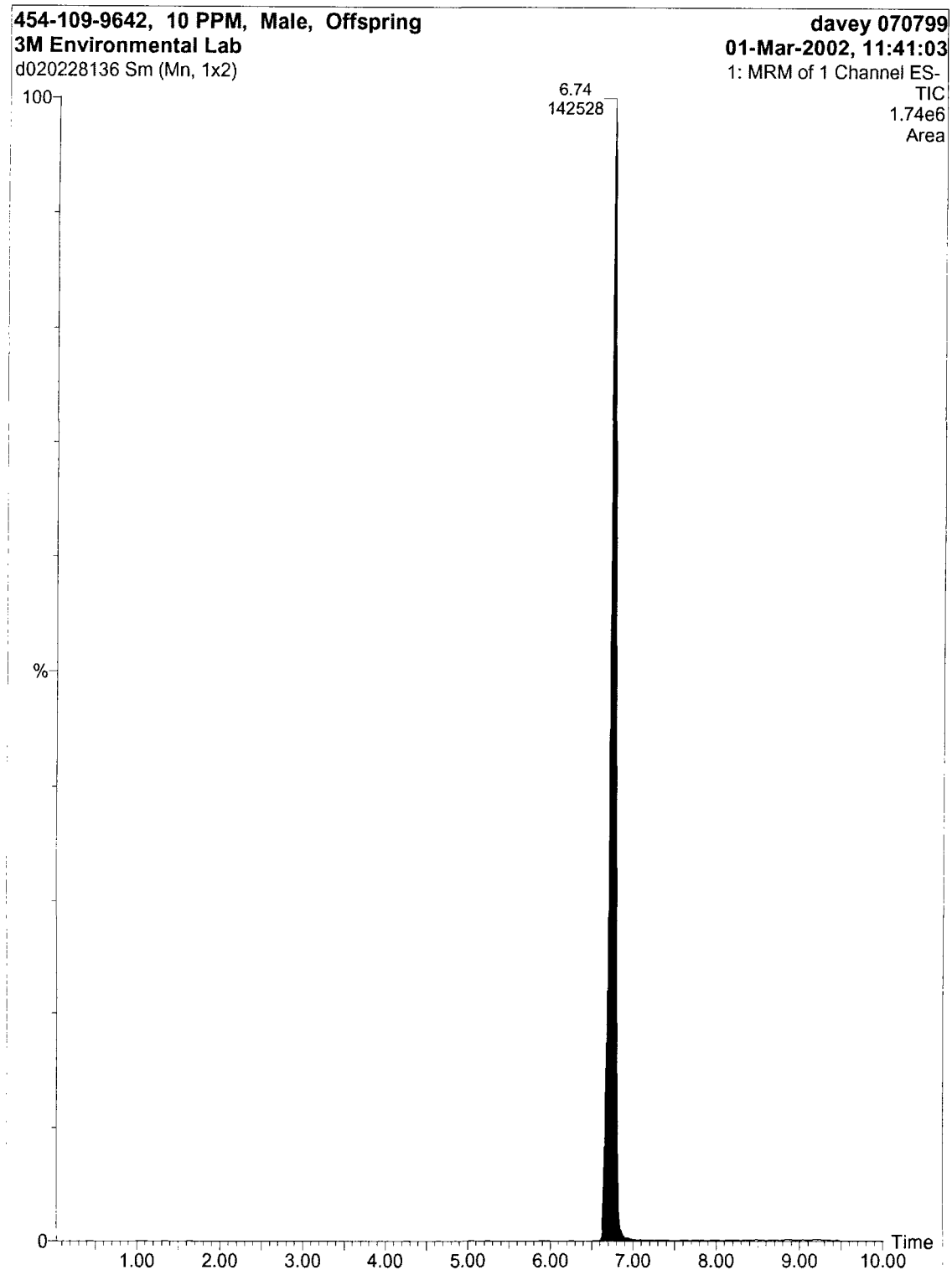






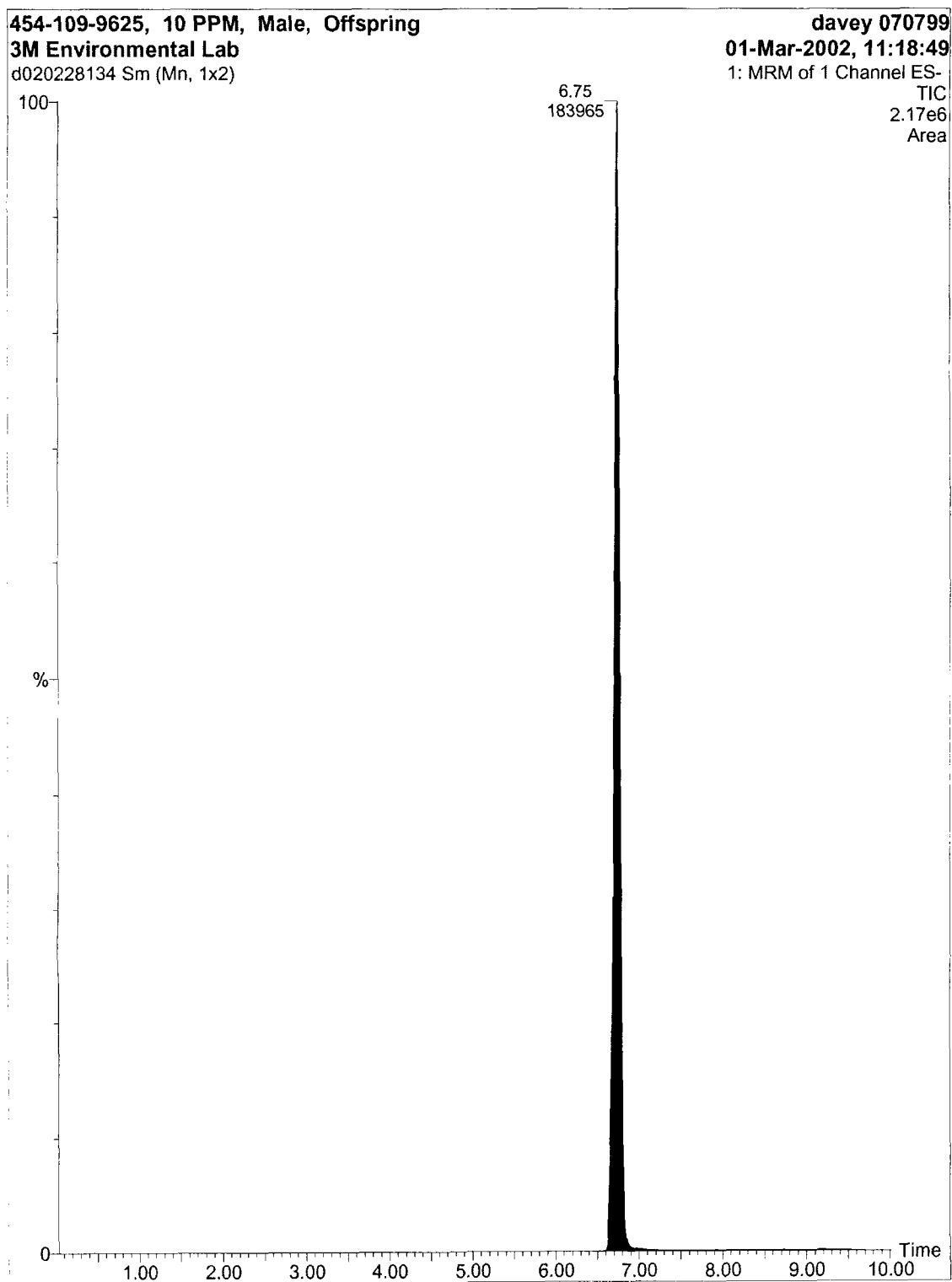


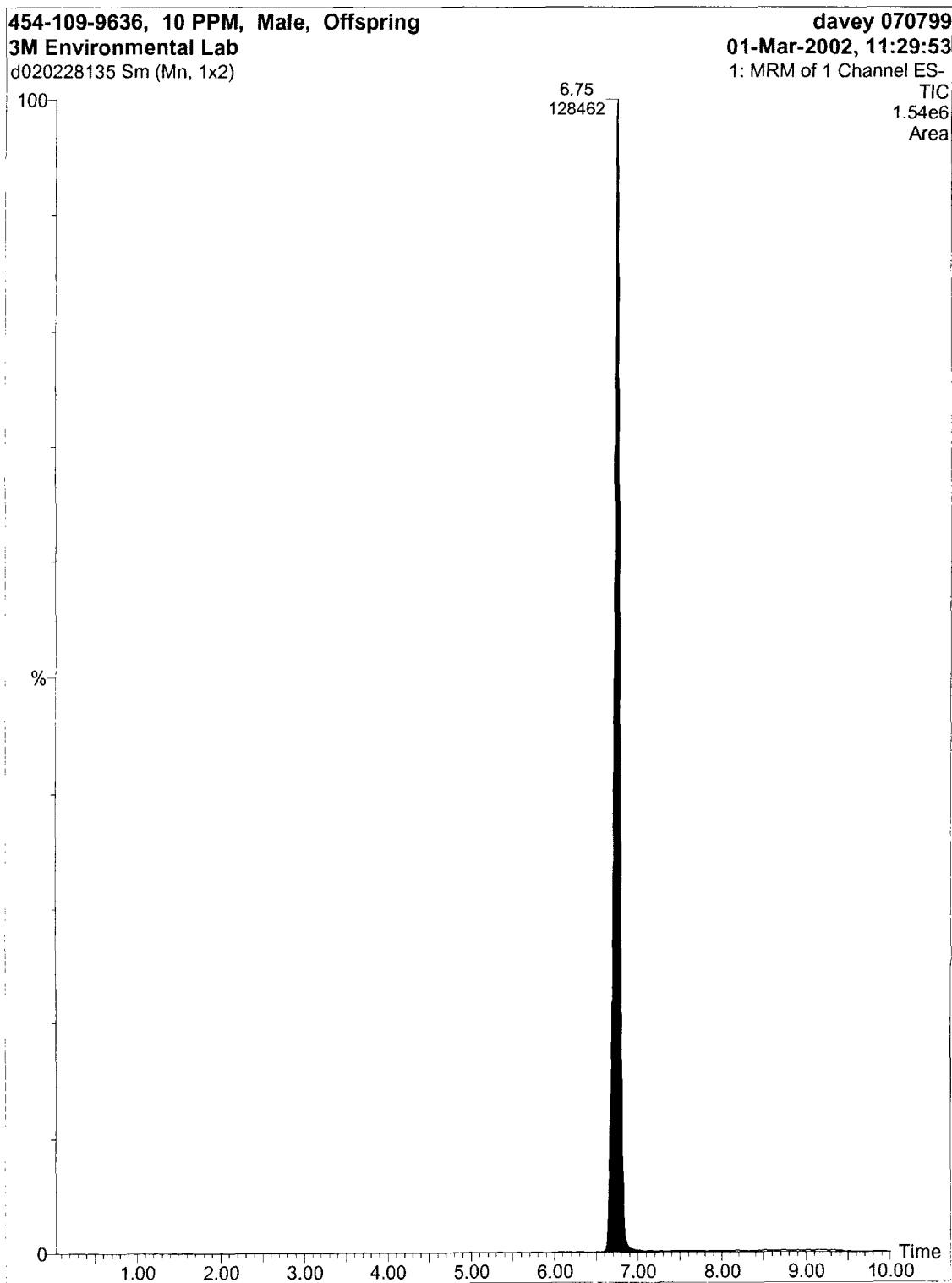
Dosed Group Male Offspring Sera Samples



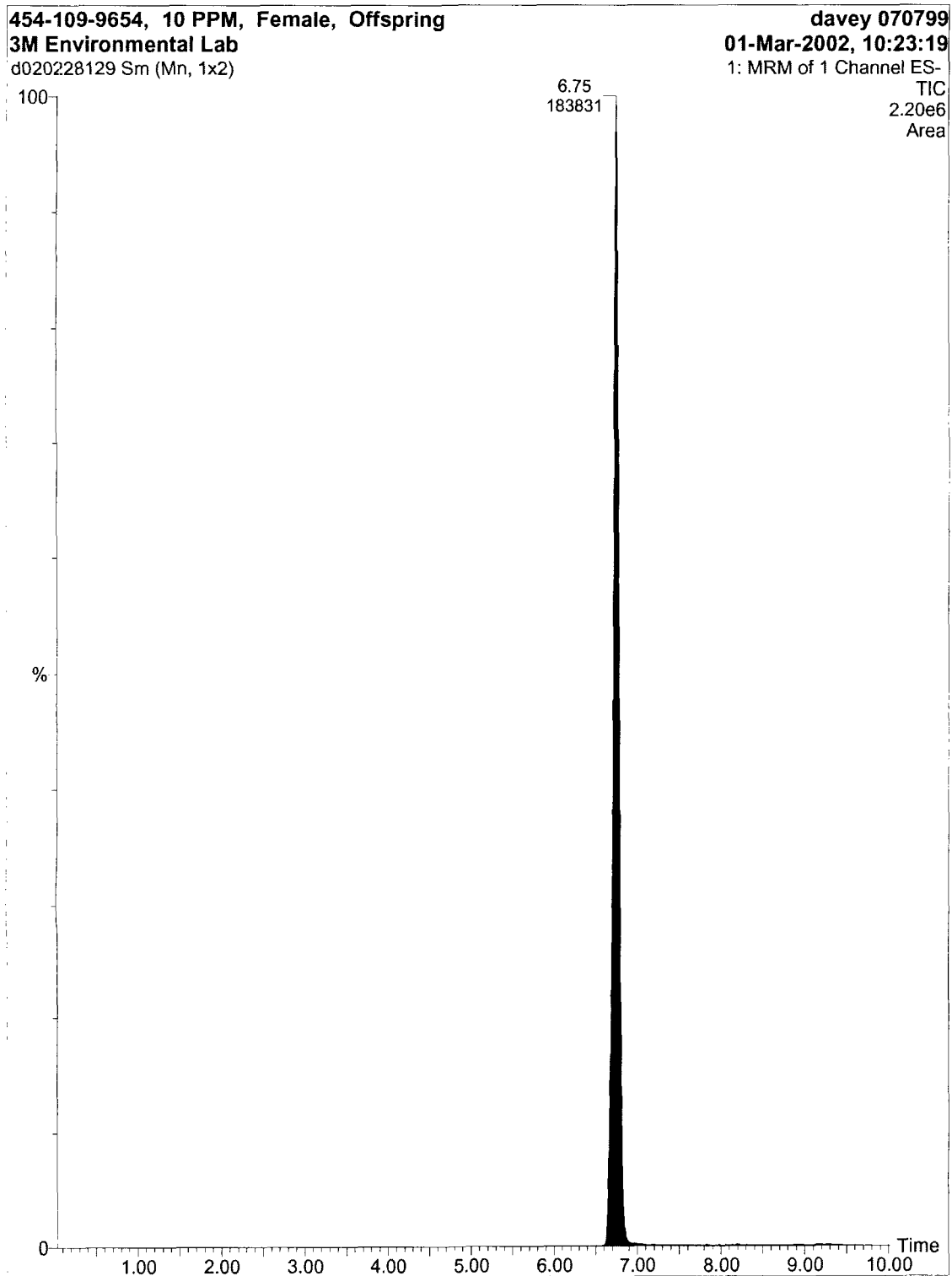
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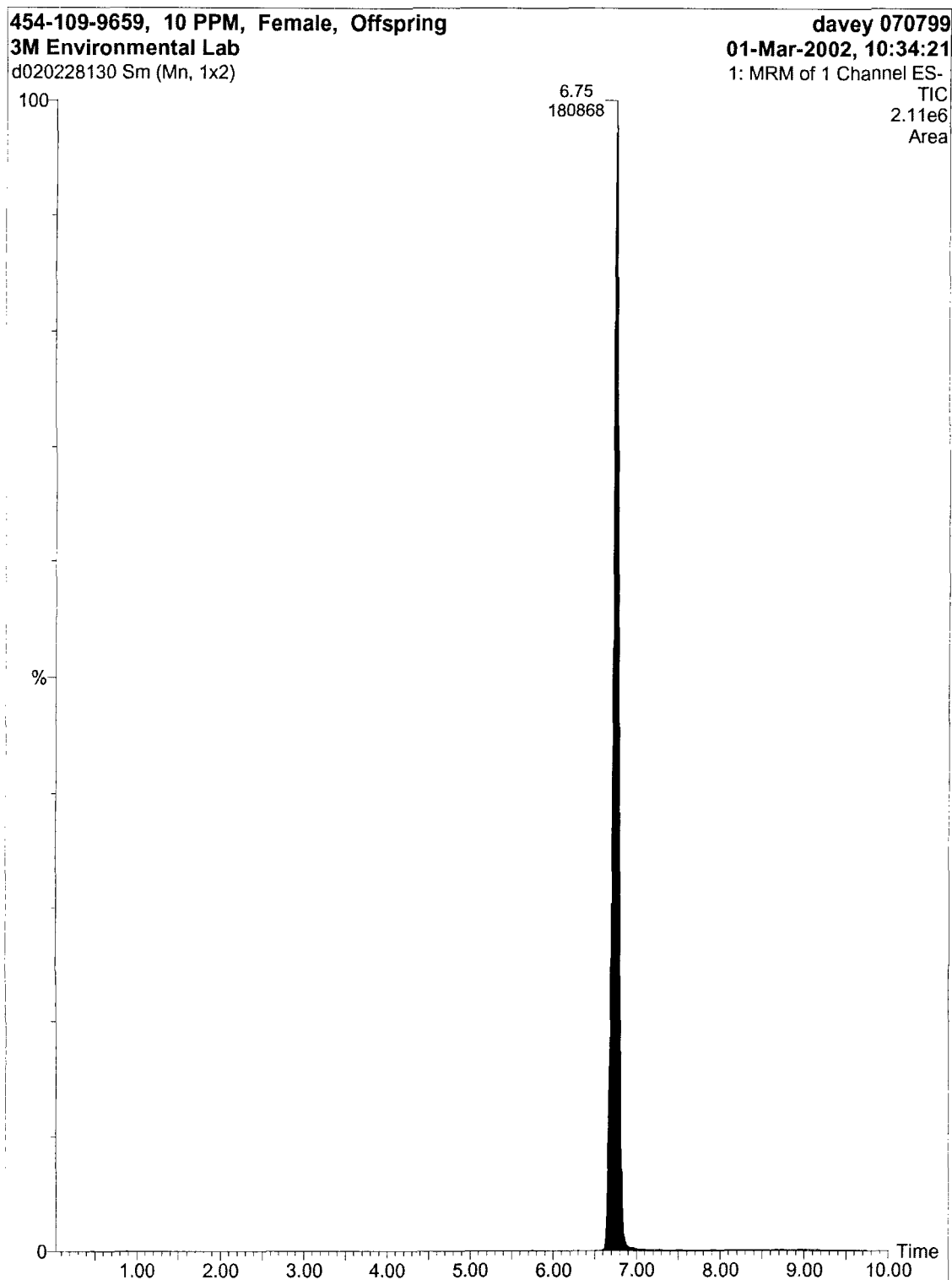
PFOS: A Reproduction Study with the Mallard

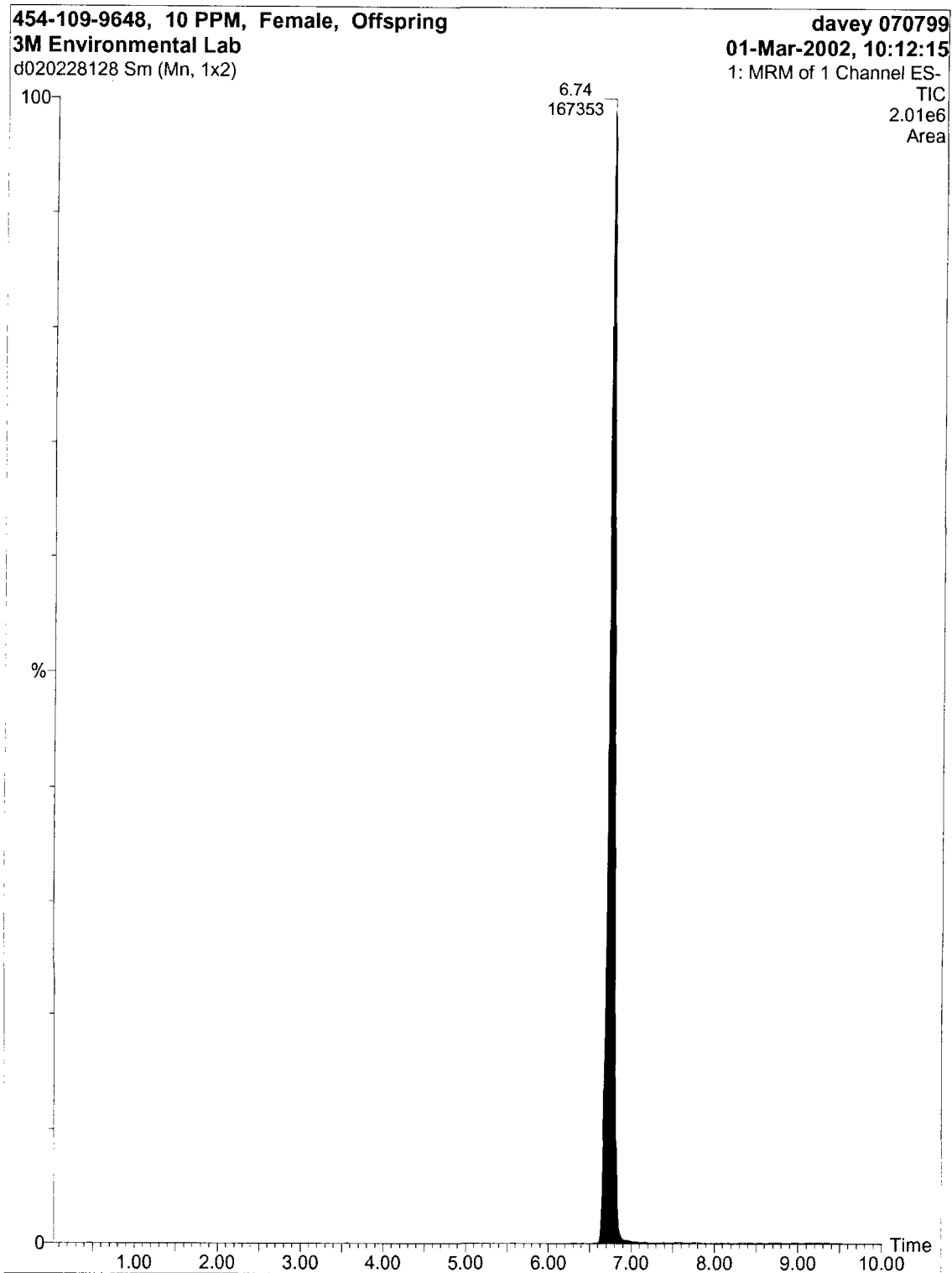




Dosed Group Female Offspring Sera Samples

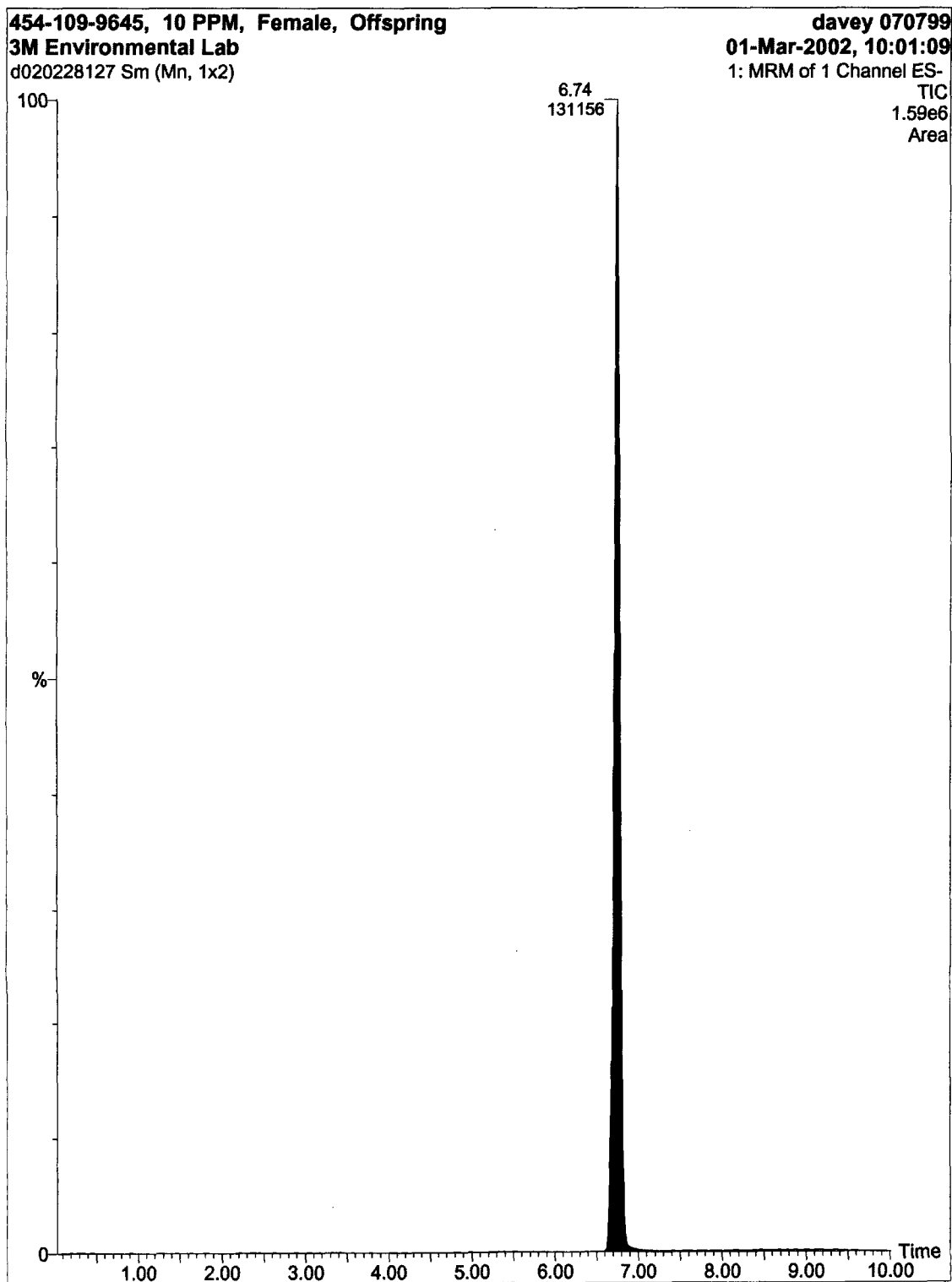


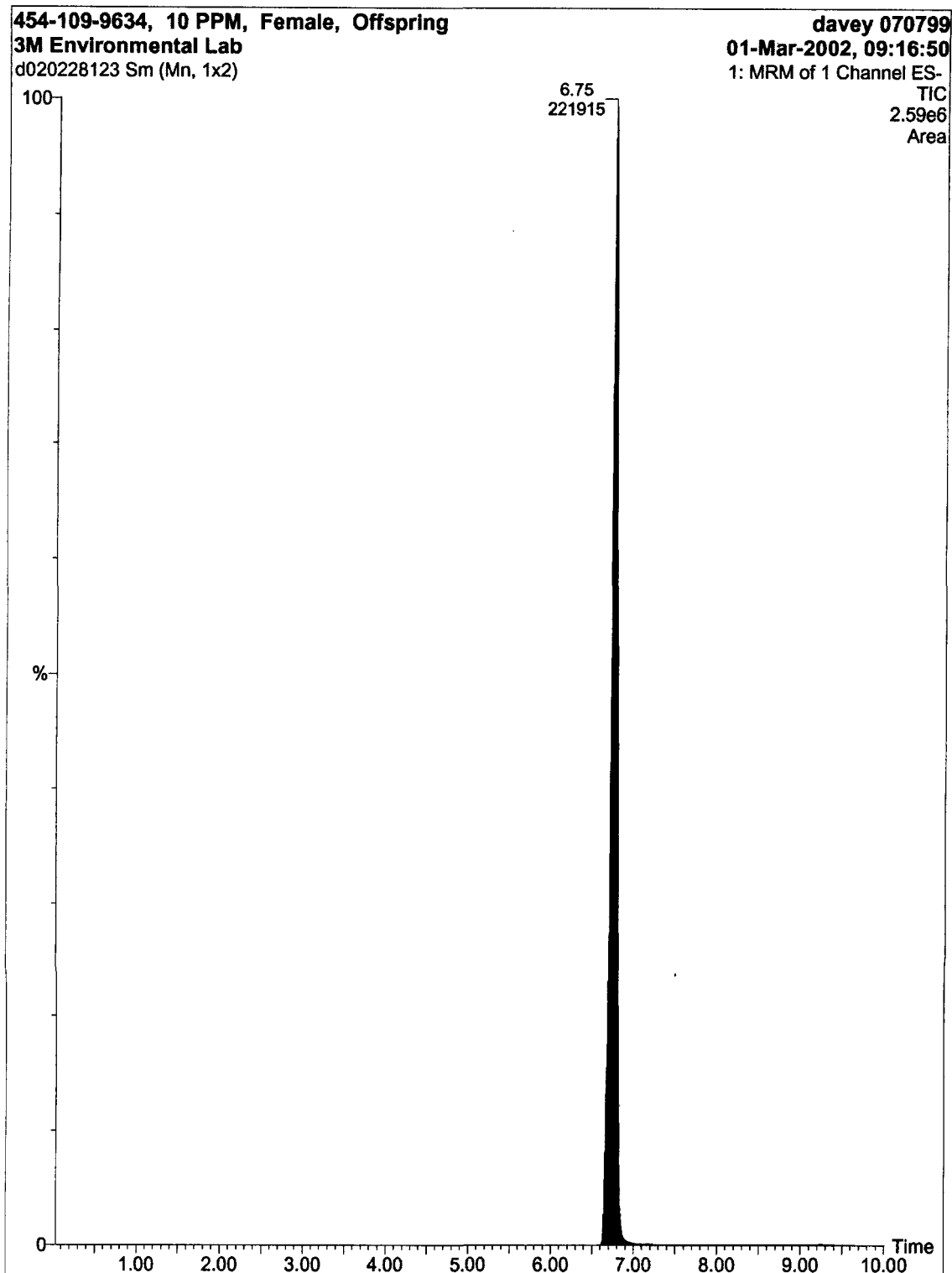




Report E01-1256

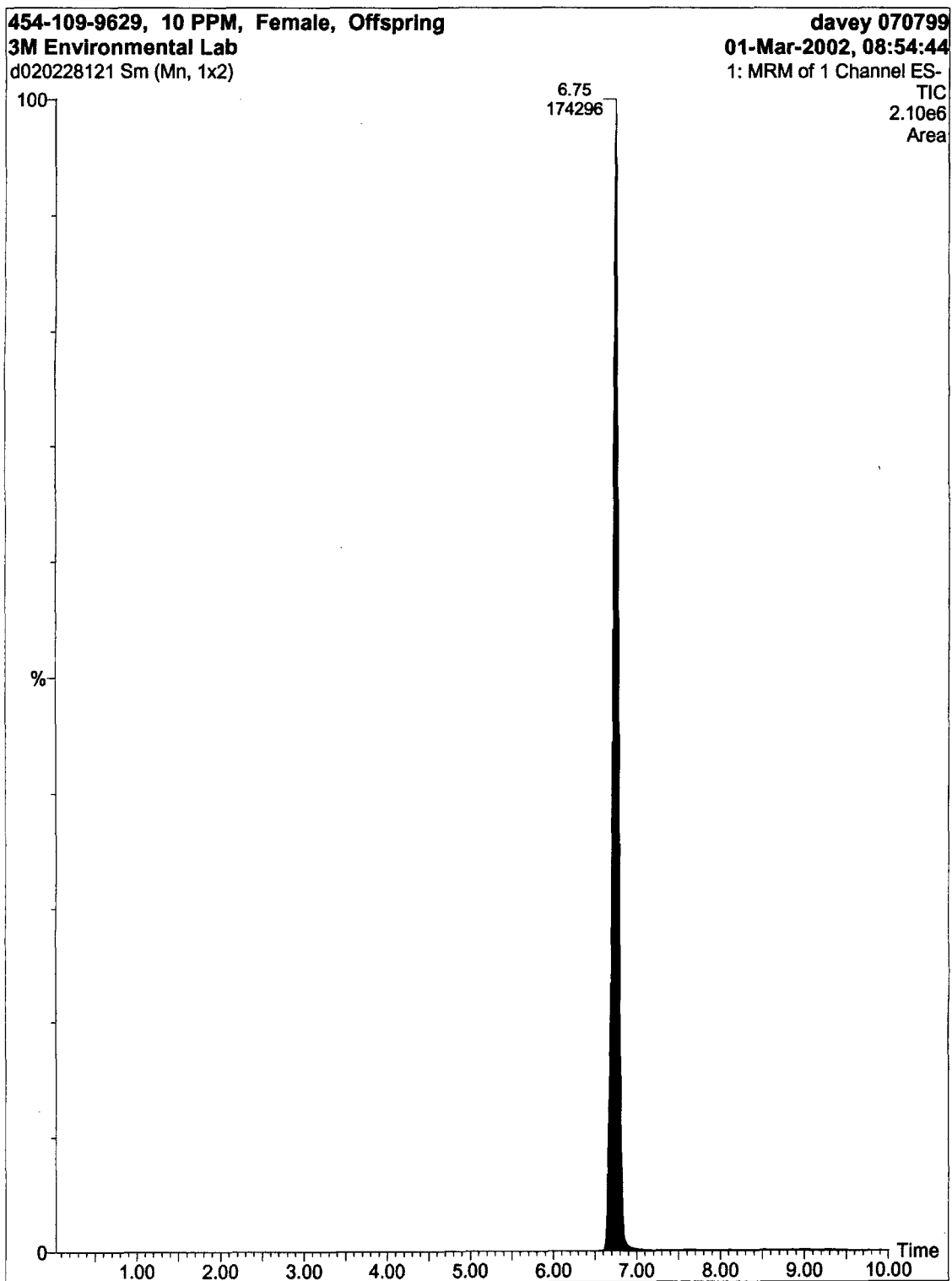
PFOS: A Reproduction Study with the Mallard





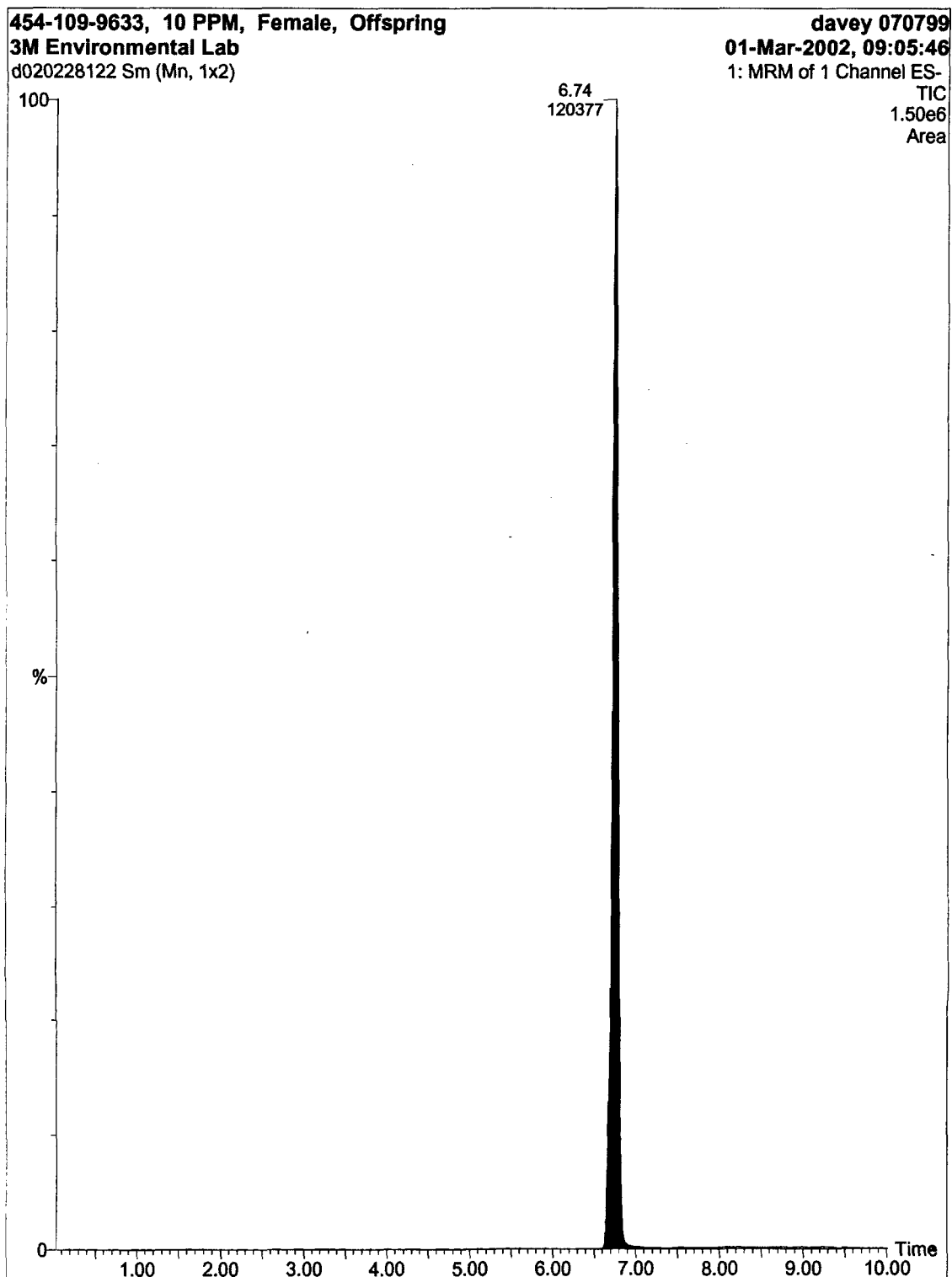
Report E01-1256

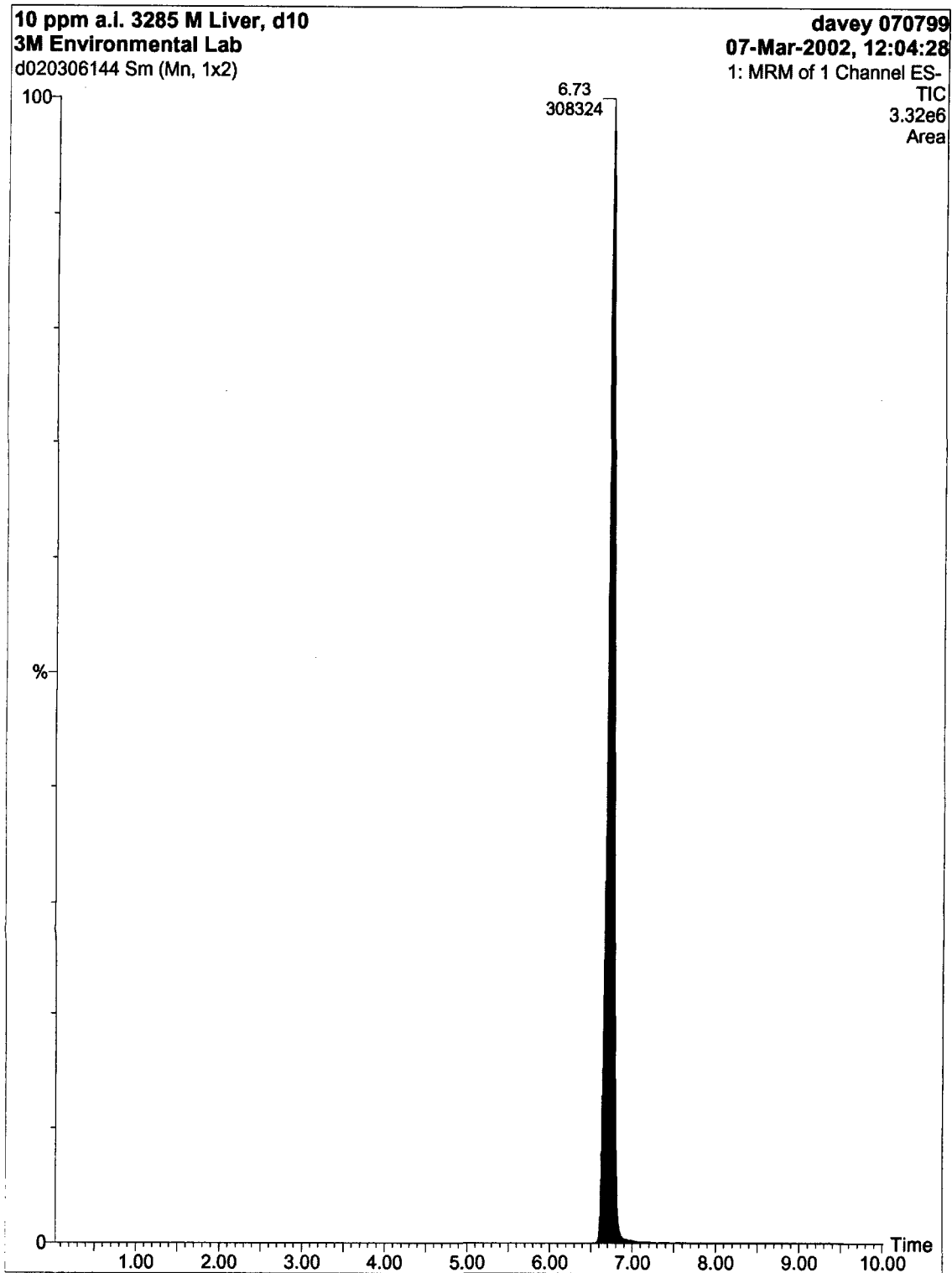
PFOS: A Reproduction Study with the Mallard



Report E01-1256

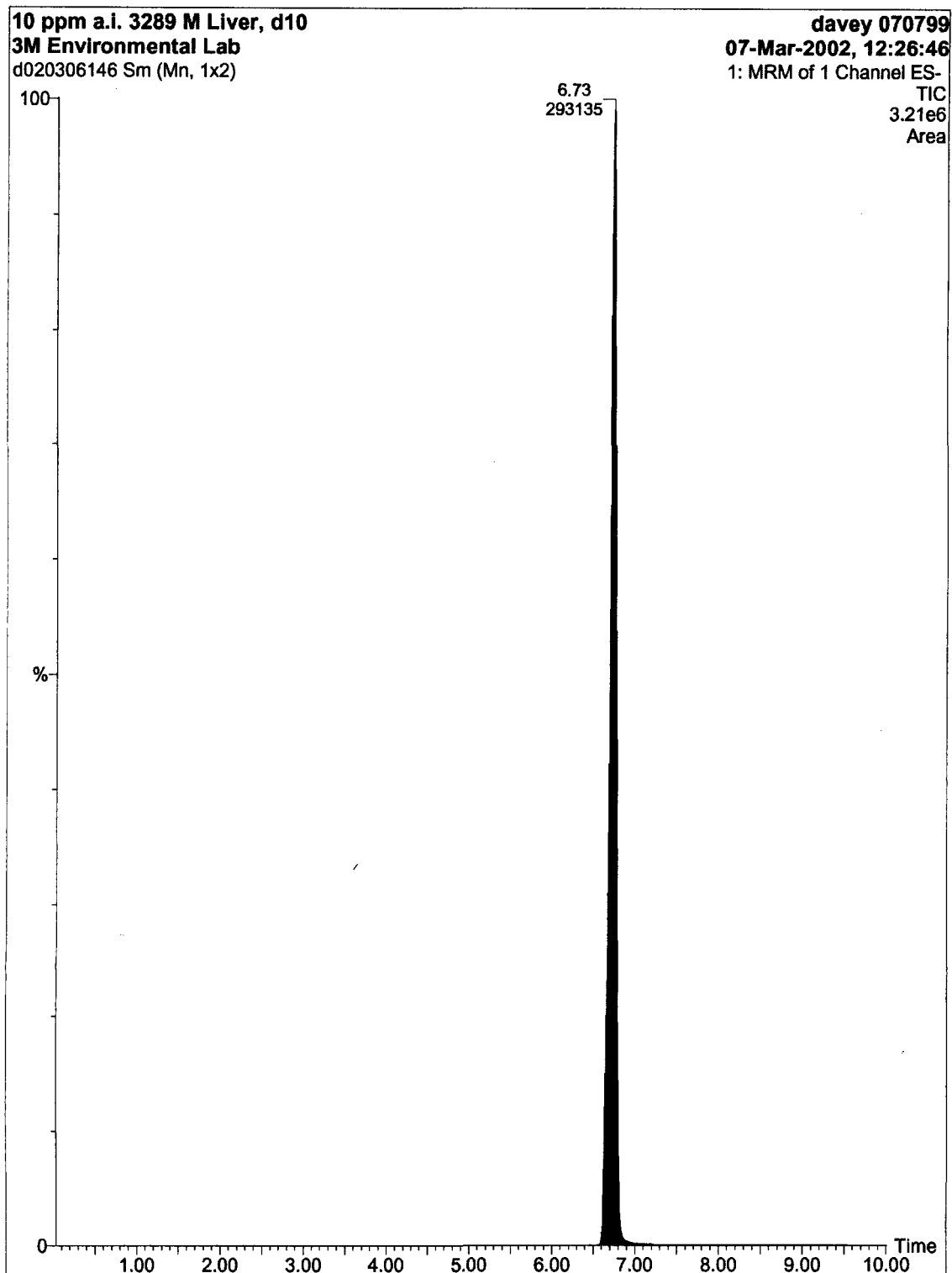
PFOS: A Reproduction Study with the Mallard



Dosed Group Male Liver Samples

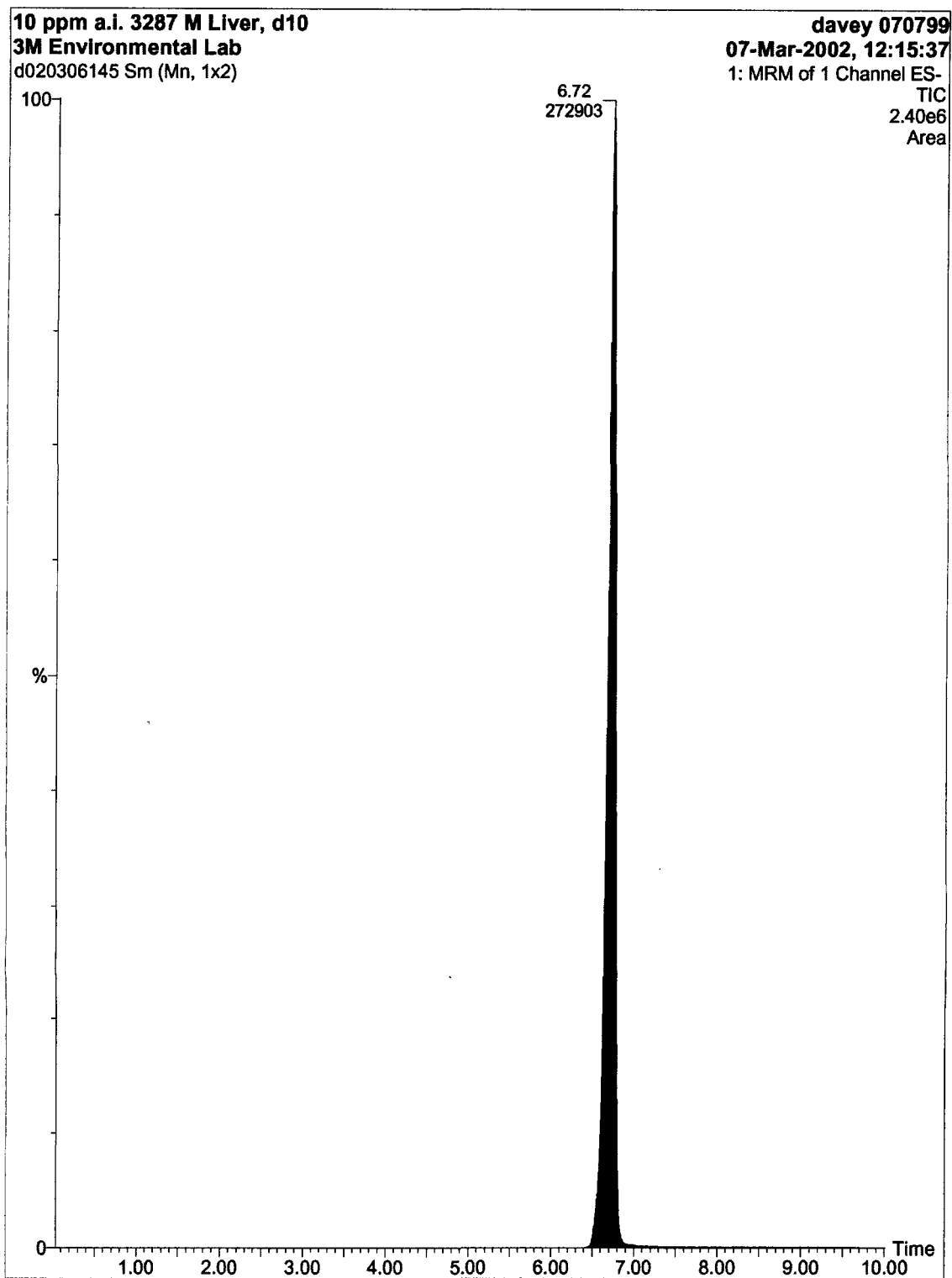
Report E01-1256

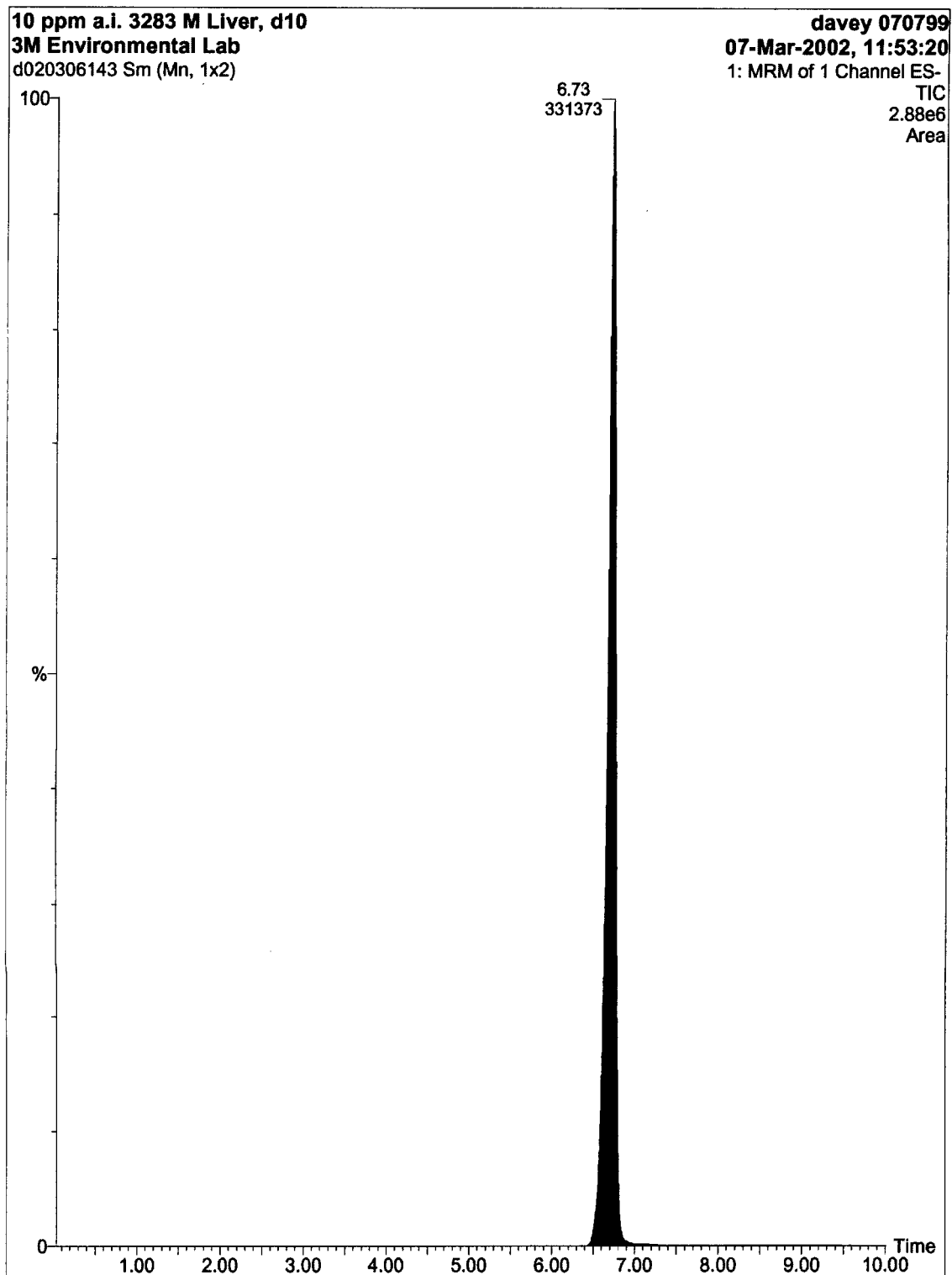
PFOS: A Reproduction Study with the Mallard

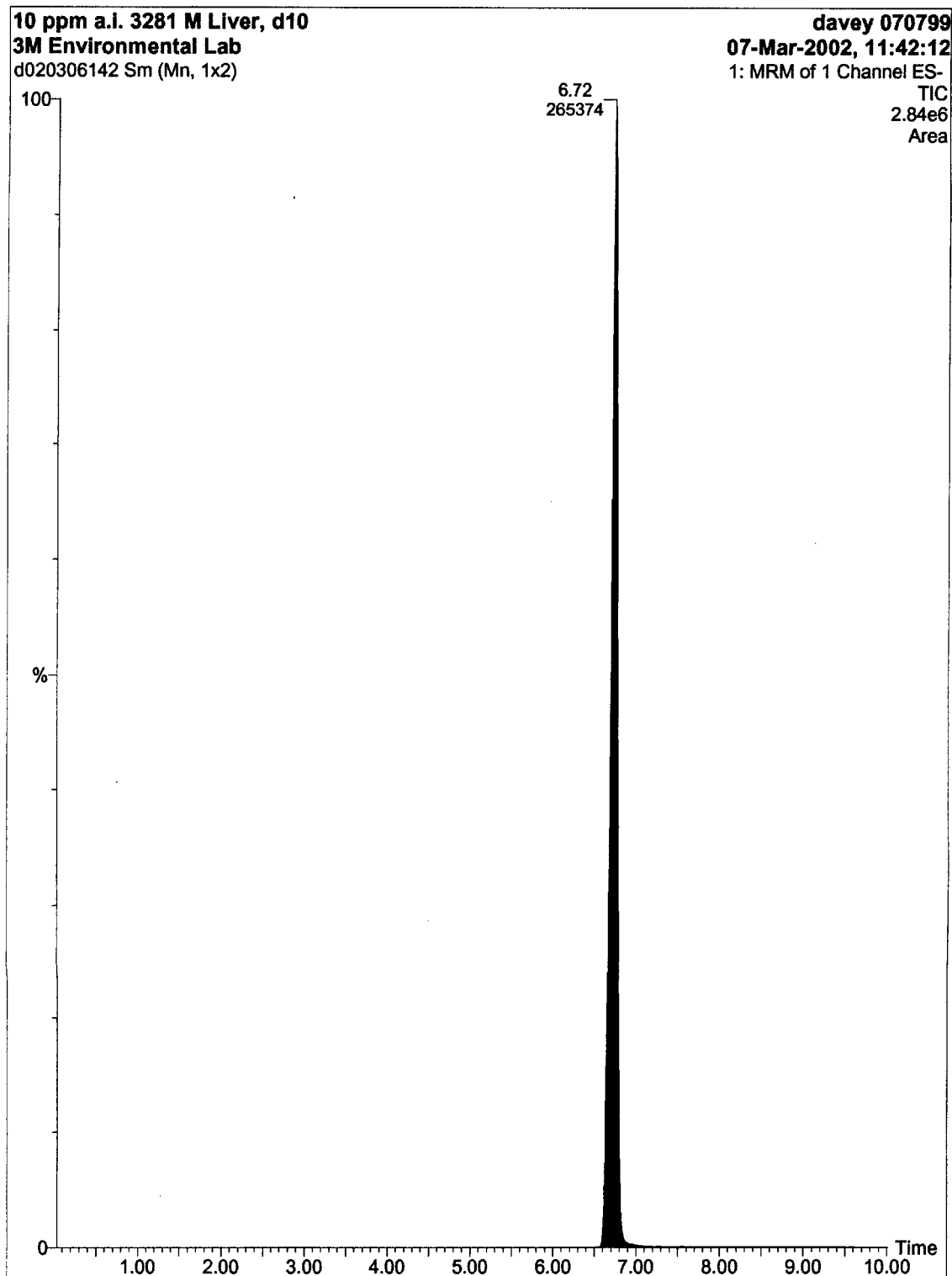


Report E01-1256

PFOS: A Reproduction Study with the Mallard

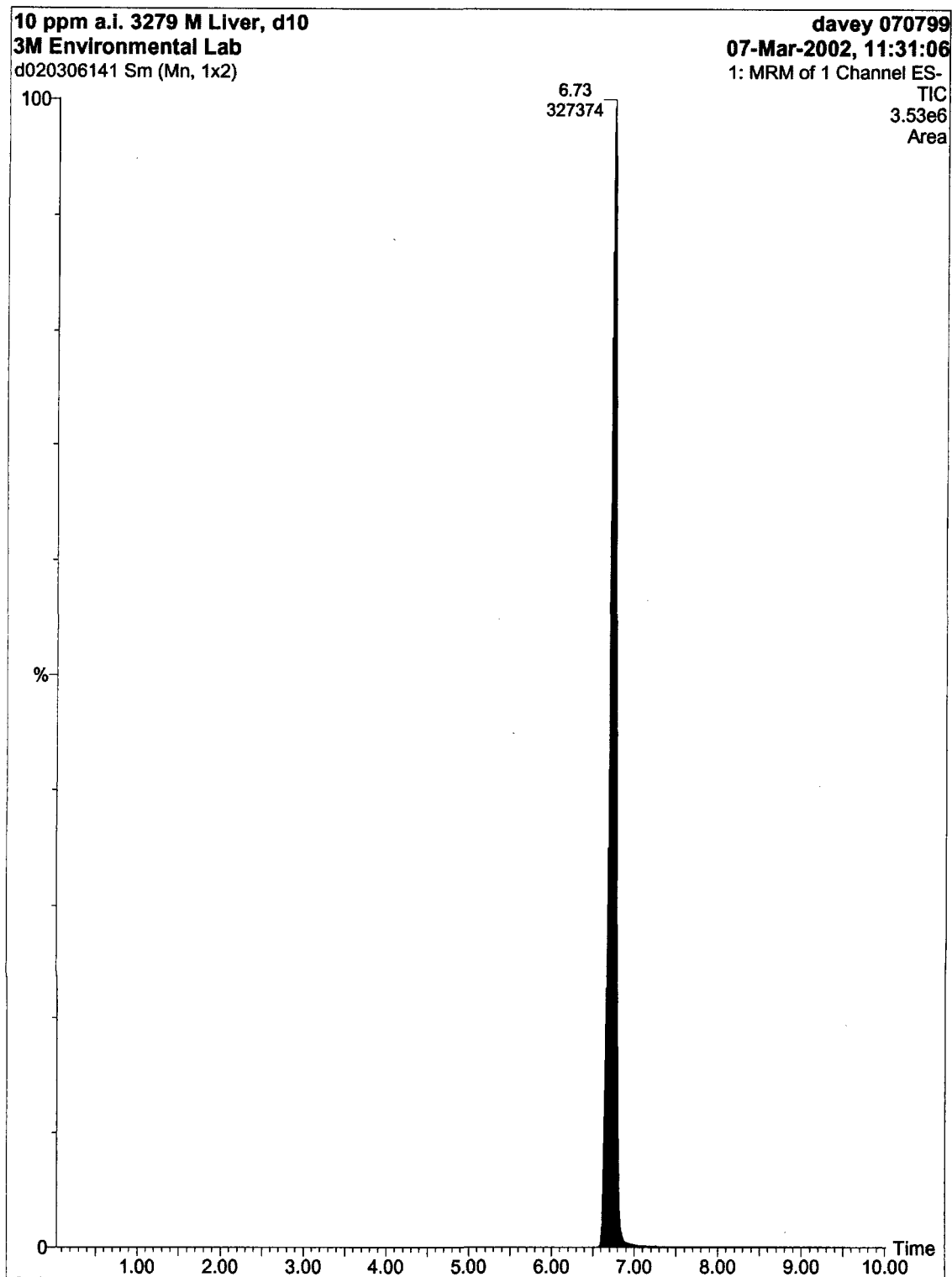






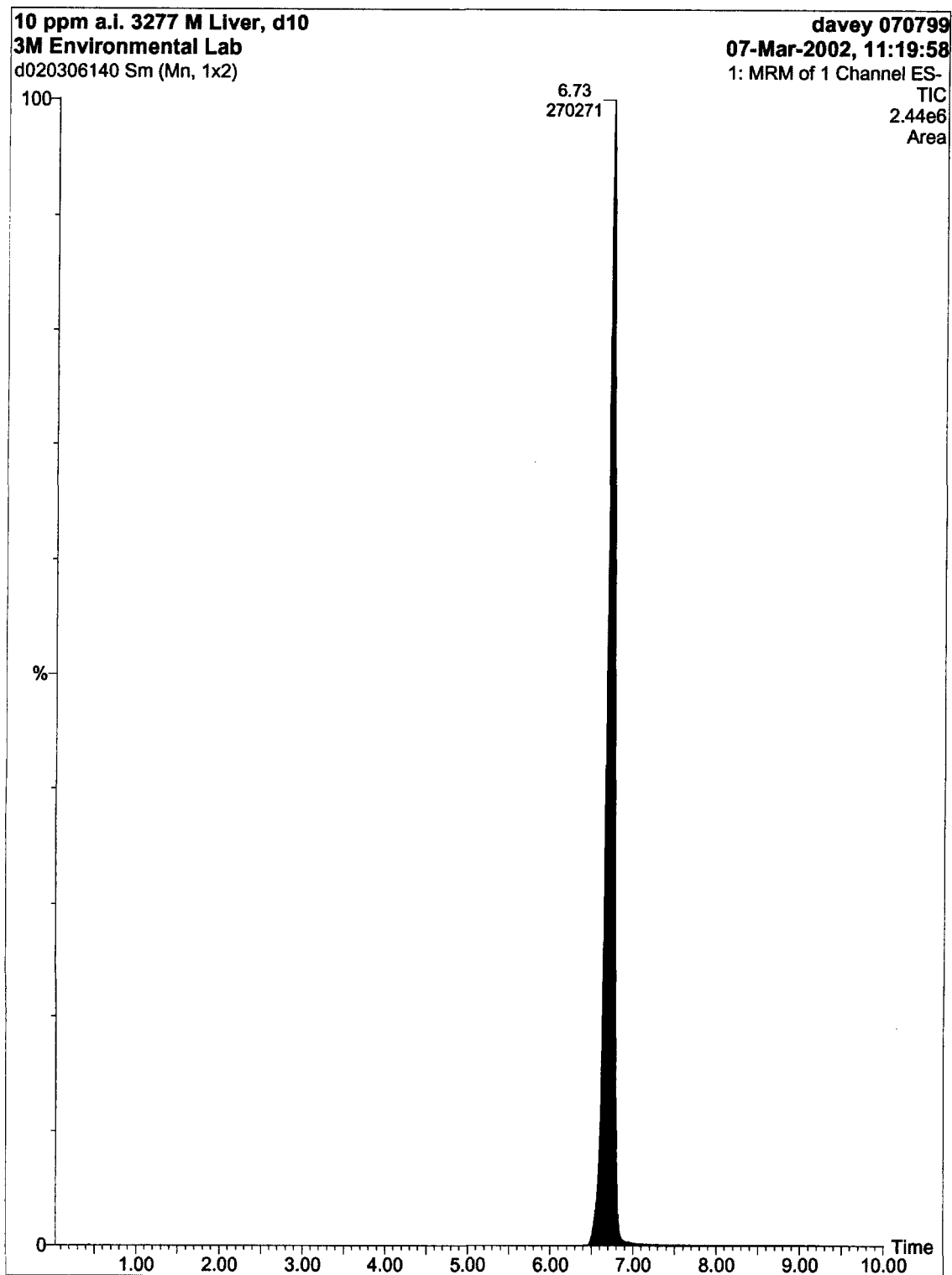
Report E01-1256

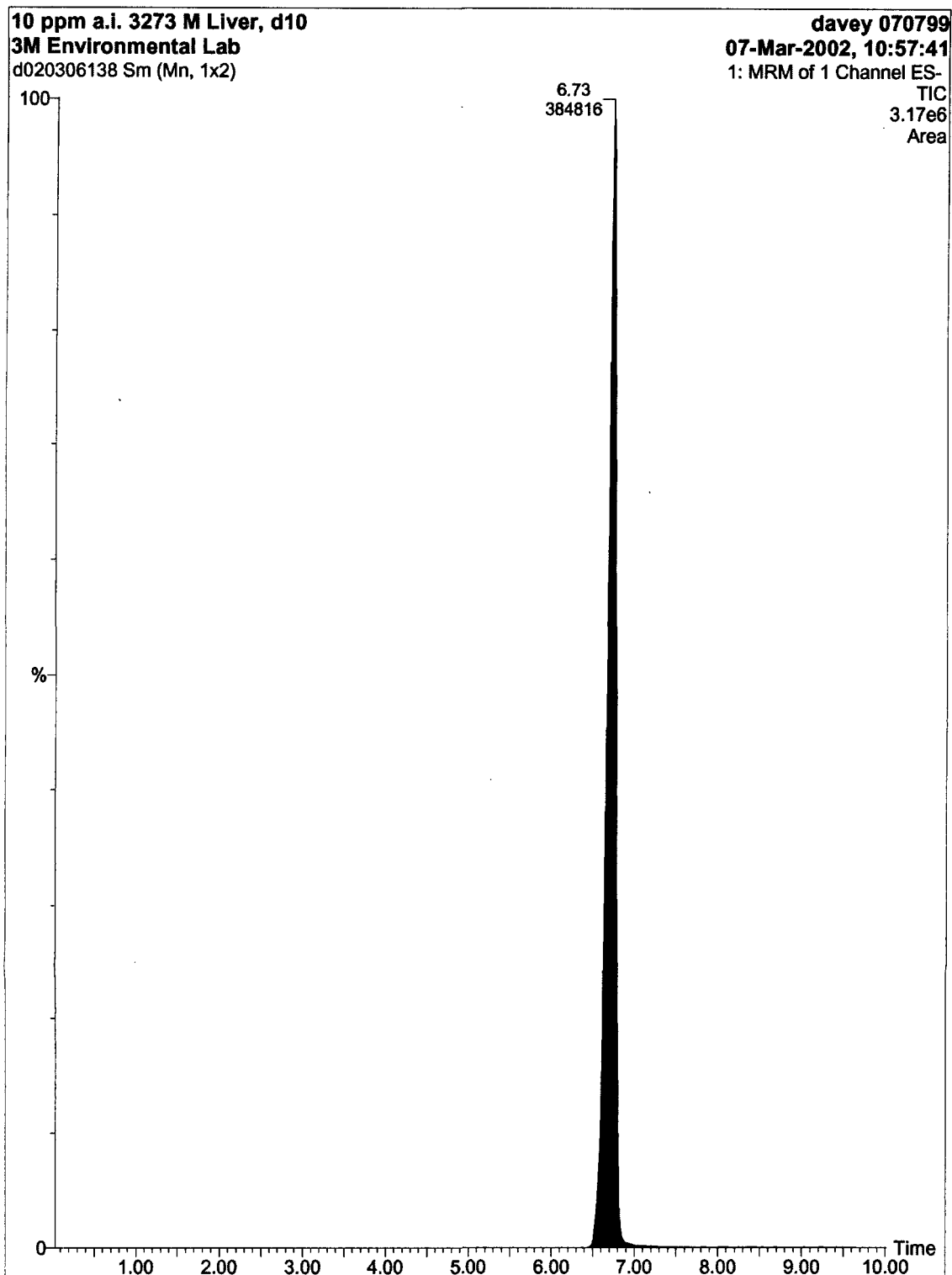
PFOS: A Reproduction Study with the Mallard



Report E01-1256

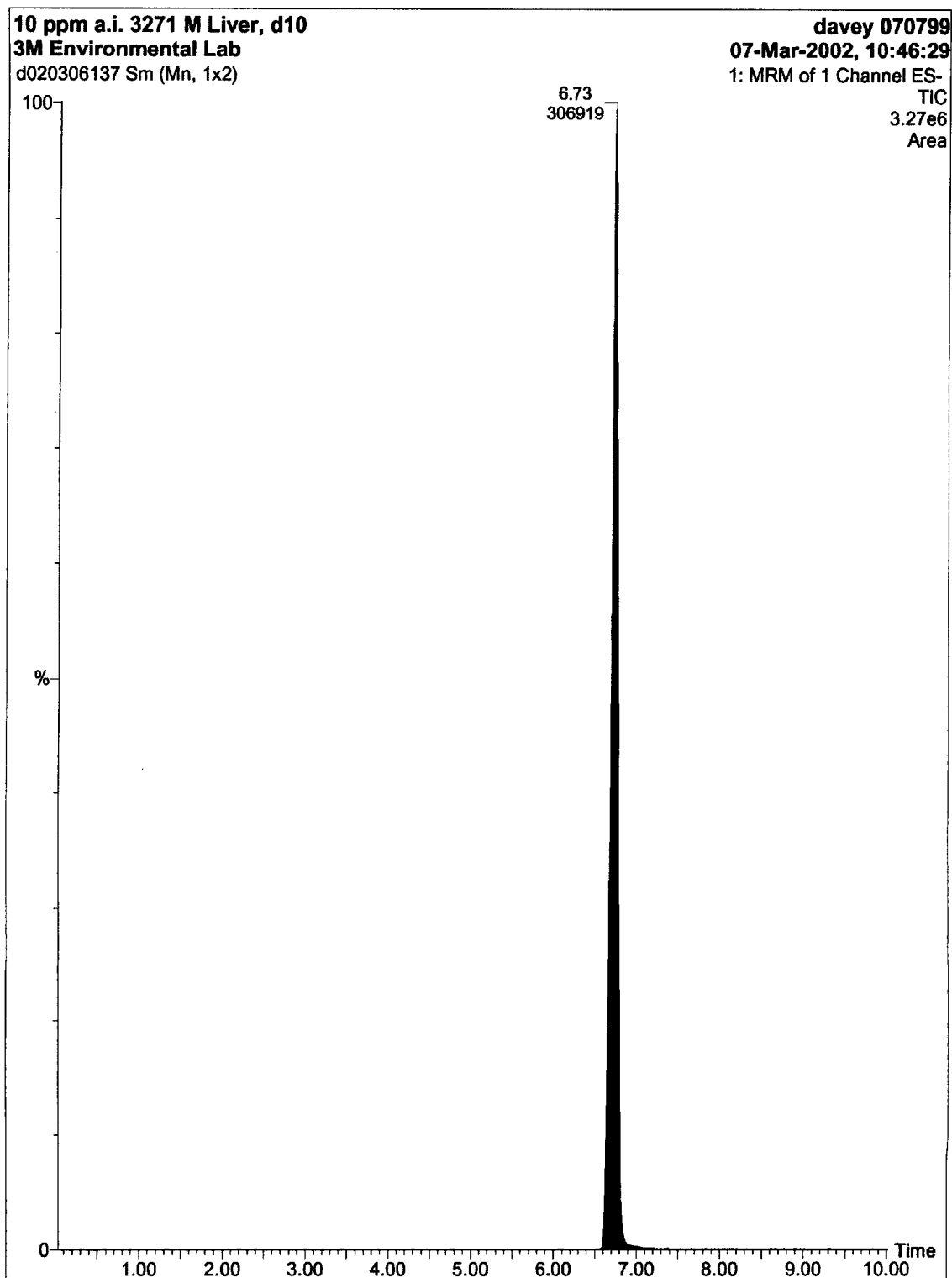
PFOS: A Reproduction Study with the Mallard

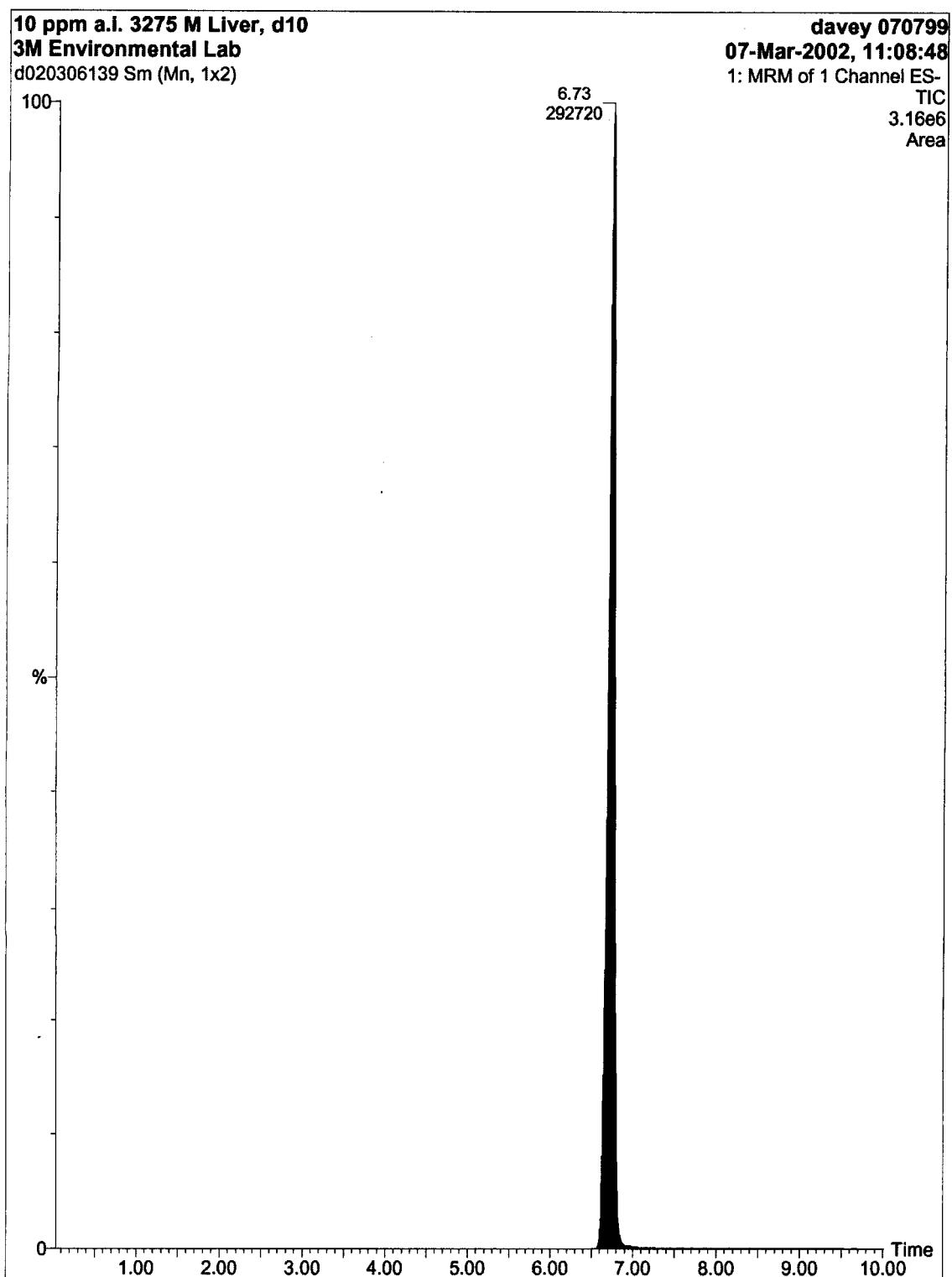


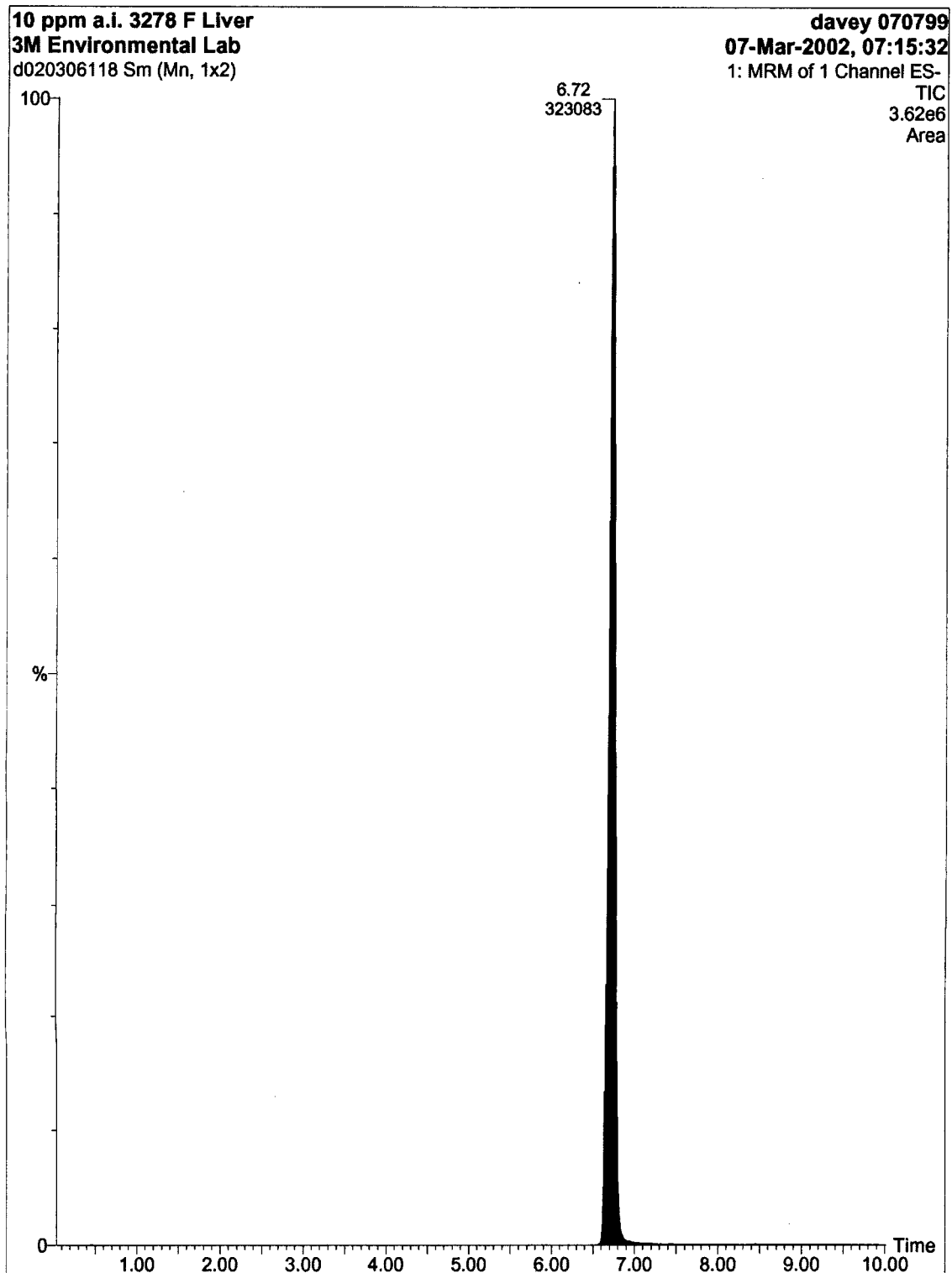


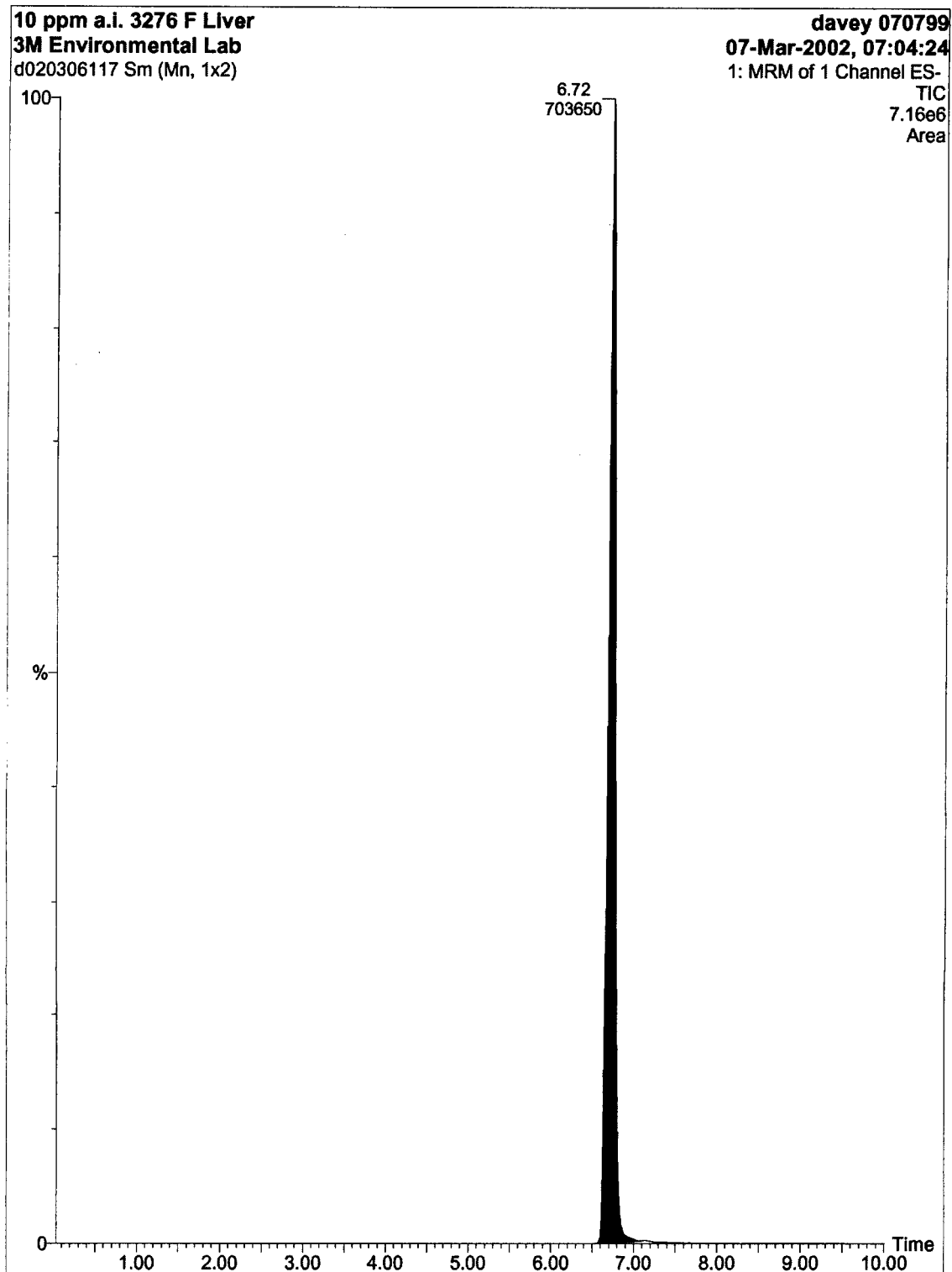
Report E01-1256

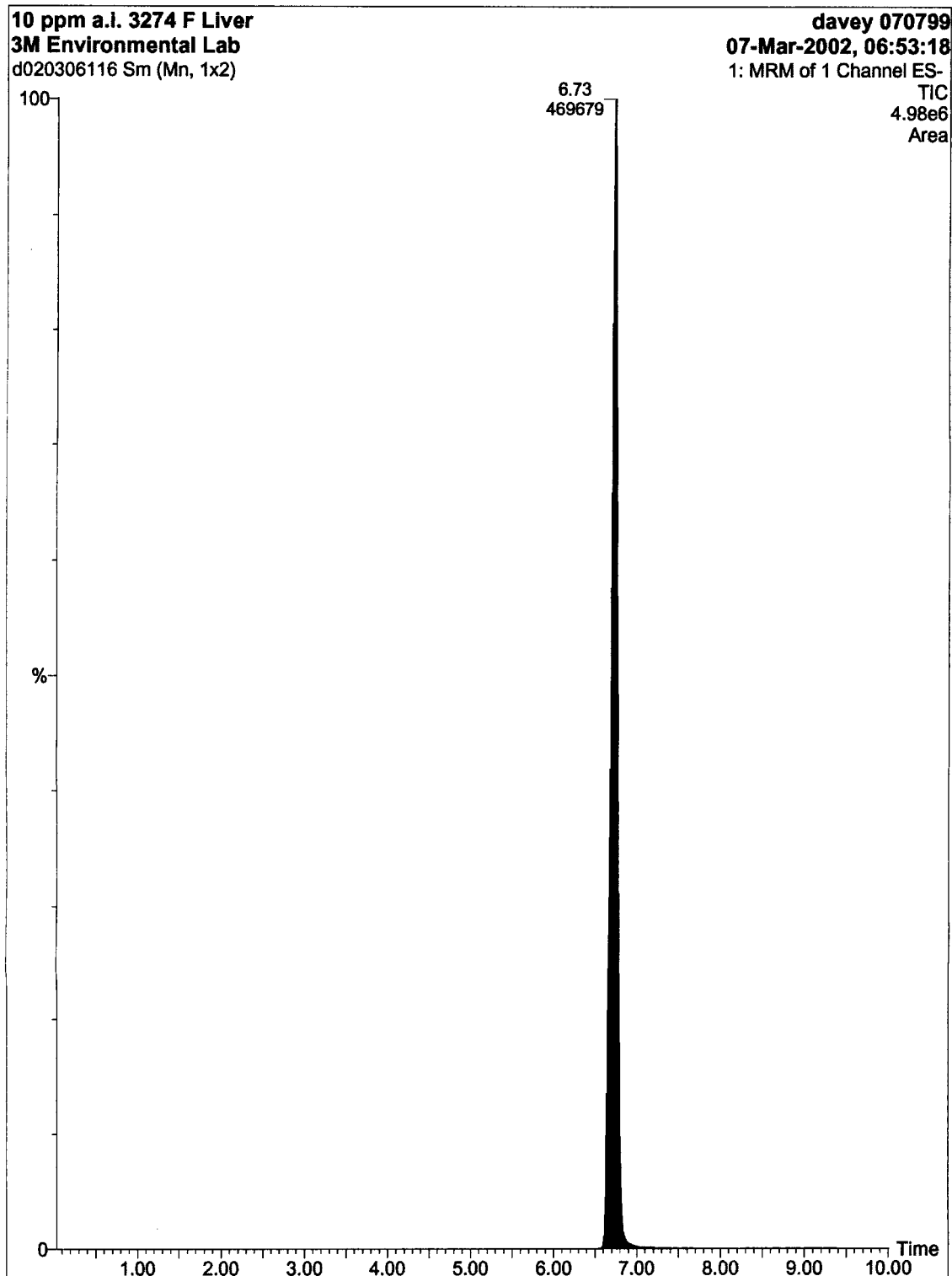
PFOS: A Reproduction Study with the Mallard

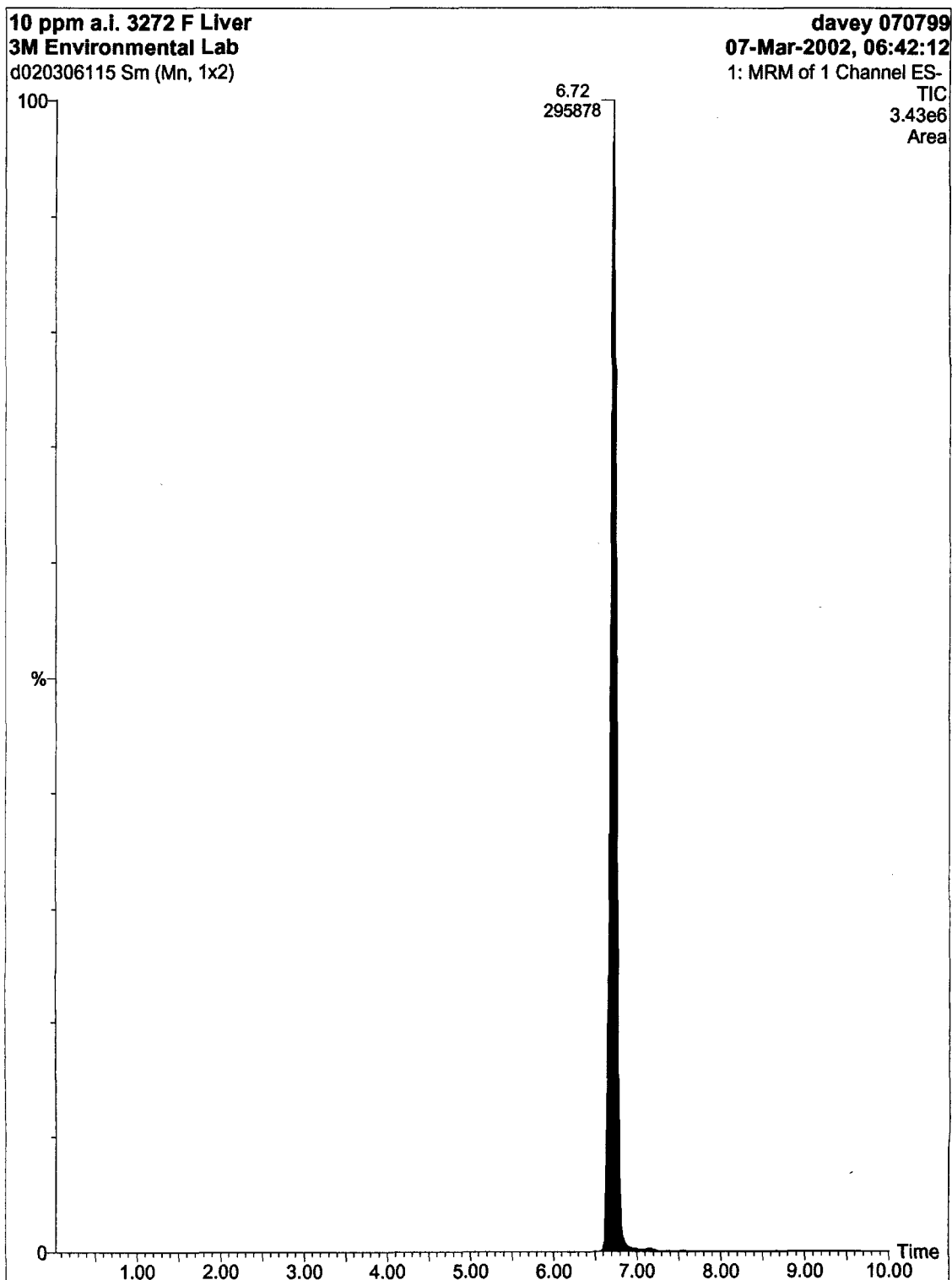




Dosed Group Female Liver Samples

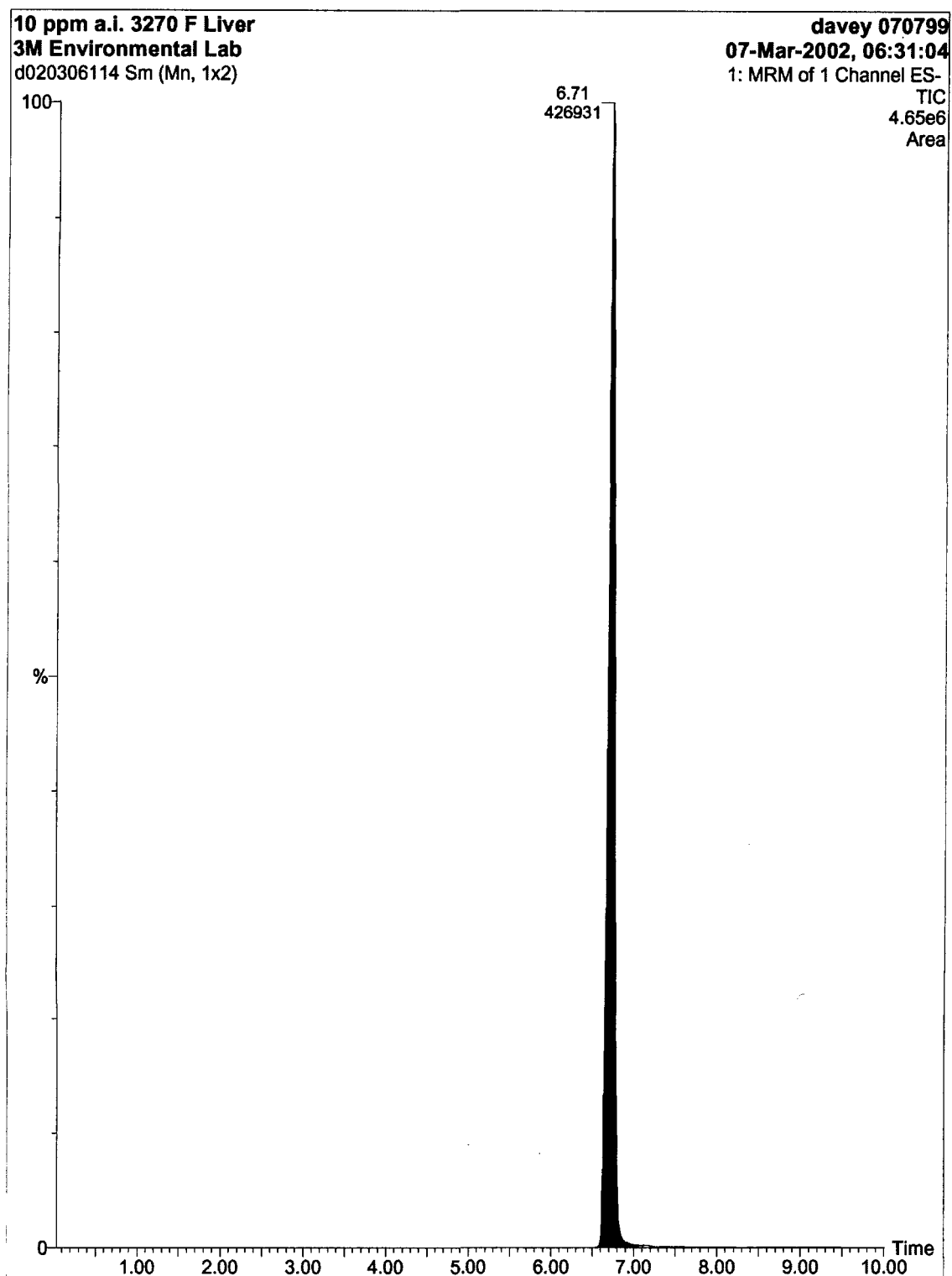


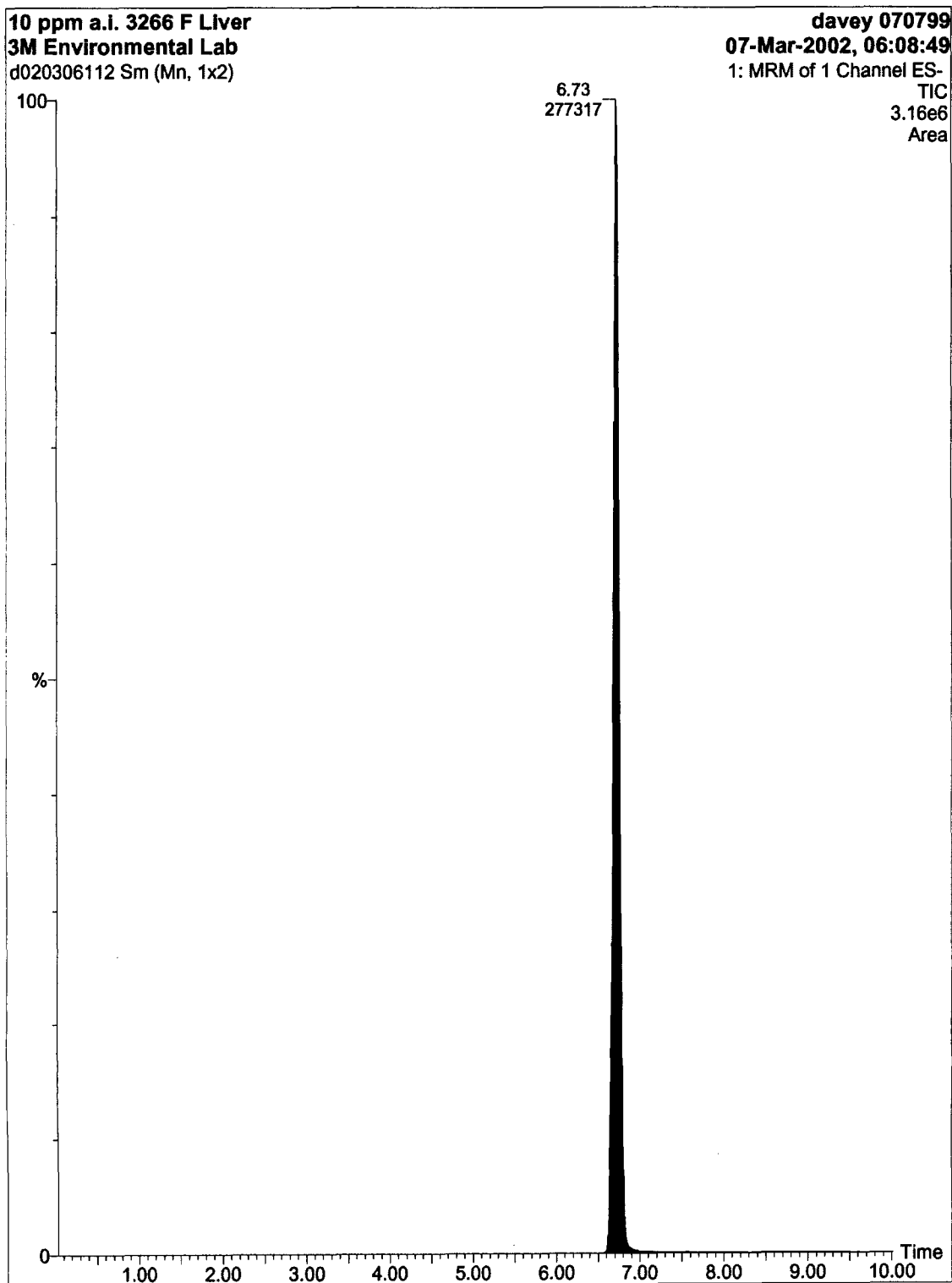


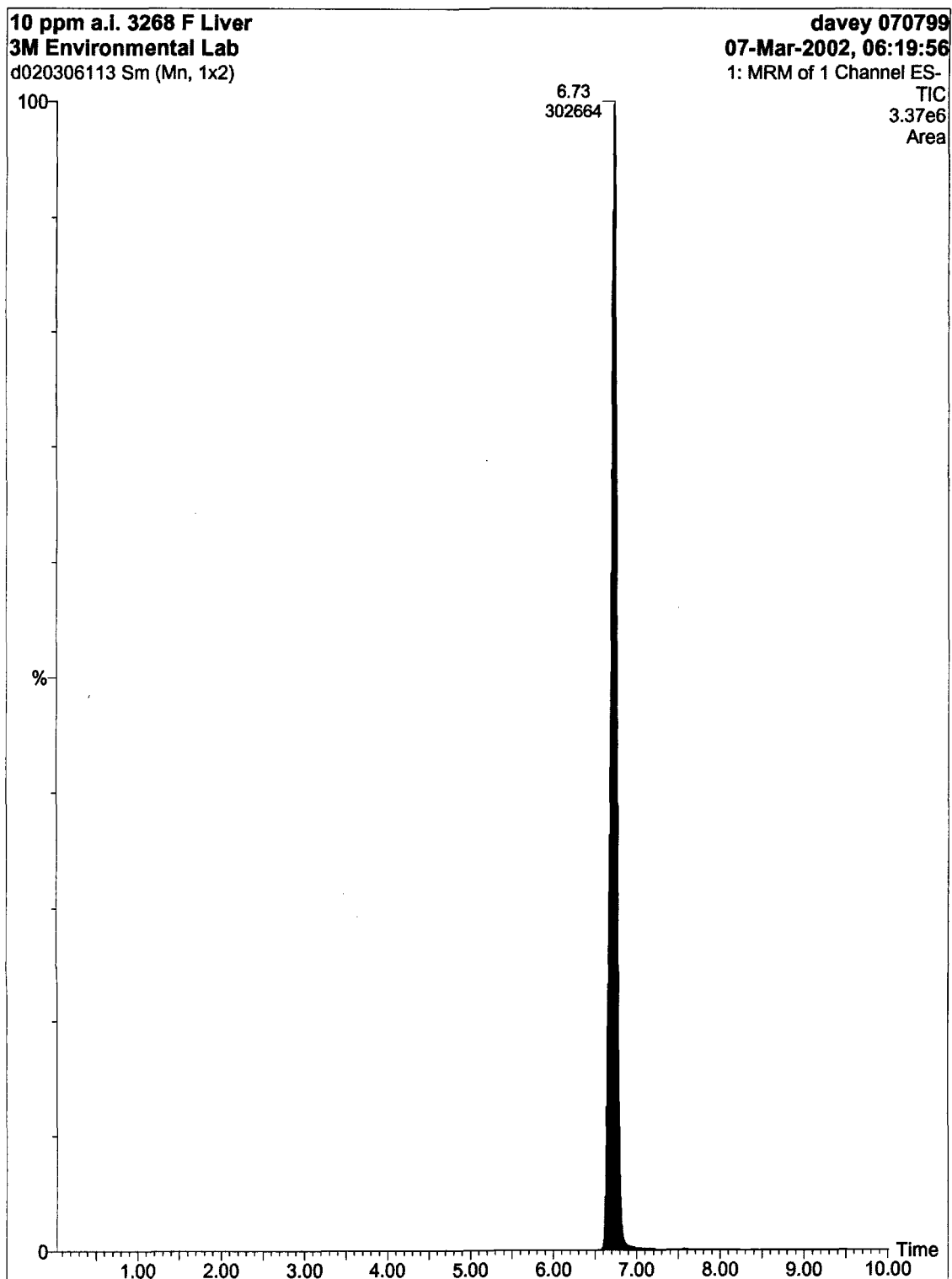


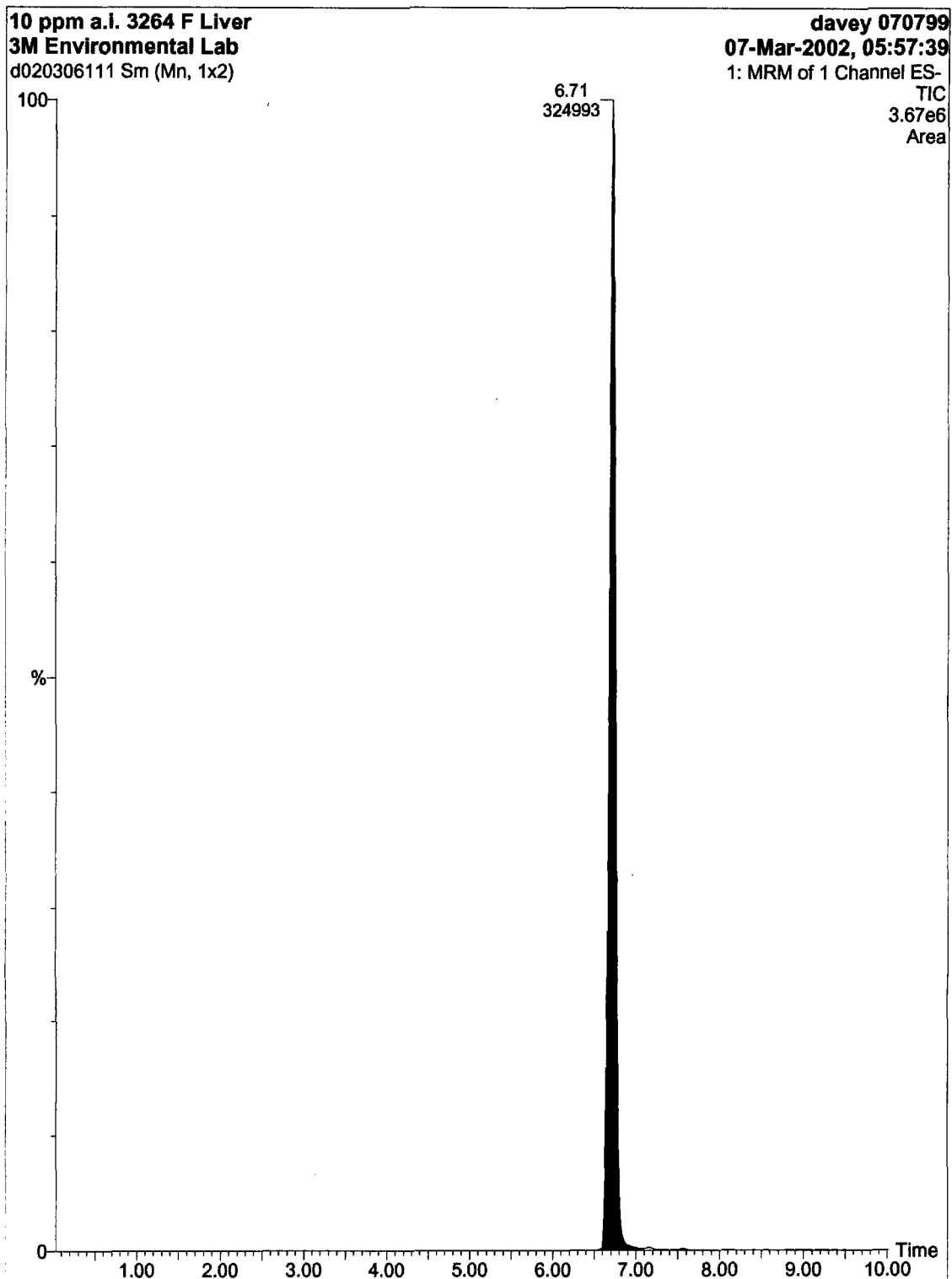
Report E01-1256

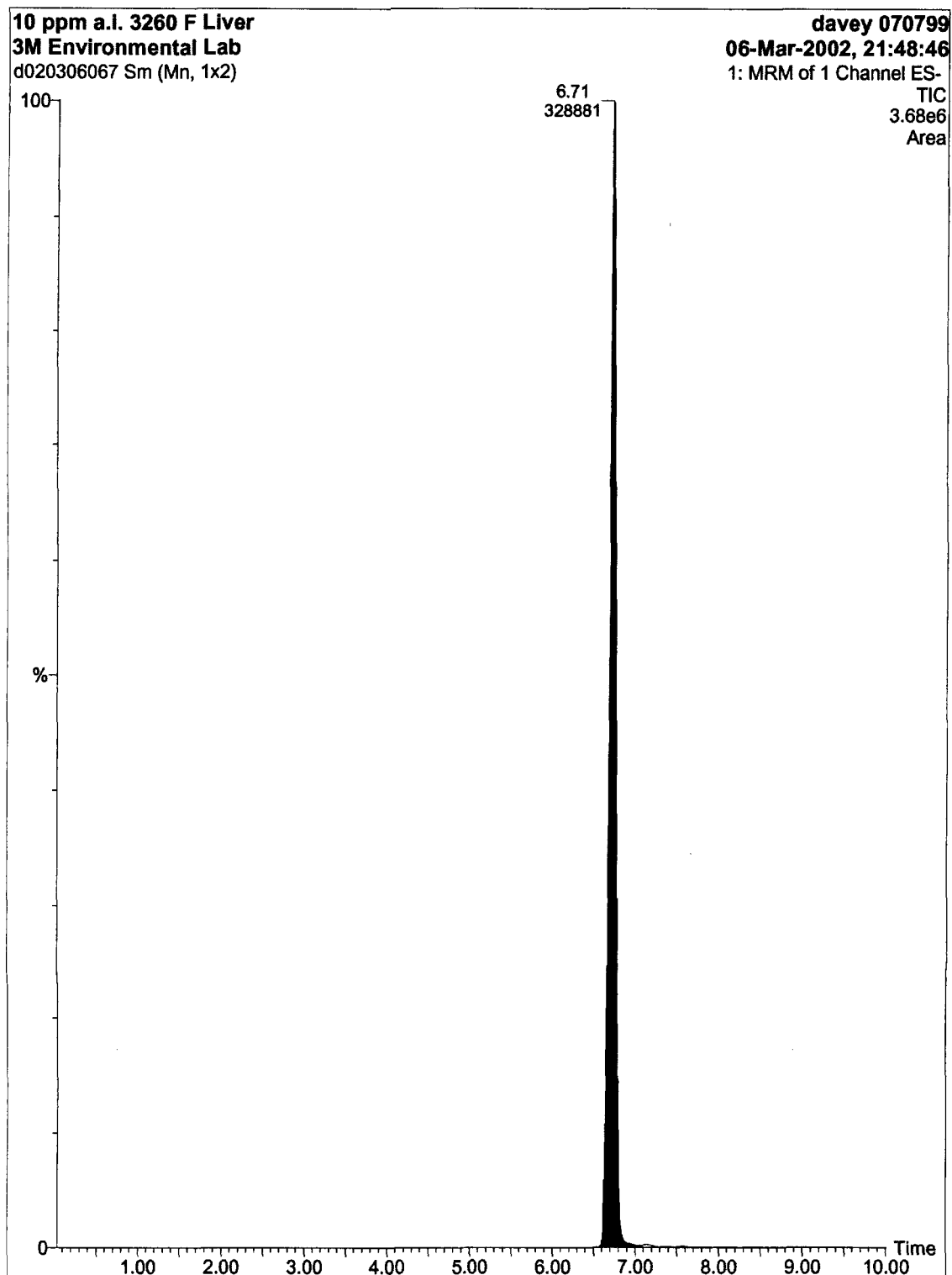
PFOS: A Reproduction Study with the Mallard

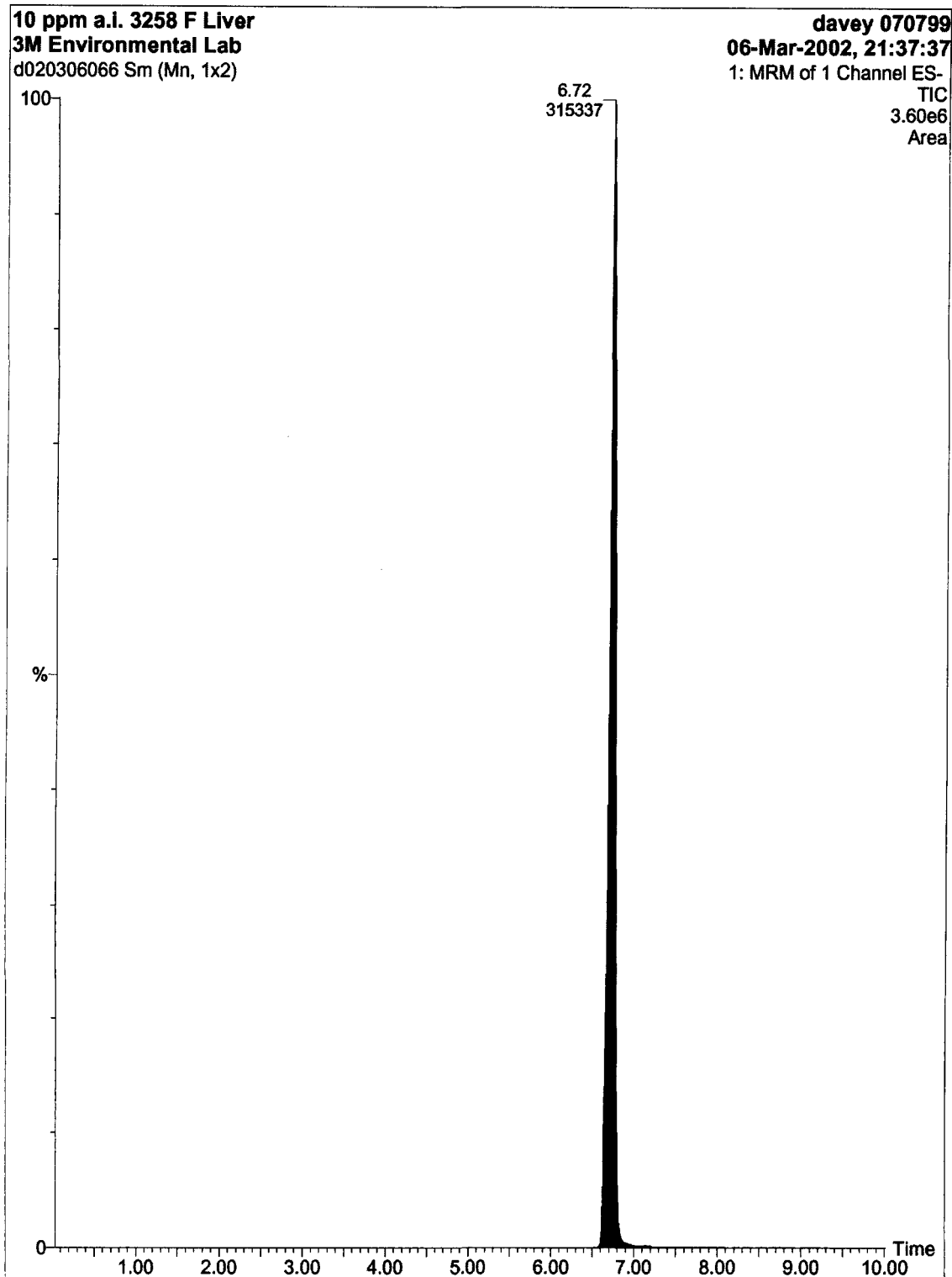


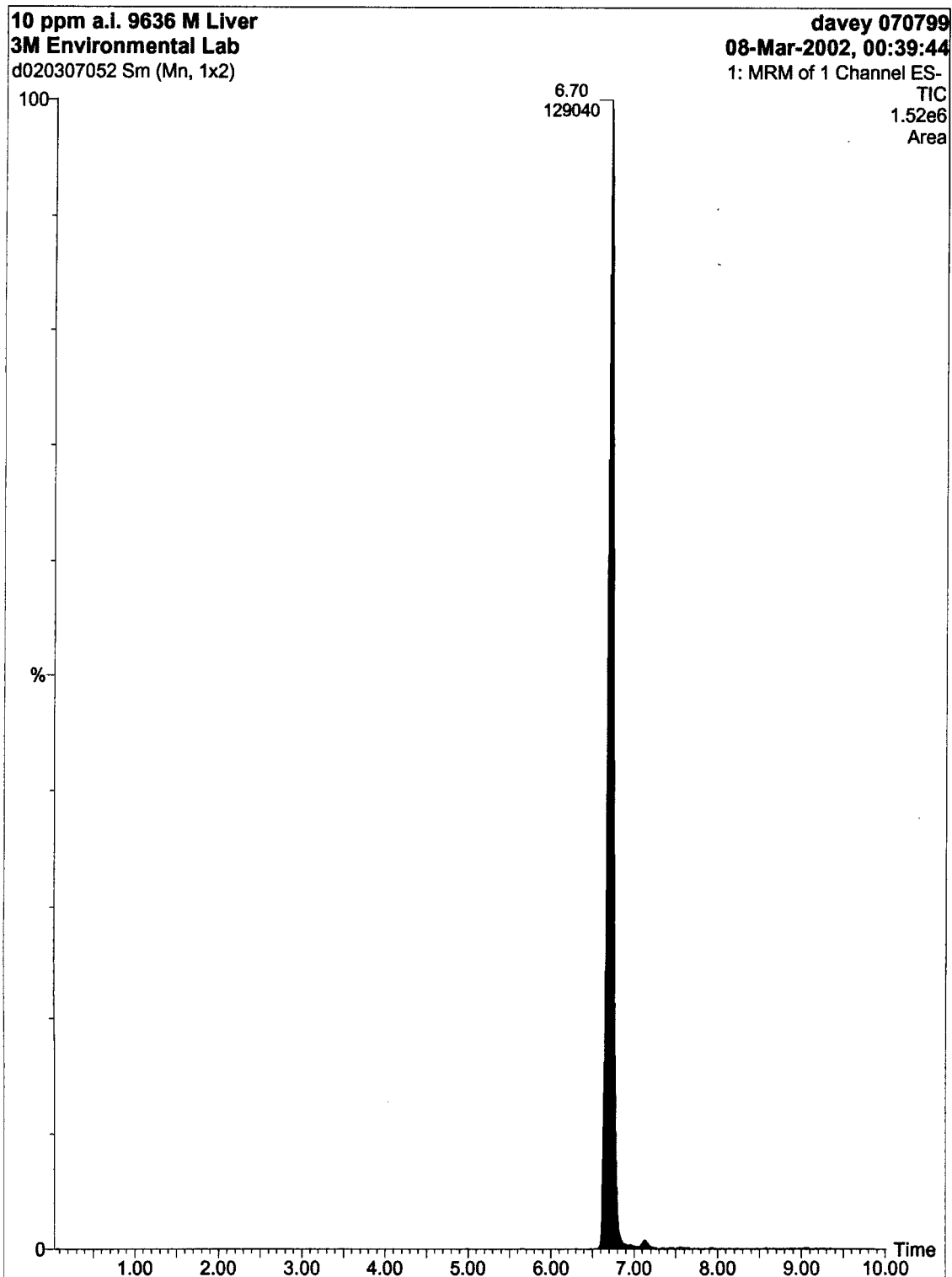






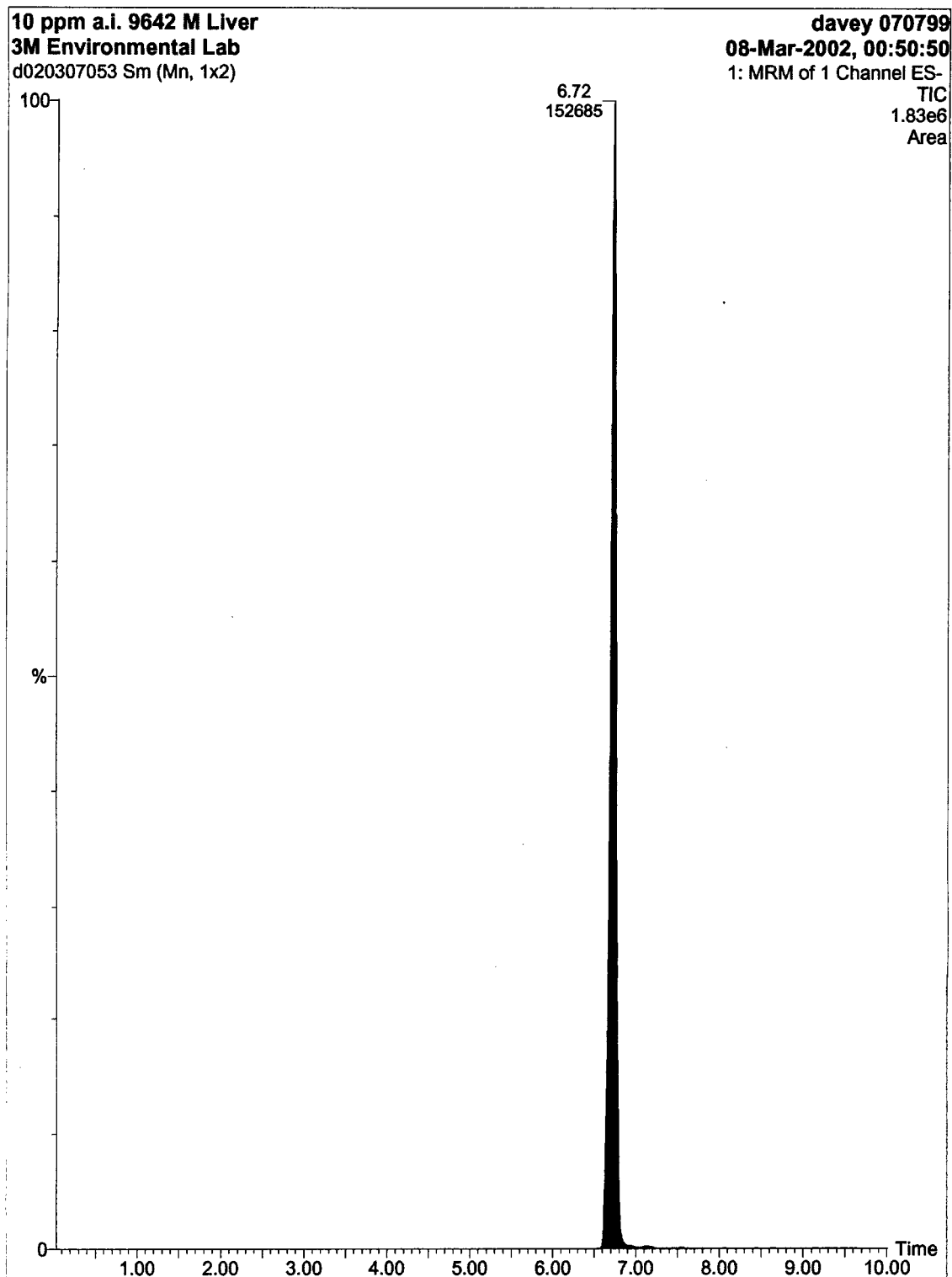




Dosed Group Male Offspring Liver Samples

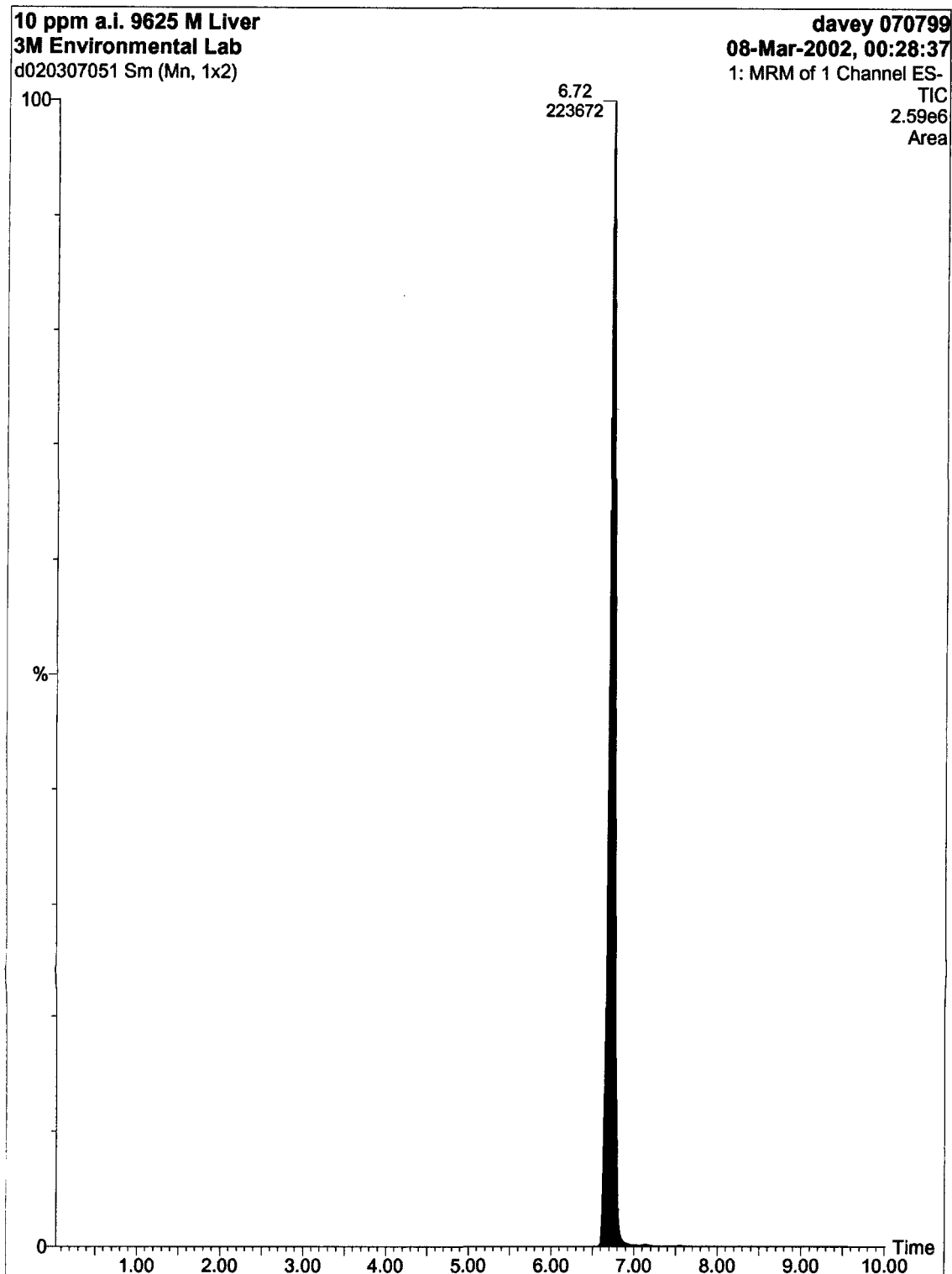
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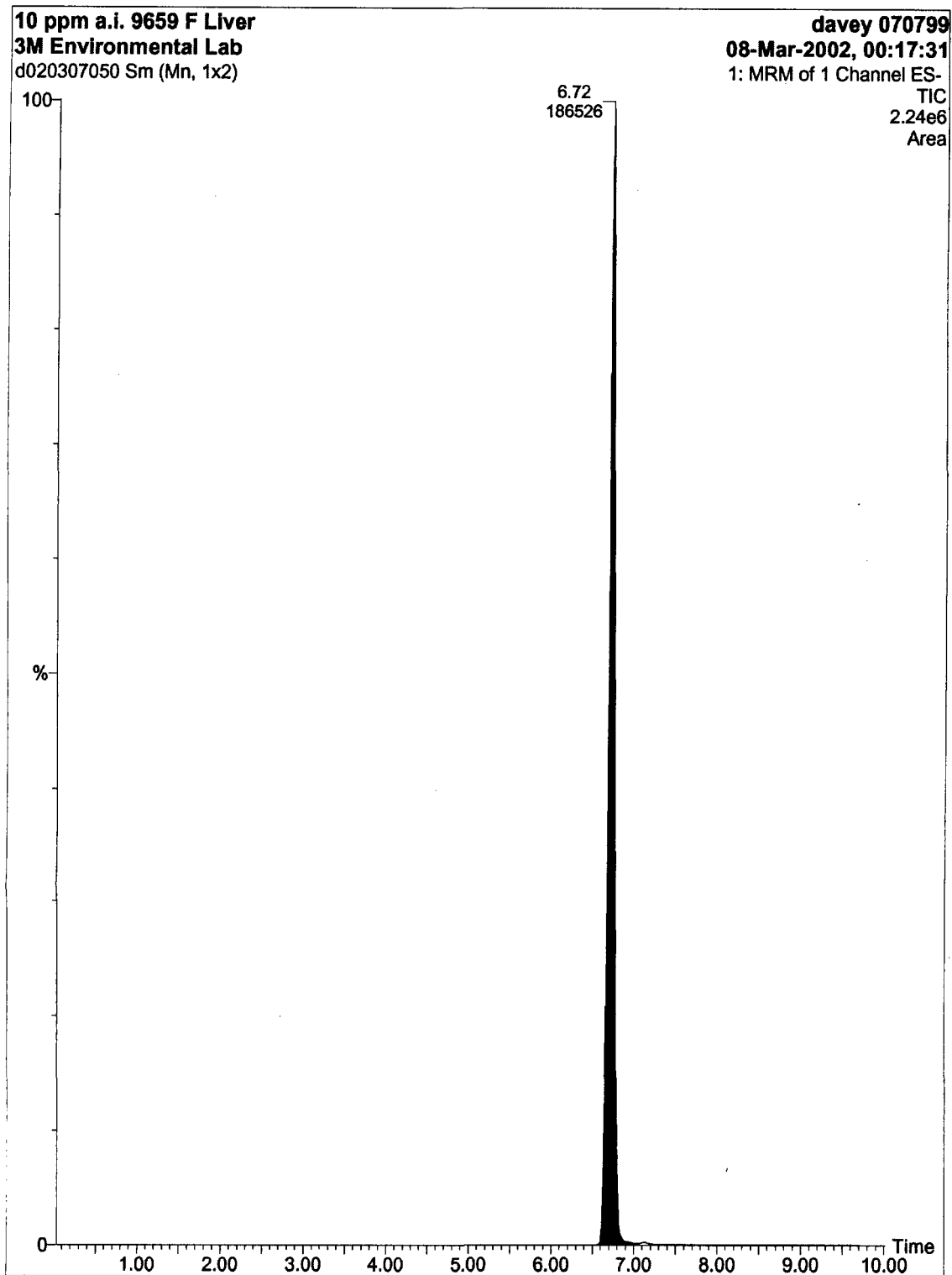
PFOS: A Reproduction Study with the Mallard

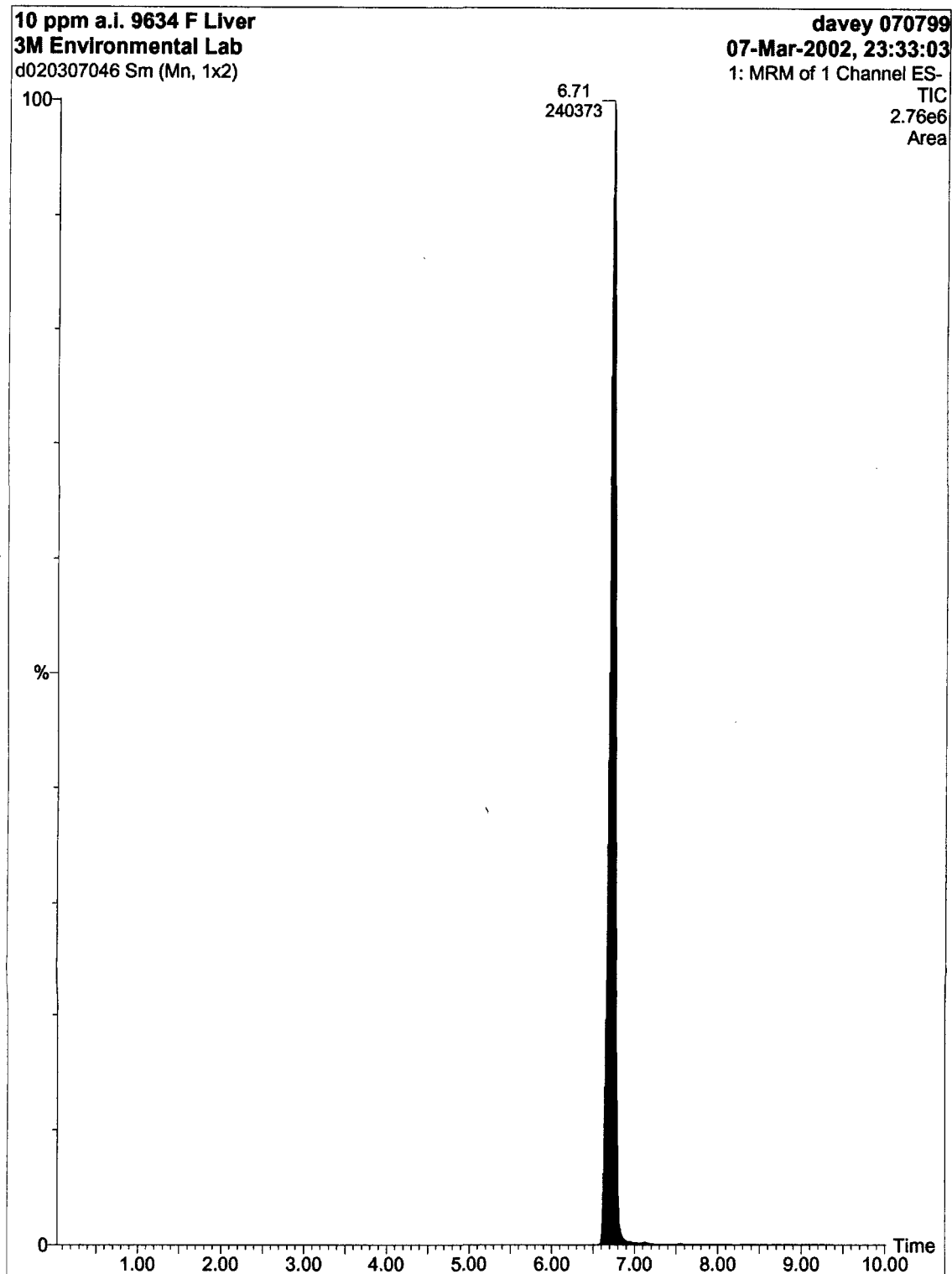


Report E01-1256

PFOS: A Reproduction Study with the Mallard

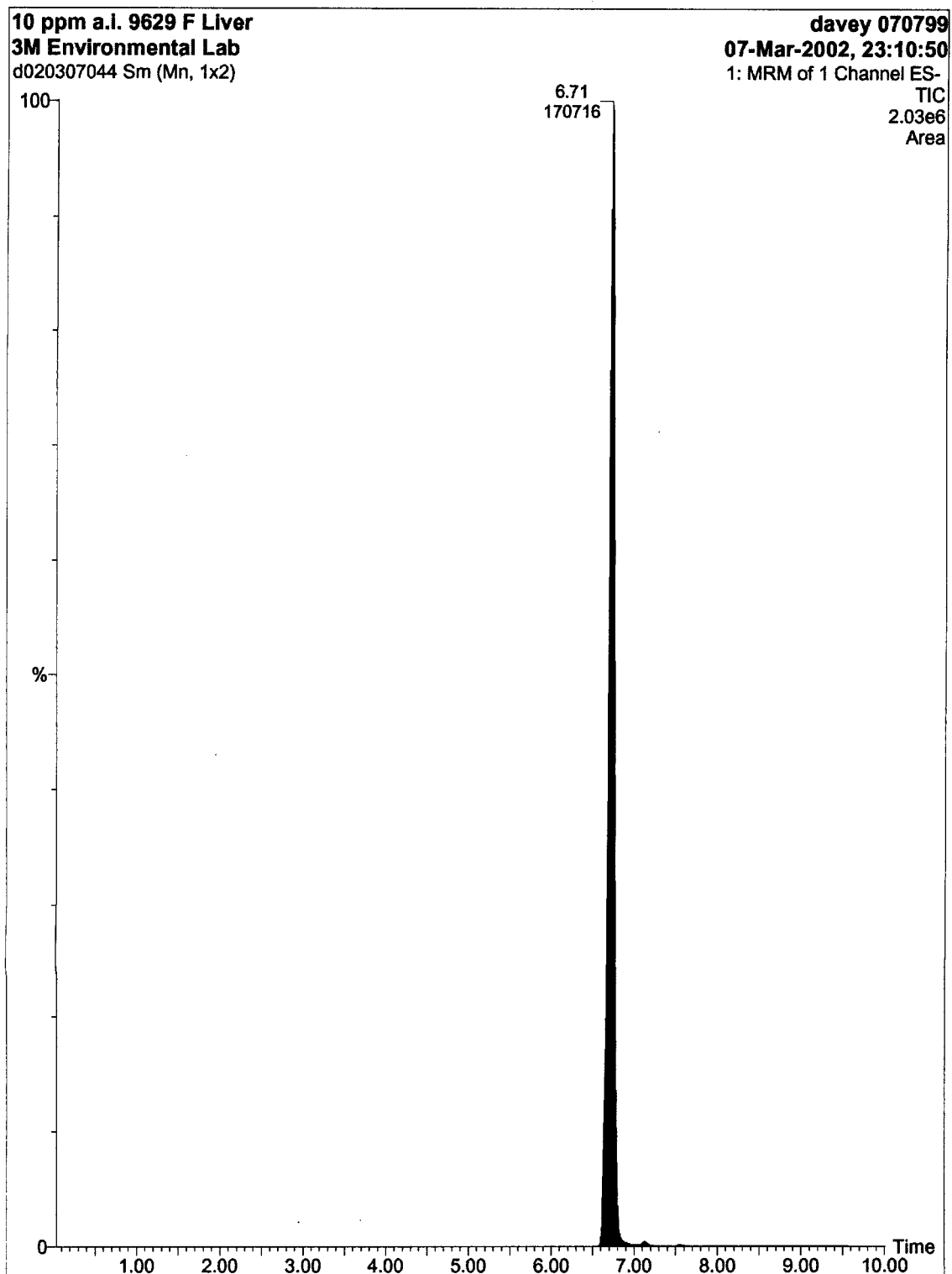


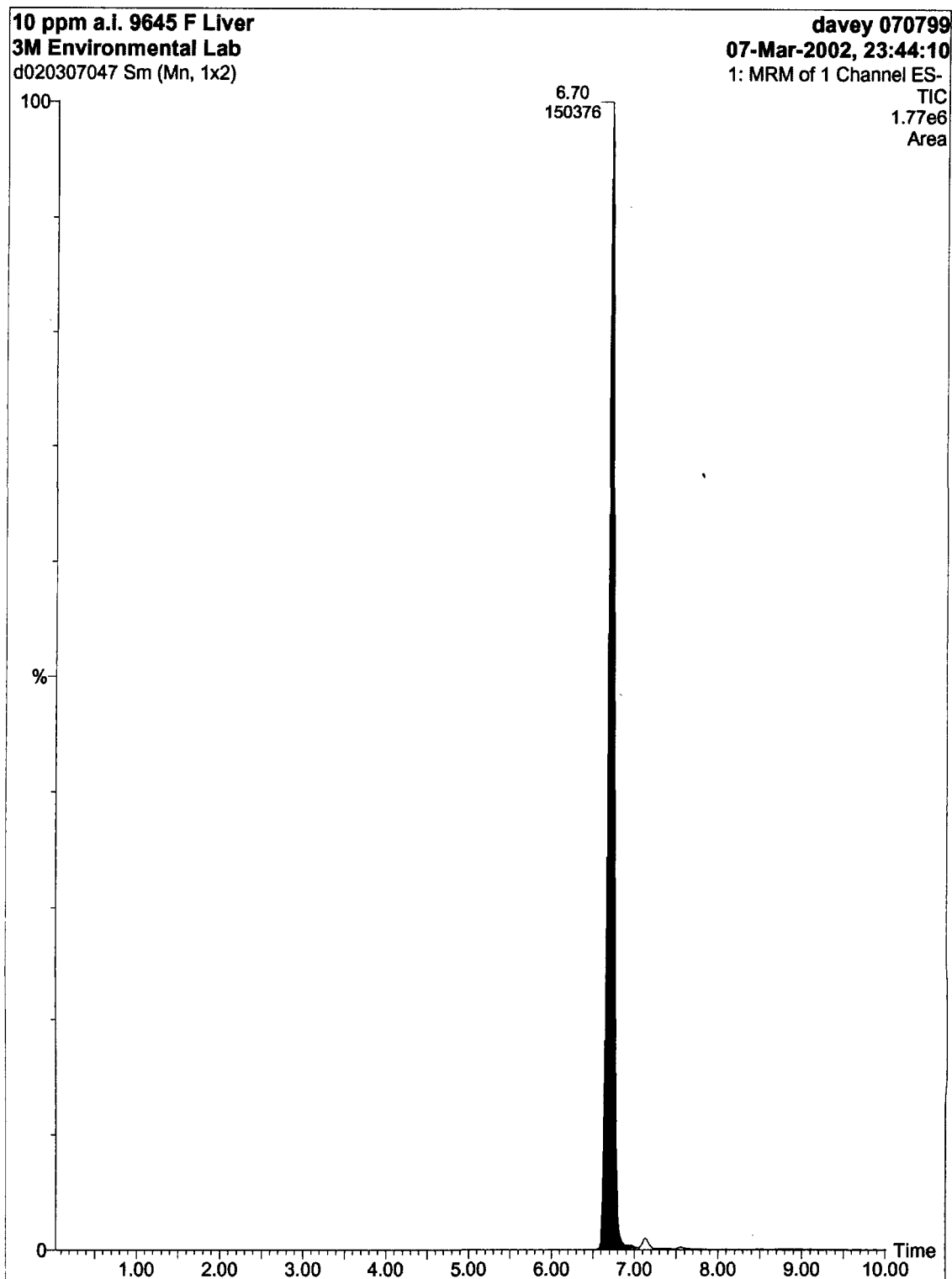
Dosed Group Female Offspring Liver Samples

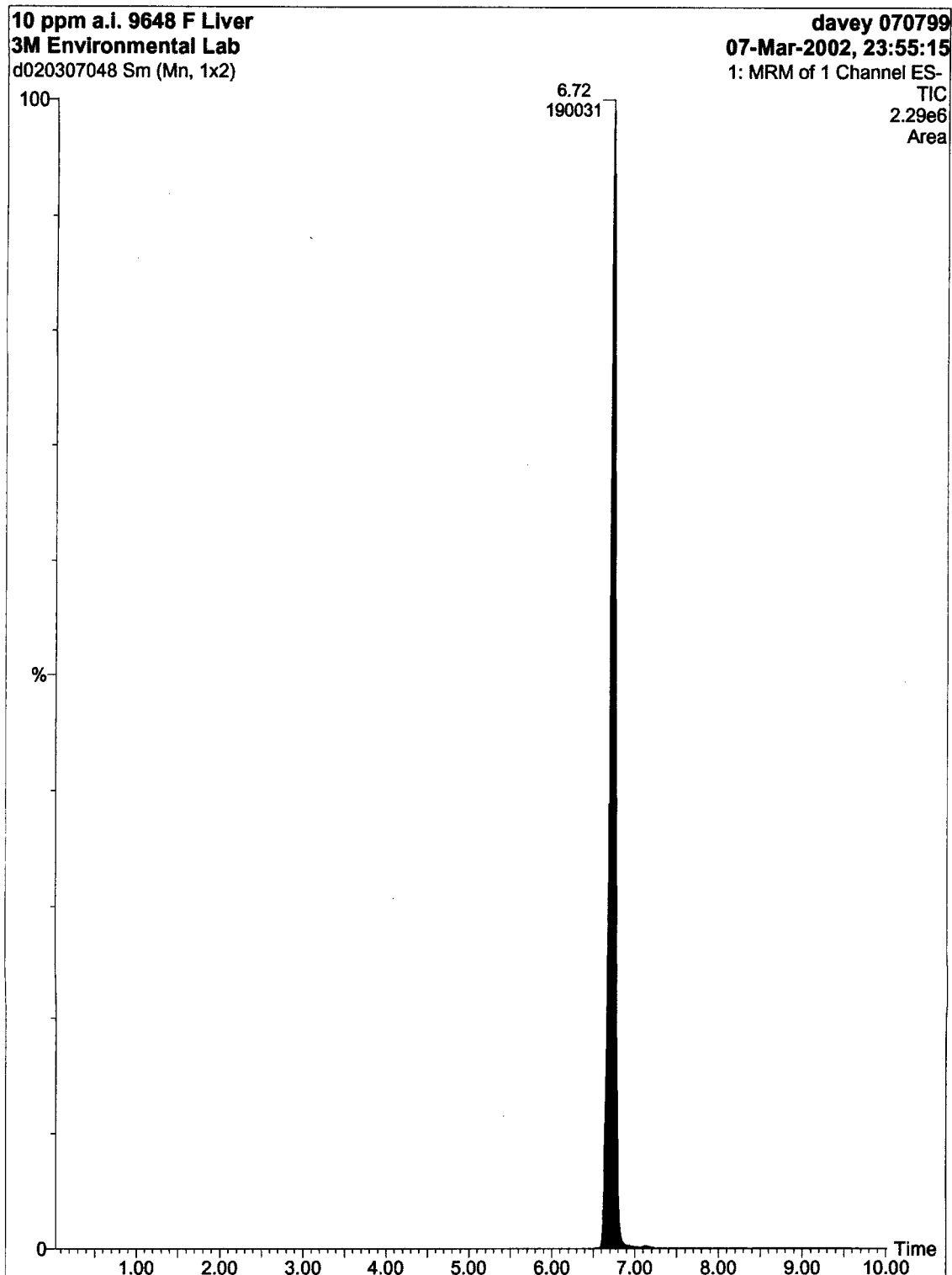


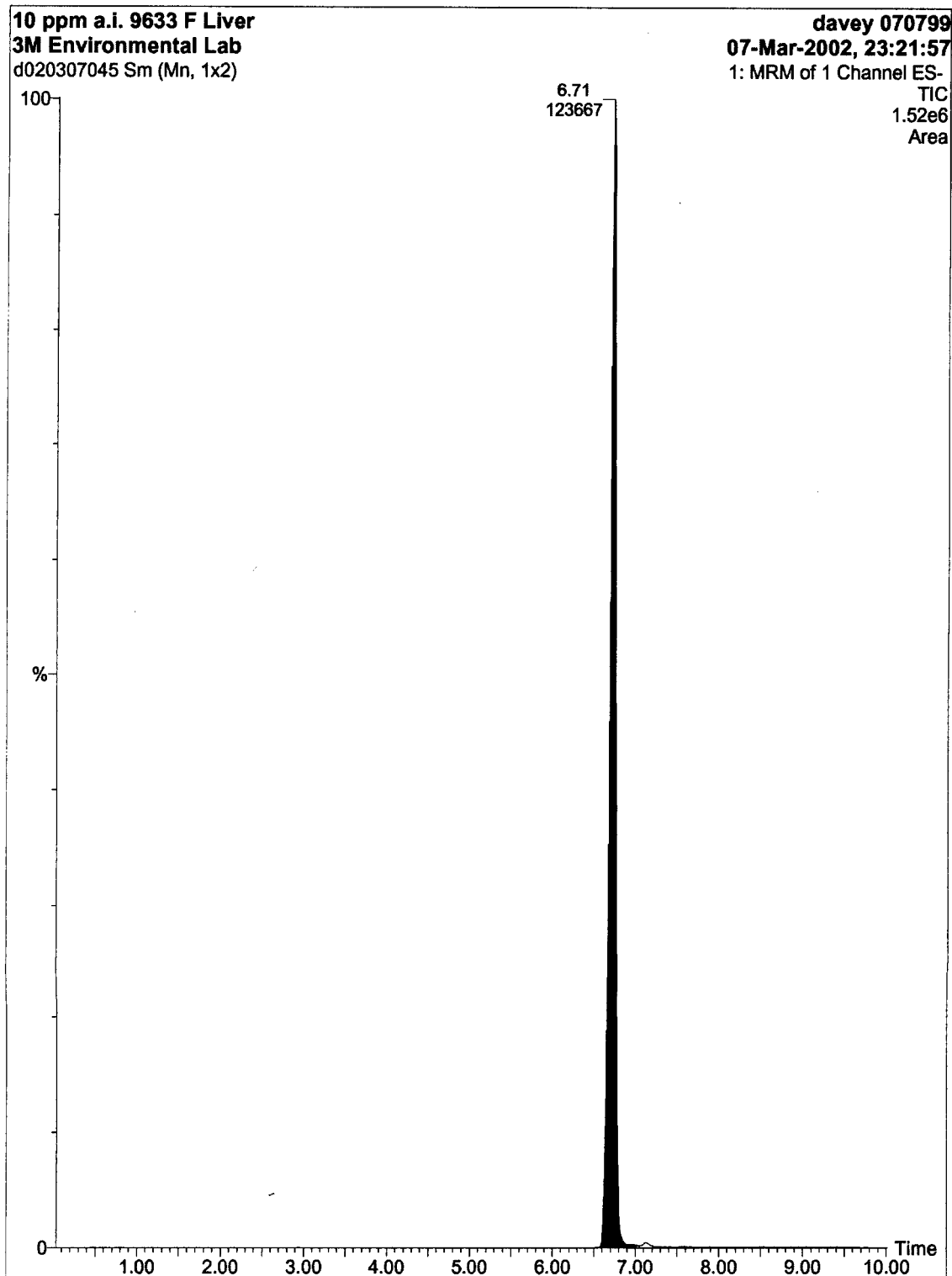
Report E01-1256

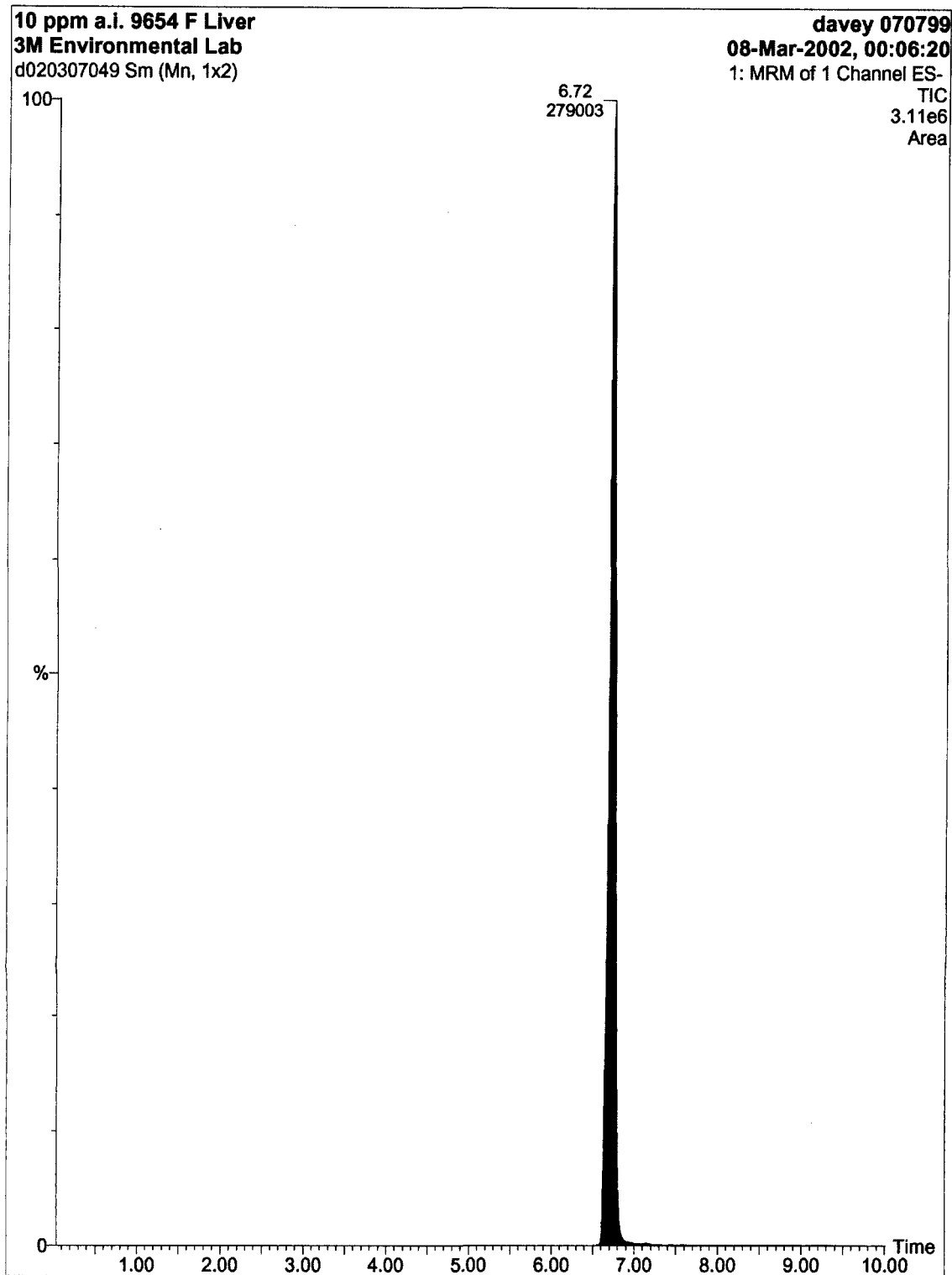
PFOS: A Reproduction Study with the Mallard

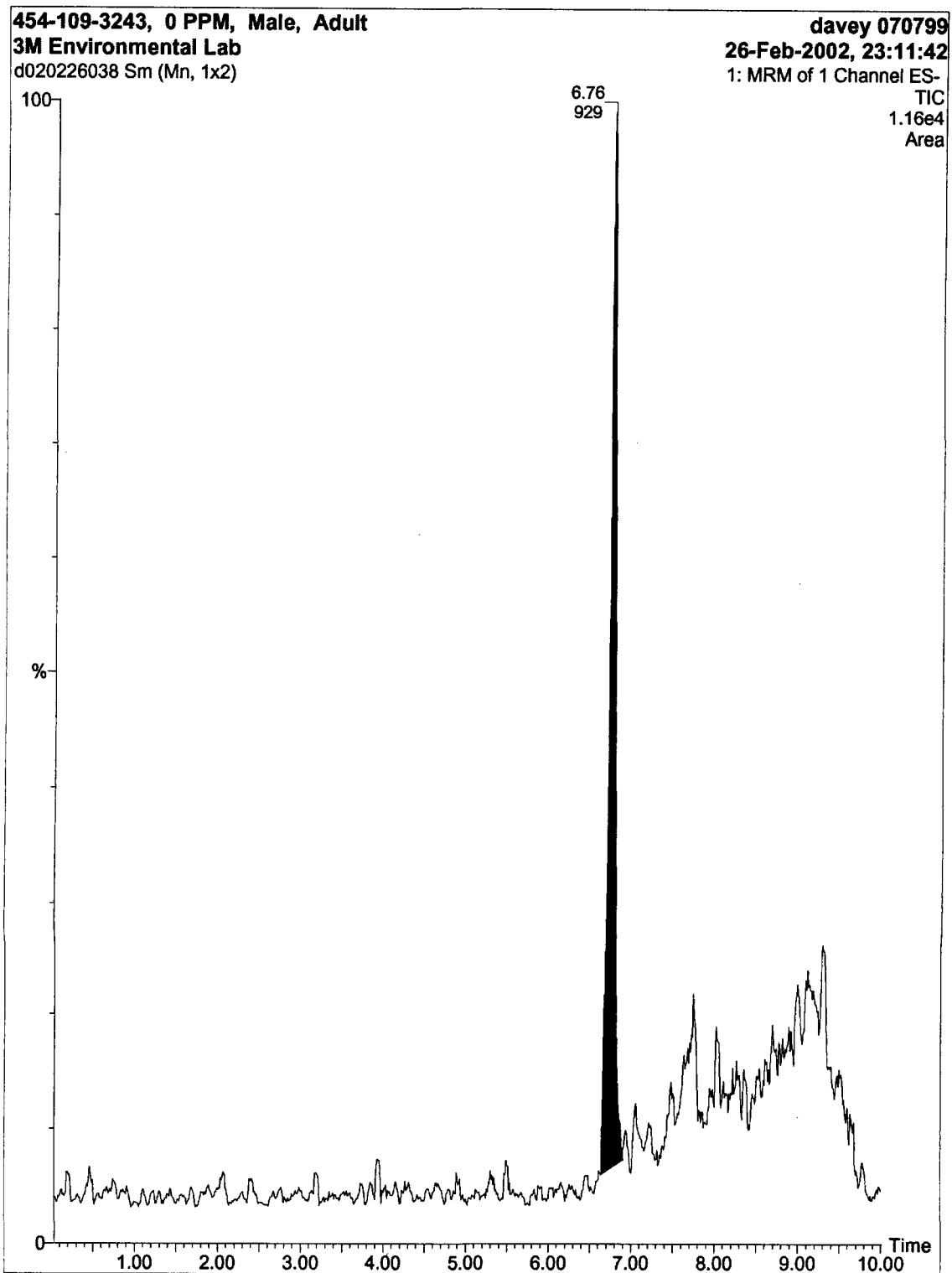


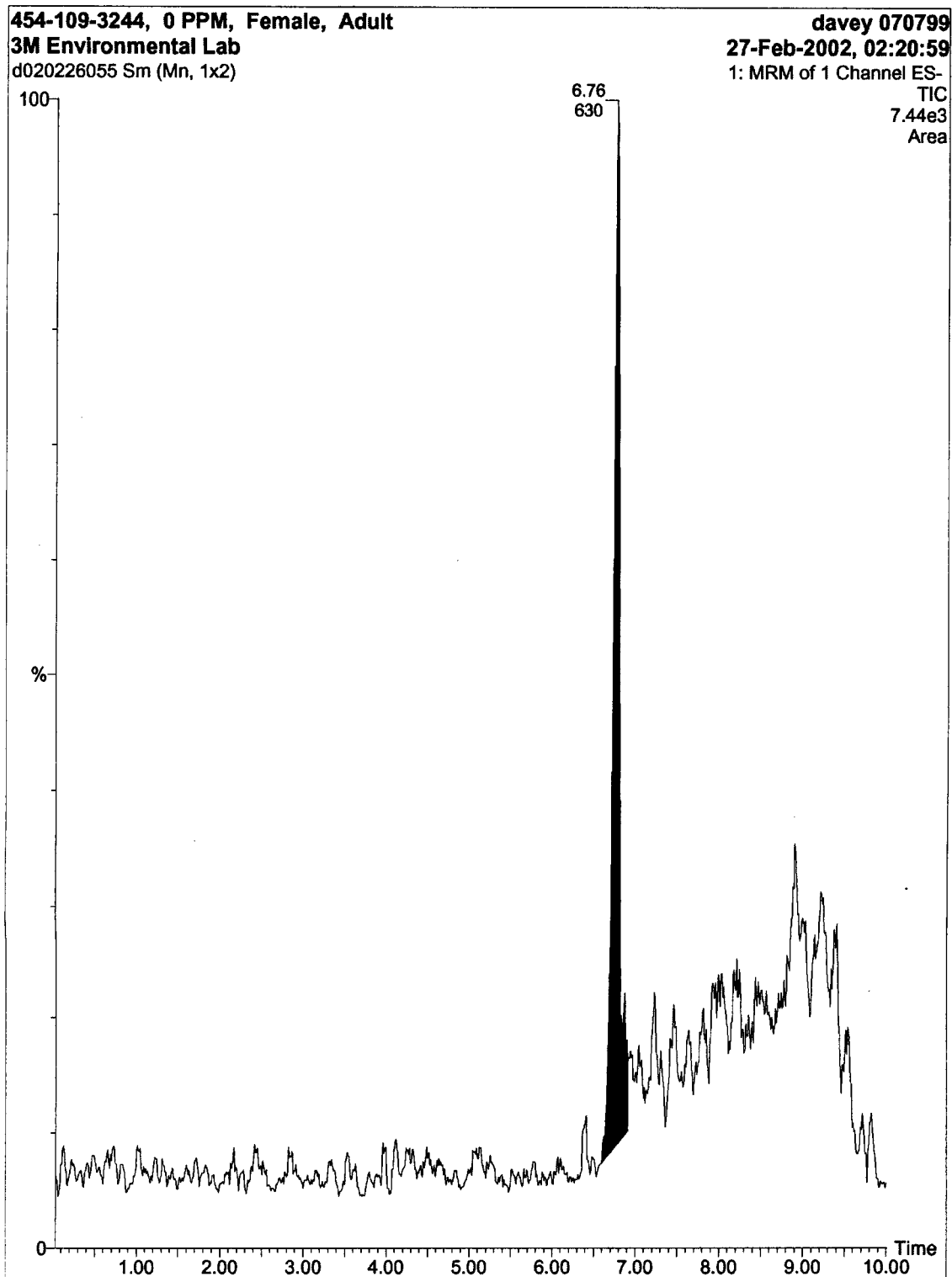


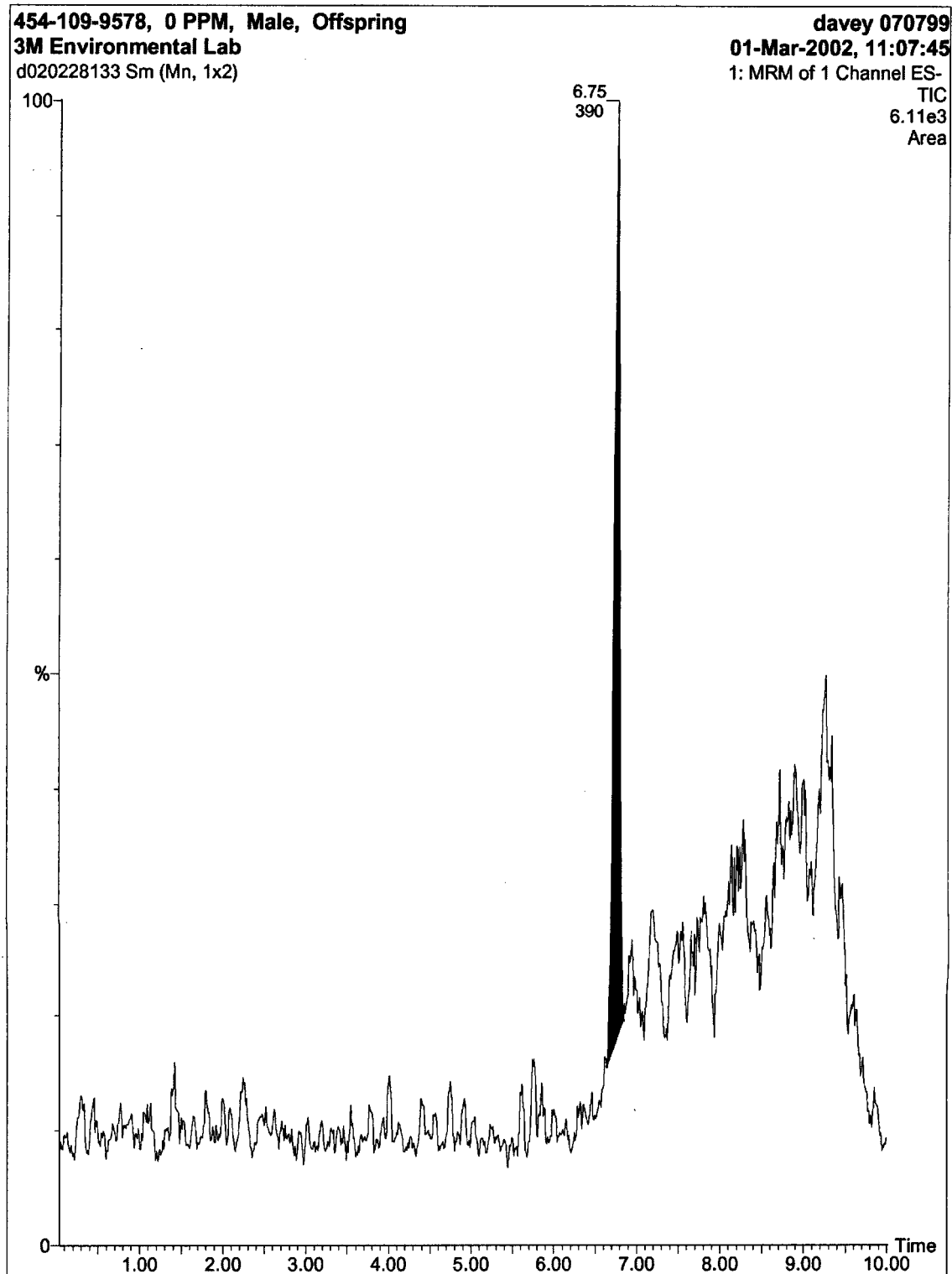


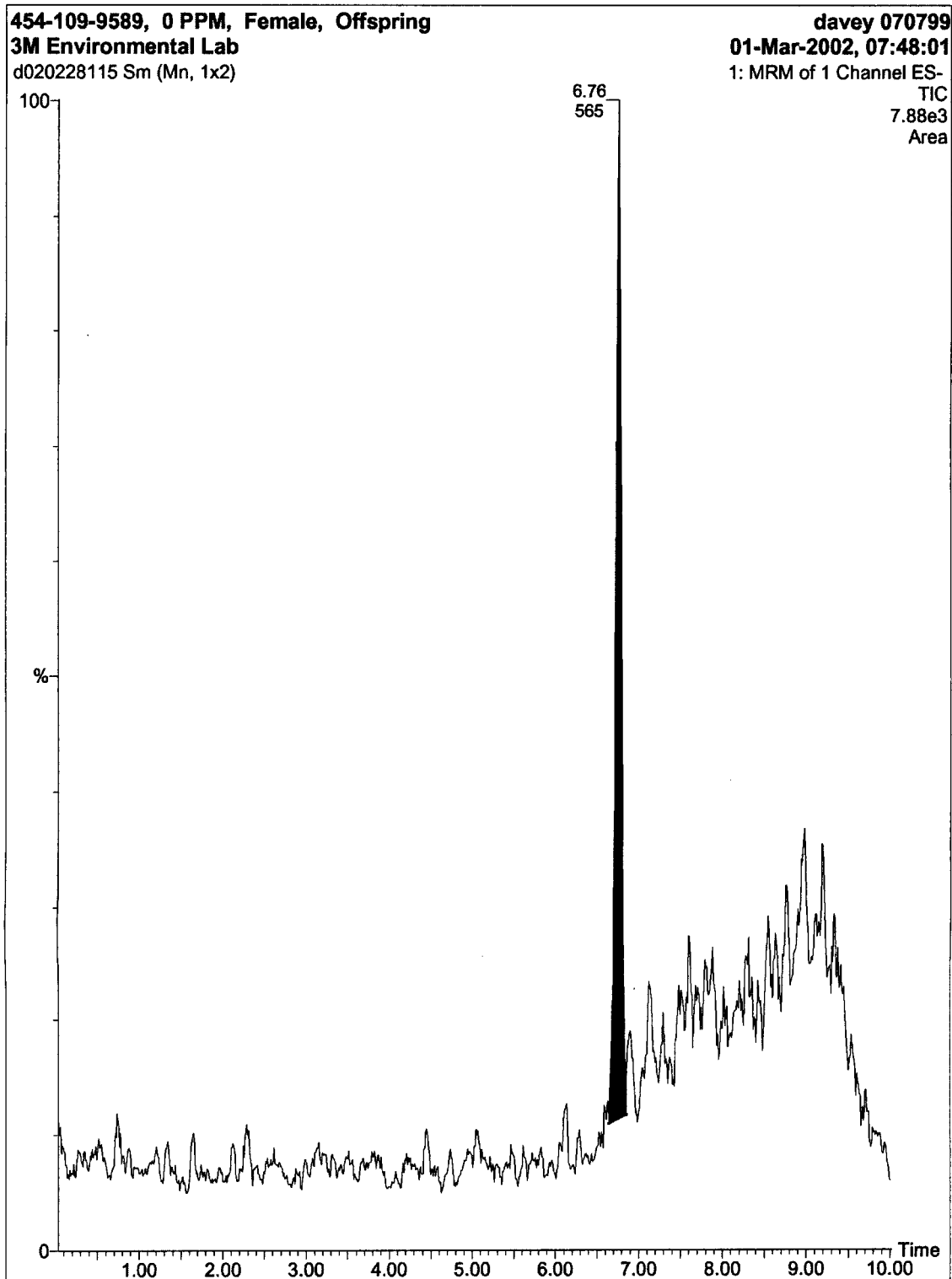


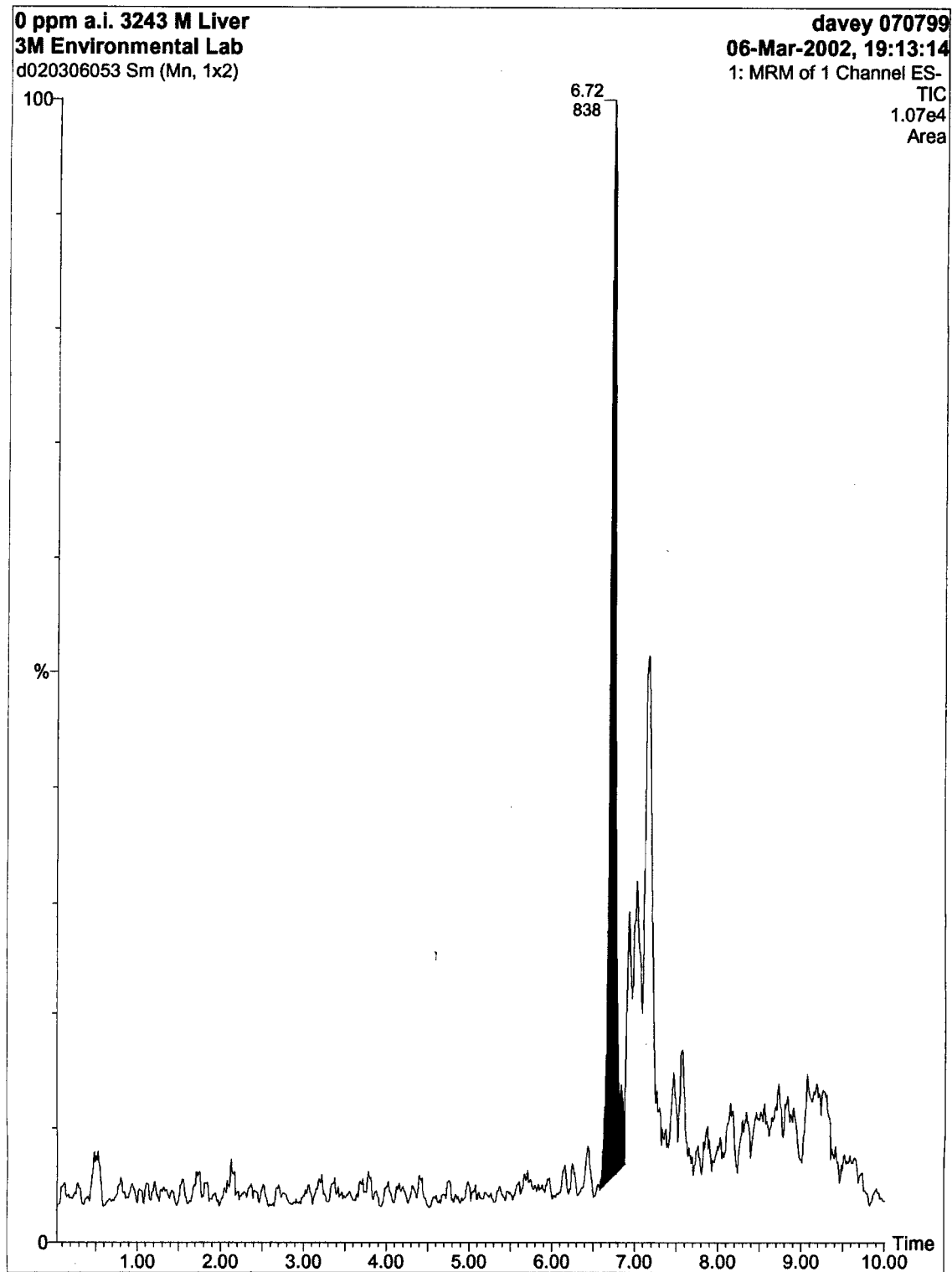


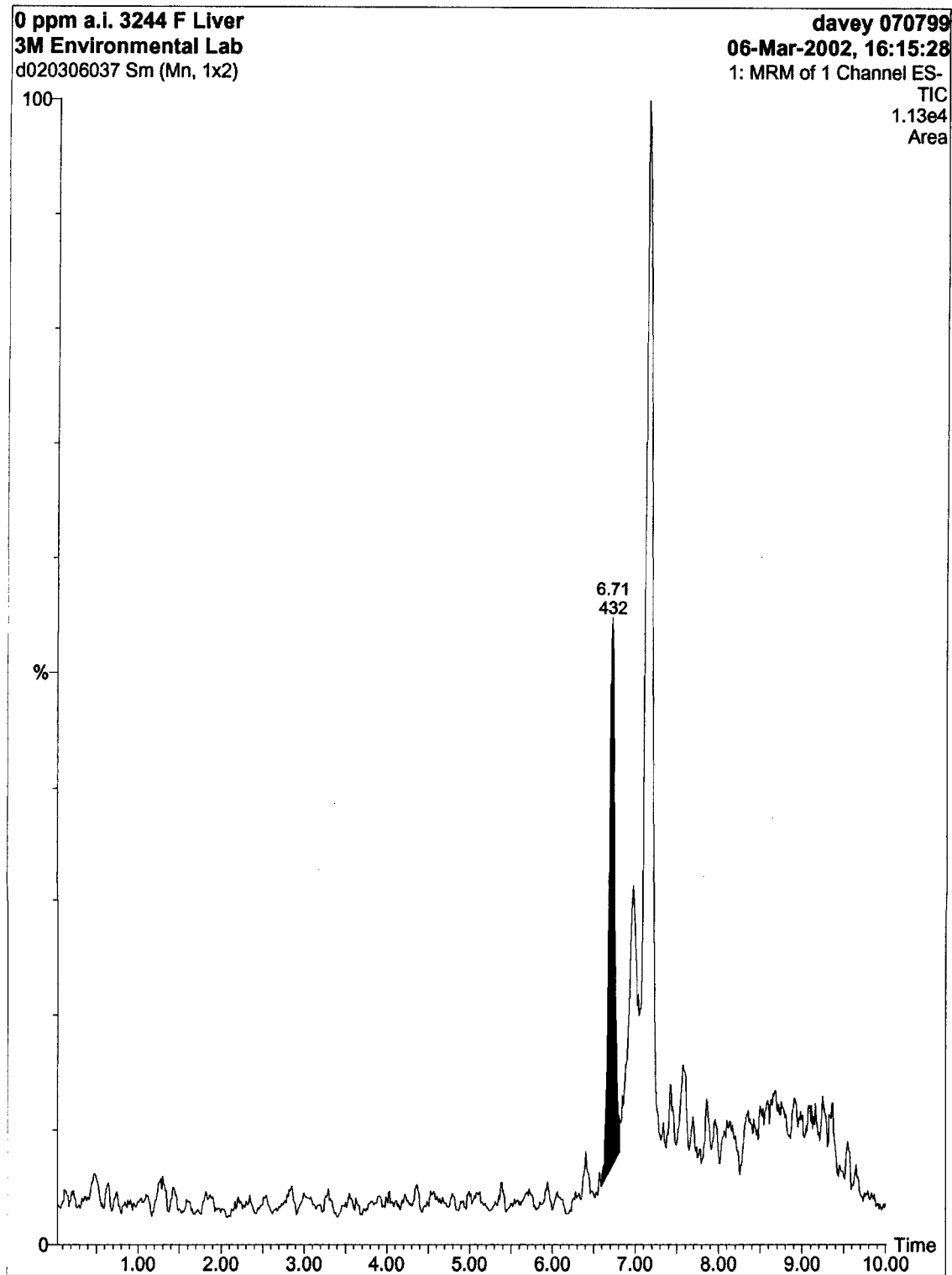
Control Group Male Sera Sample

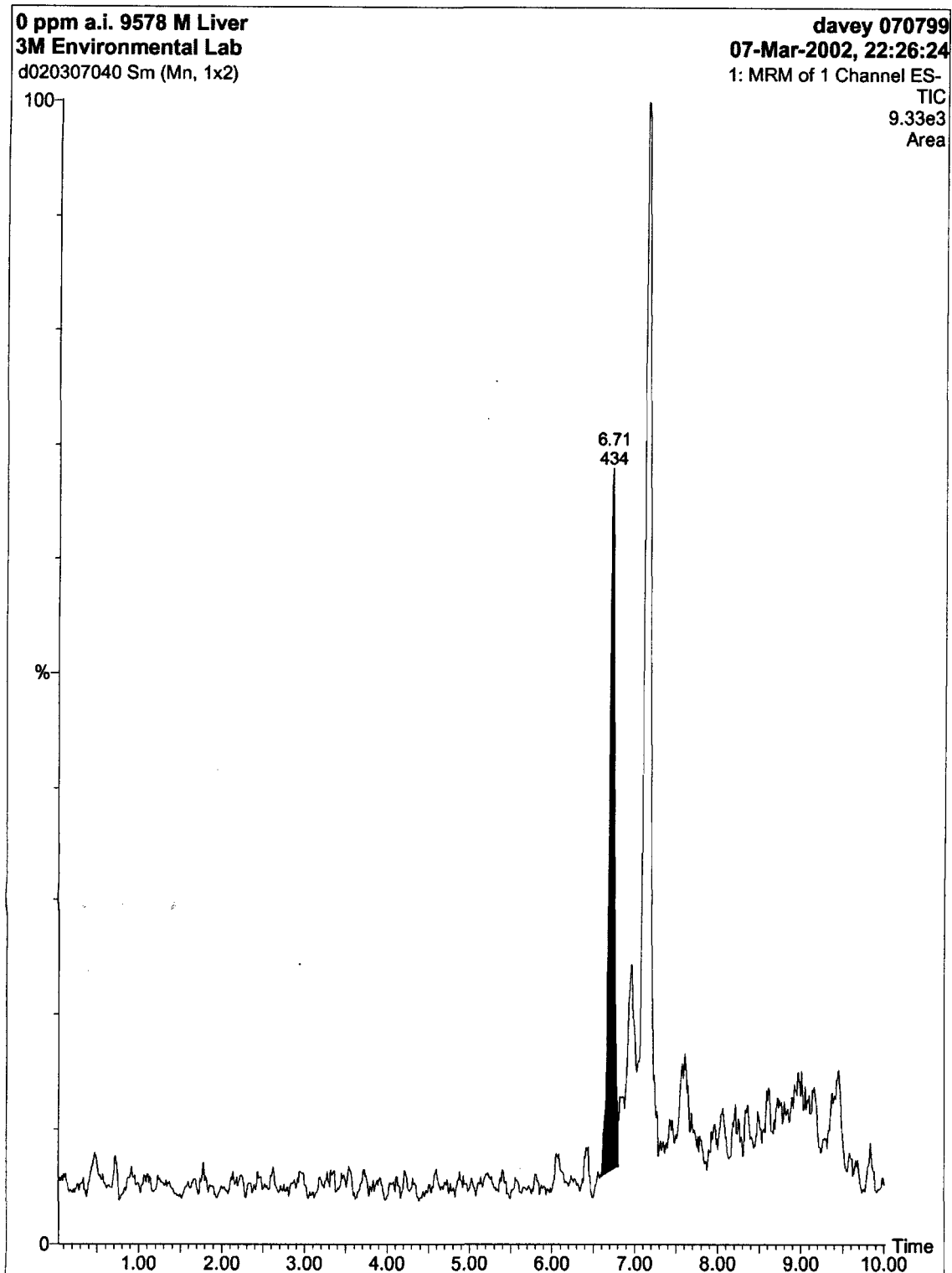
Control Group Female Sera Sample

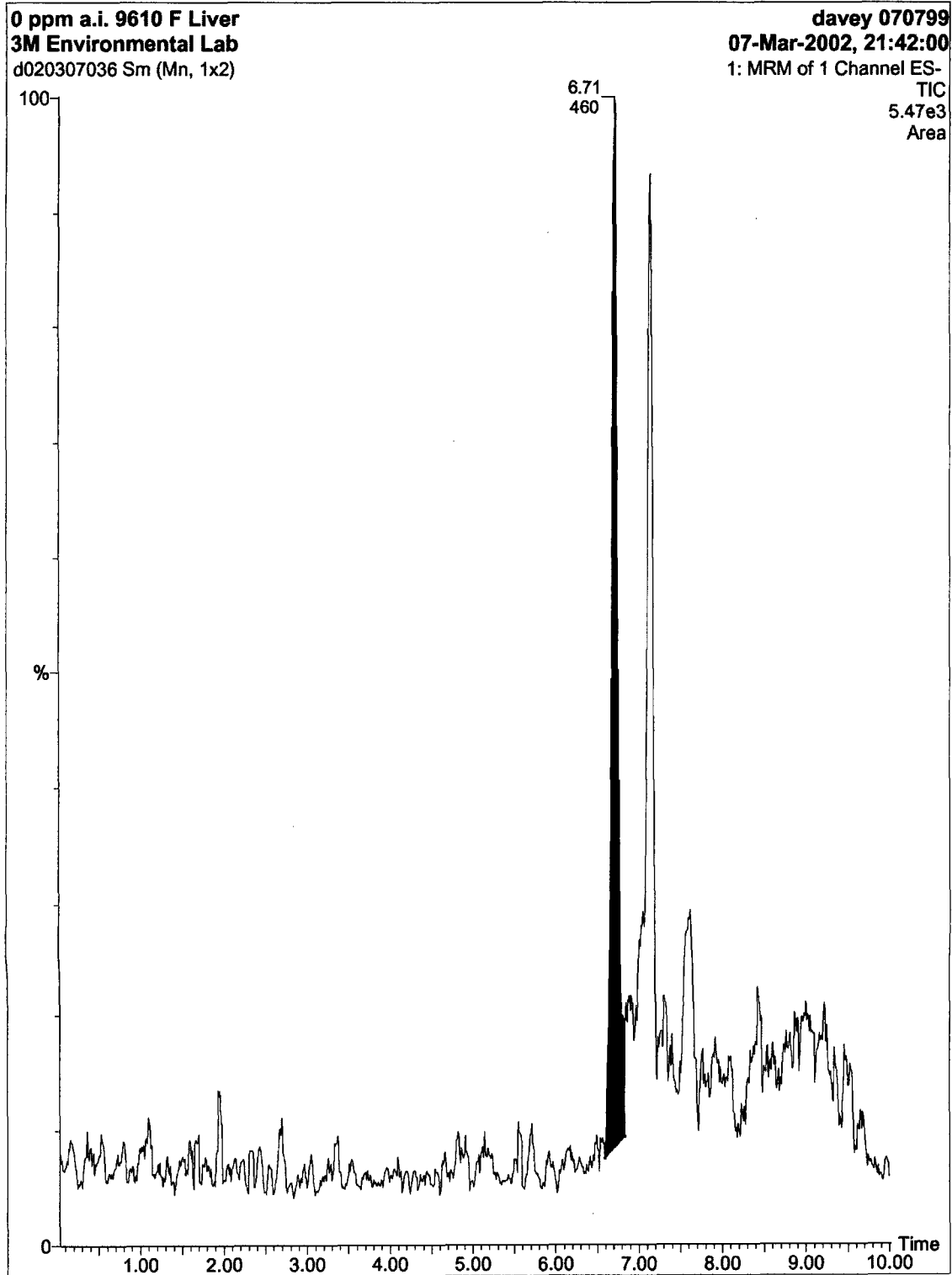
Control Group Male Offspring Sera Sample

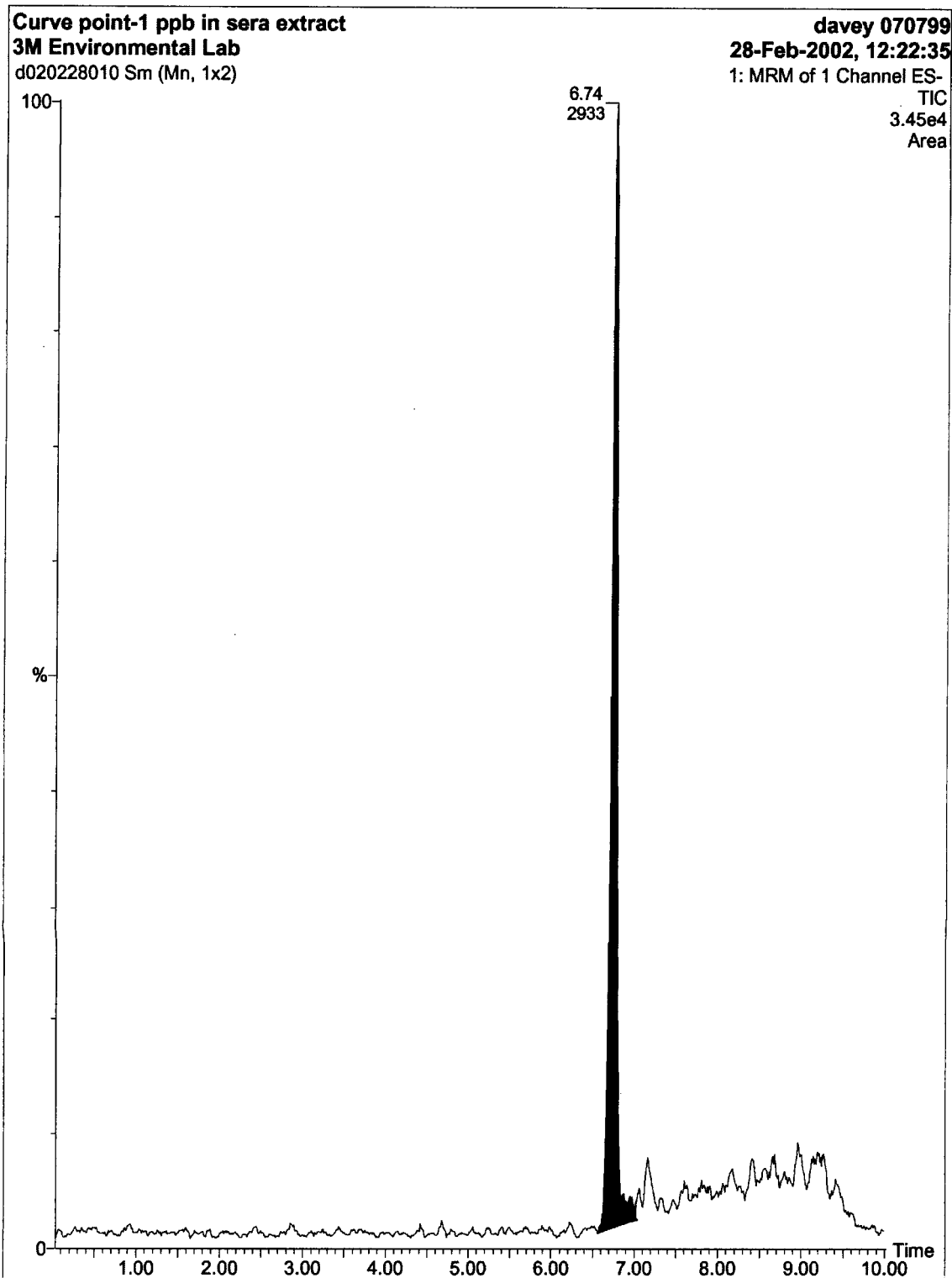
Control Group Female Offspring Sera Sample

Control Group Male Liver Sample

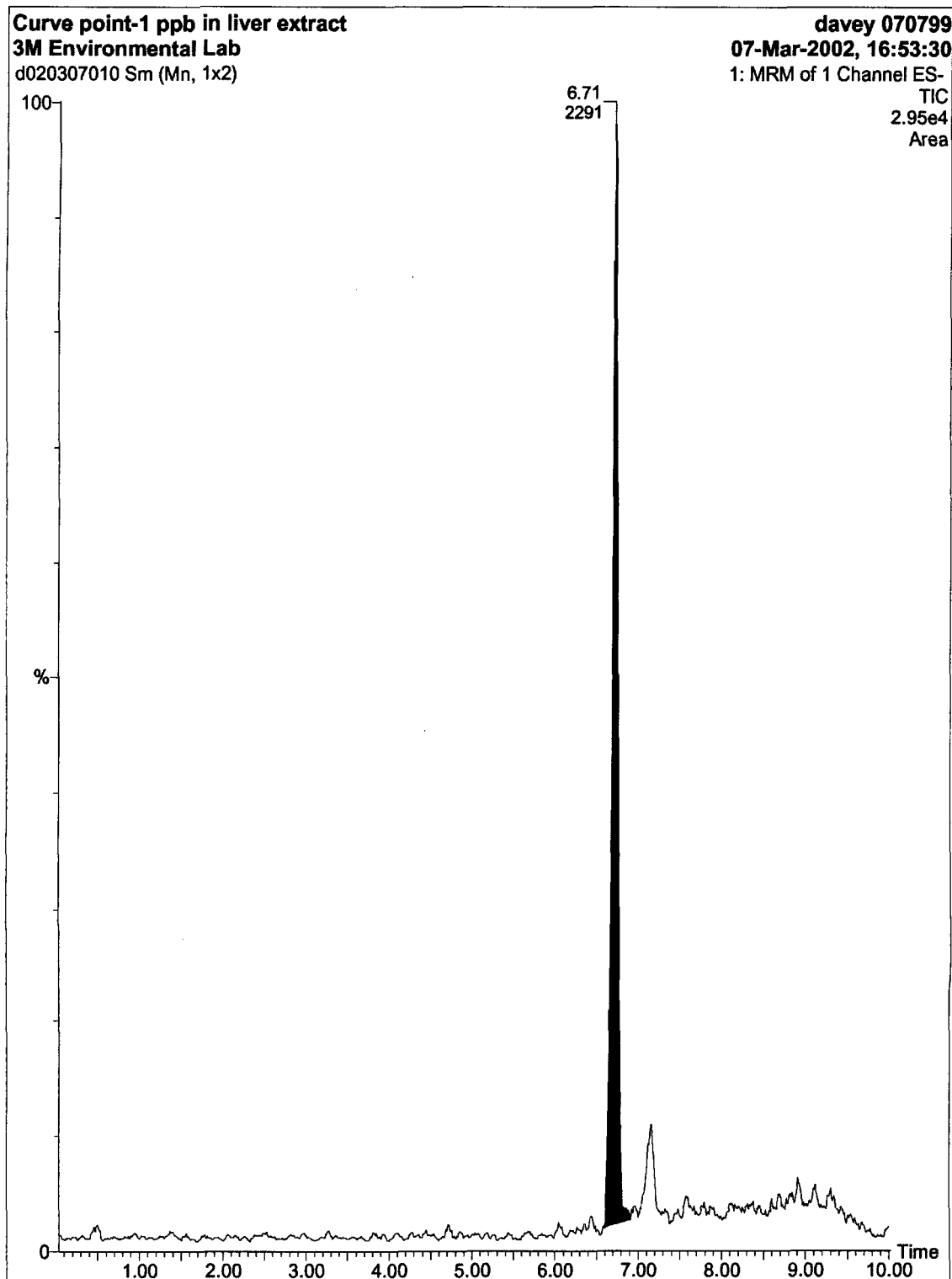
Control Group Female Liver Sample

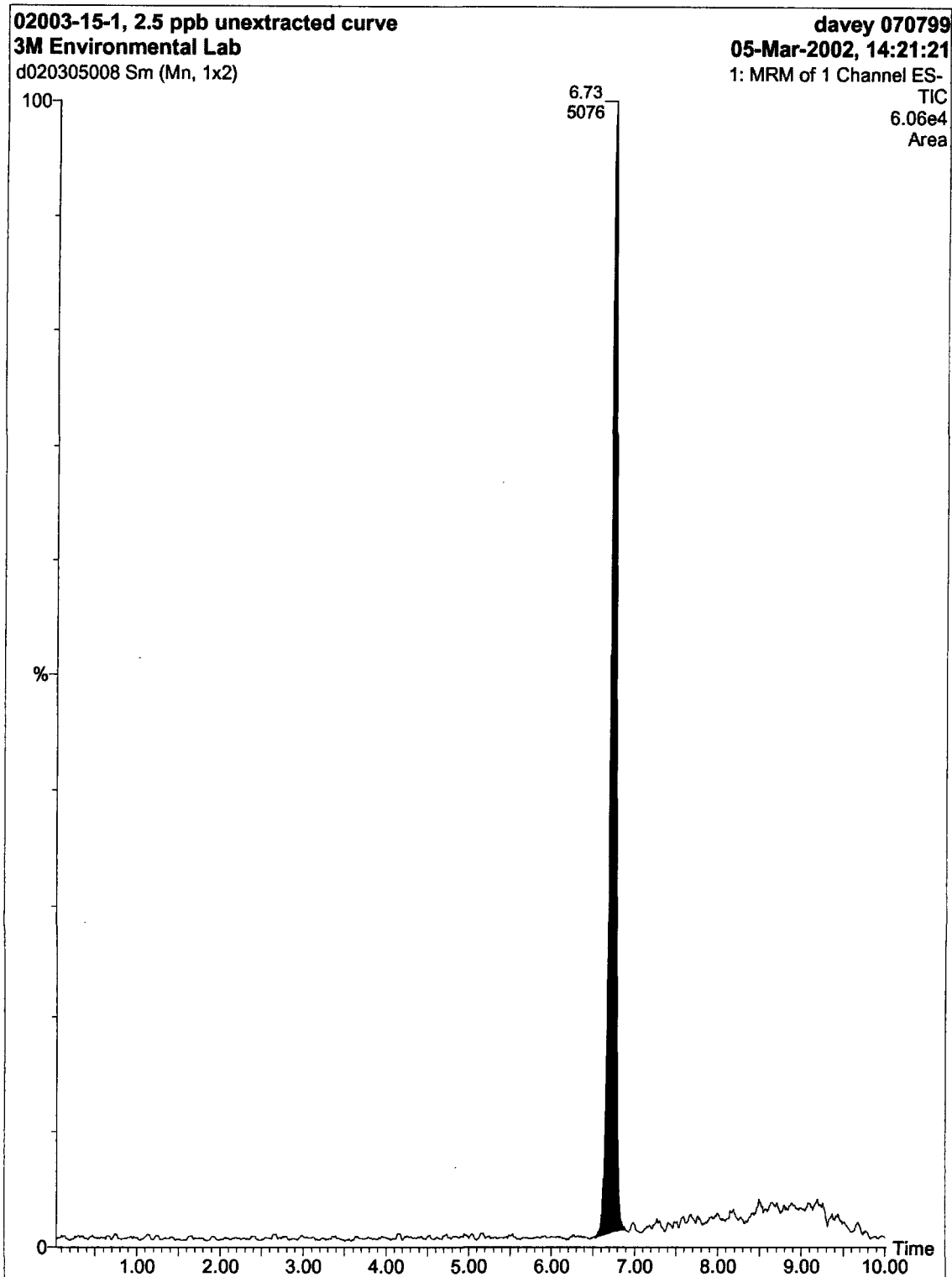
Control Group Male Offspring Liver Sample

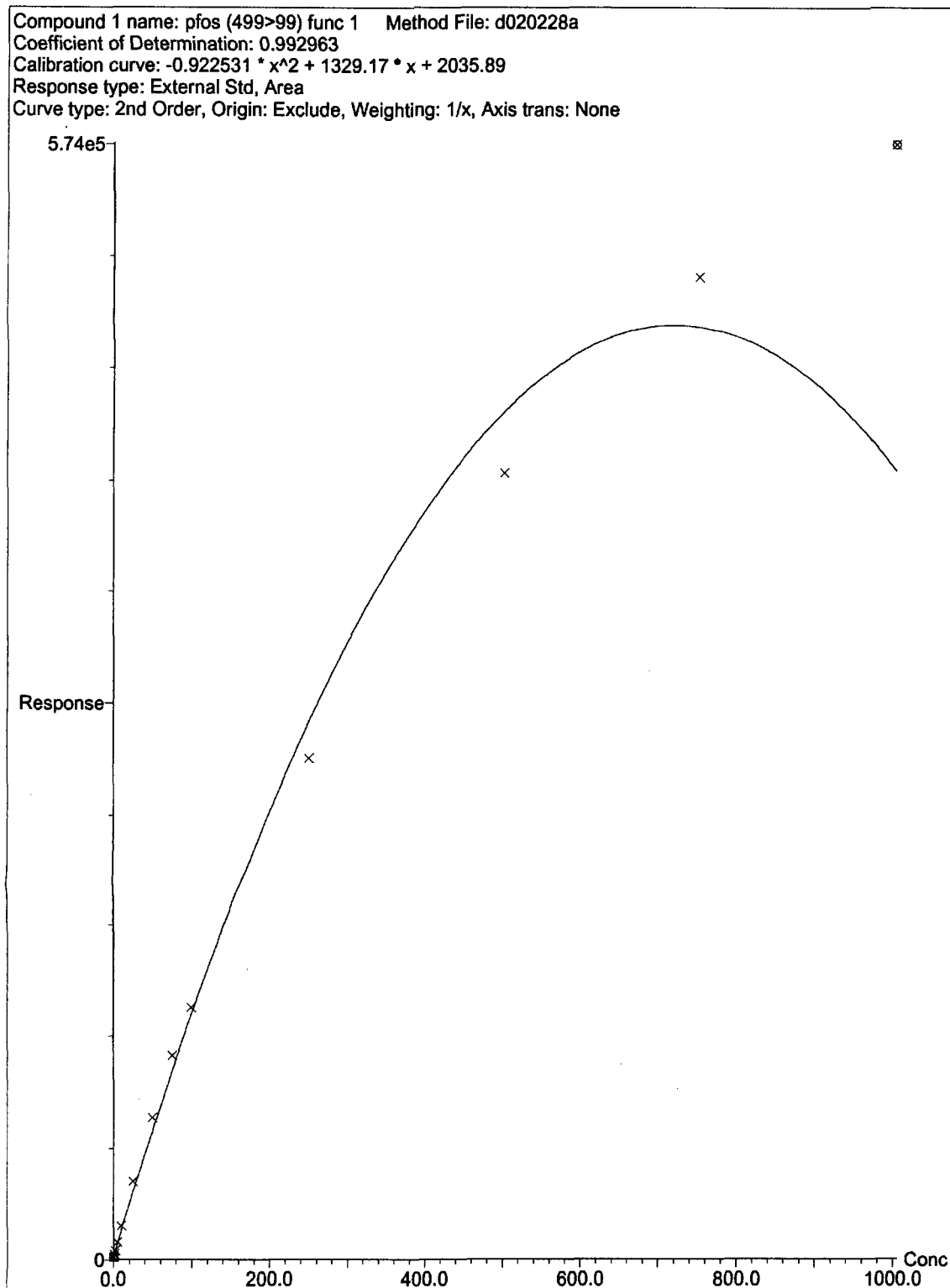
Control Group Female Offspring Liver Sample

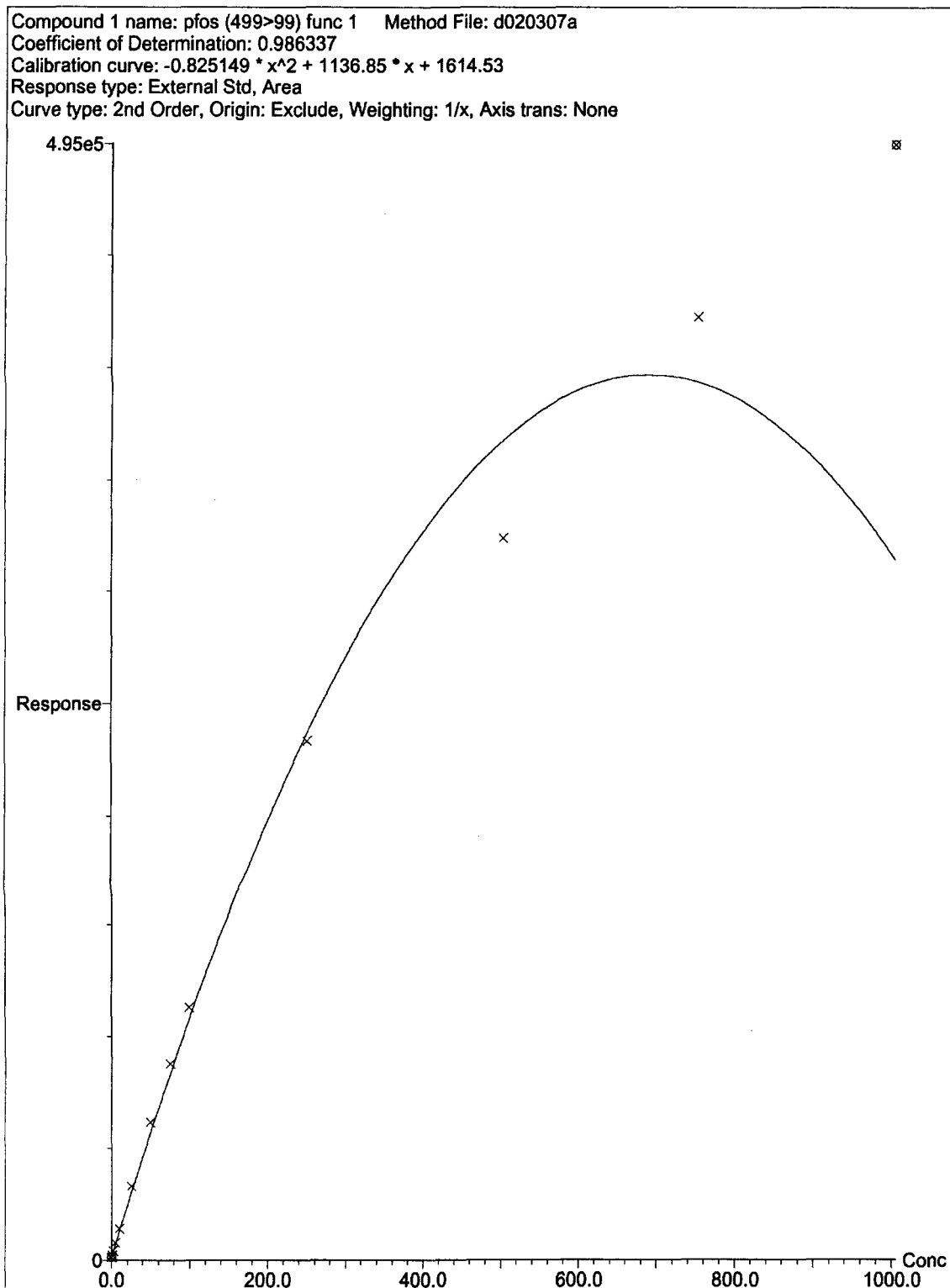
Extracted Sera Calibration Curve Point

Extracted Liver Calibration Curve Point



Unextracted Calibration Curve Point

Extracted Sera Calibration Curve

Extracted Liver Calibration Curve

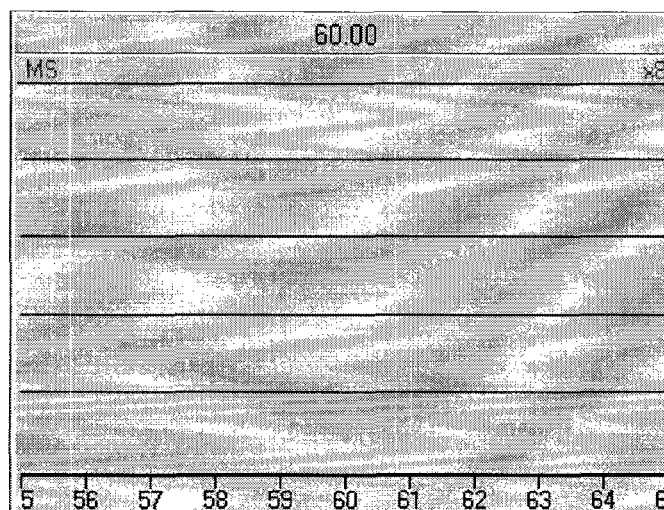
Mass Spectrometer Tune Settings

Tuning Method Report

Page 1

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

Printed: Tue Mar 19 10:22:06 2002



MS

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

Scanning Method Report**Page 1****Method:** C:\MASSLYNX\DAVEY070799.PRO\ACQUDE\10MIN-PFOS-PFDA**Last Modified:** Mon Jan 28 15:20:24 2002**Printed:** Tue Mar 19 08:38:22 2002

Solvent Delay (mins) : 0.00**Function : 1** MRM of 1 Mass Pair (ESP-)**Inter Channel Delay (Secs) :** 0.03**Span (Daltons) :** 0.00**Start Time (Mins) :** 0.00**End Time (Mins) :** 10.00**Repeats :** 1

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

Function : 2 MRM of 1 Mass Pair (ESP-)**Inter Channel Delay (Secs) :** 0.03**Span (Daltons) :** 0.00**Start Time (Mins) :** 0.00**End Time (Mins) :** 10.00**Repeats :** 1

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

ATTACHMENT D: SAMPLE PREPARATION SHEETS

sample number	description	Amount of liver weighed (g)	weighed by/date	Homogenized w/ water to (ml)	homogenized by/date
E01-1326-29473	0 ppm a.i. 3217 M Liver	1.1169g	2/22/02 OK	10	2-25-02 [initials]
E01-1326-29482	0 ppm a.i. 3218 F Liver	1.0095g			
E01-1326-29574	0 ppm a.i. 3219 M Liver	1.1847g			
E01-1326-29583	0 ppm a.i. 3220 F Liver	1.0944g			
E01-1326-29592	0 ppm a.i. 3221 M Liver	1.1181g			
E01-1326-29601	0 ppm a.i. 3222 F Liver	1.0435g			
E01-1326-29610	0 ppm a.i. 3223 M Liver	1.0788g			
E01-1326-29619	0 ppm a.i. 3224 F Liver	1.0425g			
E01-1326-29628	0 ppm a.i. 3225 M Liver	1.1043g			
E01-1326-29637	0 ppm a.i. 3226 F Liver	1.0703g			
E01-1326-29646	0 ppm a.i. 3227 M Liver	1.0486g			
E01-1326-29655	0 ppm a.i. 3228 F Liver	1.0526g			
E01-1326-29664	0 ppm a.i. 3229 M Liver	1.0336g			
E01-1326-29673	0 ppm a.i. 3230 F Liver	1.0411g			
E01-1326-29682	0 ppm a.i. 3231 M Liver	1.0165g			
E01-1326-29691	0 ppm a.i. 3232 F Liver	1.1704g			
E01-1326-29700	0 ppm a.i. 3233 M Liver	1.0395g			
E01-1326-29709	0 ppm a.i. 3234 F Liver	1.1290g			
E01-1326-29718	0 ppm a.i. 3235 M Liver	1.0373g			
E01-1326-29727	0 ppm a.i. 3236 F Liver	1.0800g			
E01-1326-29736	0 ppm a.i. 3237 M Liver	1.1679g			
E01-1326-29745	0 ppm a.i. 3238 F Liver	1.1170g			
E01-1326-29754	0 ppm a.i. 3239 M Liver	1.1493g			
E01-1326-29763	0 ppm a.i. 3240 F Liver	1.0086g			
E01-1326-29772	0 ppm a.i. 3241 M Liver	1.0619g			
E01-1326-29781	0 ppm a.i. 3242 F Liver	1.0907g			
E01-1326-29790	0 ppm a.i. 3243 M Liver	1.0263g			
E01-1326-29799	0 ppm a.i. 3244 F Liver	1.0291g			
E01-1326-29808	0 ppm a.i. 3245 M Liver	1.1541g			
E01-1326-29817	0 ppm a.i. 3246 F Liver	1.1294g			
E01-1326-29826	0 ppm a.i. 3247 M Liver	1.0813g			
E01-1326-29835	0 ppm a.i. 3248 F Liver	1.1075g			
E01-1326-29844	0 ppm a.i. 3249 M Liver	1.0263g			
E01-1326-29853	0 ppm a.i. 3250 F Liver	1.1709g			
E01-1326-29862	0 ppm a.i. 3251 M Liver	1.0088g			
E01-1326-29871	0 ppm a.i. 3252 F Liver	1.0767g			
E01-1326-29880	0 ppm a.i. 3253 M Liver	1.0294g			
E01-1326-29889	0 ppm a.i. 3254 F Liver	1.1344g			
E01-1326-29898	0 ppm a.i. 3255 M Liver	1.0726g			
E01-1326-29907	0 ppm a.i. 3256 F Liver	1.0669g			
E01-1326-29916	10 ppm a.i. 3257 M Liver	1.0654g			
E01-1326-29925	10 ppm a.i. 3258 F Liver	1.0034g			
E01-1326-29934	10 ppm a.i. 3259 M Liver	1.1316g			
E01-1326-29943	10 ppm a.i. 3260 F Liver	1.1331g			
E01-1326-29952	10 ppm a.i. 3261 M Liver	1.1330g			
E01-1326-29961	10 ppm a.i. 3262 F Liver	1.1294g			
E01-1326-29970	10 ppm a.i. 3263 M Liver	1.1404g			
E01-1326-29980	10 ppm a.i. 3264 F Liver	1.1829g			
E01-1326-29989	10 ppm a.i. 3265 M Liver	1.1480g			

sample number	description	Amount of liver weighed (g)	weighed by/date	Homogenized w/ water to (ml)	homogenized by/date
E01-1326-29998	10 ppm a.i. 3266 F Liver	1.0487g	2-22-02 OK	10	2-26-02 (h)
E01-1326-30007	10 ppm a.i. 3267 M Liver	1.0620g			
E01-1326-30016	10 ppm a.i. 3268 F Liver	1.1214g			
E01-1326-30025	10 ppm a.i. 3269 M Liver	1.1087g			
E01-1326-30034	10 ppm a.i. 3270 F Liver	1.0751g			
E01-1326-30043	10 ppm a.i. 3271 M Liver	1.0313g			
E01-1326-30052	10 ppm a.i. 3272 F Liver	1.0658g			
E01-1326-30061	10 ppm a.i. 3273 M Liver	1.1399g			
E01-1326-30070	10 ppm a.i. 3274 F Liver	1.1226g			
E01-1326-30079	10 ppm a.i. 3275 M Liver	1.1143g			
E01-1326-30088	10 ppm a.i. 3276 F Liver	1.0953g			
E01-1326-30097	10 ppm a.i. 3277 M Liver	1.0062g			
E01-1326-30106	10 ppm a.i. 3278 F Liver	1.1956g			
E01-1326-30115	10 ppm a.i. 3279 M Liver	1.0380g			
E01-1326-30124	10 ppm a.i. 3280 F Liver	1.0600g			
E01-1326-30133	10 ppm a.i. 3281 M Liver	1.0609g			
E01-1326-30142	10 ppm a.i. 3282 F Liver	1.0275g			
E01-1326-30151	10 ppm a.i. 3283 M Liver	1.0955g			
E01-1326-30160	10 ppm a.i. 3284 F Liver	1.0915g			
E01-1326-30169	10 ppm a.i. 3285 M Liver	1.1981g			
E01-1326-30178	10 ppm a.i. 3286 F Liver	1.0657g			
E01-1326-30187	10 ppm a.i. 3287 M Liver	1.0500g			
E01-1326-30196	10 ppm a.i. 3288 F Liver	1.0320g			
E01-1326-30205	10 ppm a.i. 3289 M Liver	1.0251g			
E01-1326-30214	10 ppm a.i. 3290 F Liver	1.0352g			
E01-1326-30223	10 ppm a.i. 3291 M Liver	1.0441g			
E01-1326-30232	10 ppm a.i. 3292 F Liver	1.0658g			
E01-1326-30241	10 ppm a.i. 3293 M Liver	1.0979g			
E01-1326-30250	10 ppm a.i. 3294 F Liver	1.0627g			
E01-1326-30259	10 ppm a.i. 3295 M Liver	1.0815g			
E01-1326-30268	10 ppm a.i. 3296 F Liver	1.1238g			
E01-1367-31295	0 ppm a.i. 9583 F Liver	1.1308g	2-22-02 OK	2-22-02 (h)	2-22-02 (h)
E01-1367-31301	0 ppm a.i. 9591 M Liver	1.0411g			
E01-1367-31307	0 ppm a.i. 9589 F Liver	1.0002g			
E01-1367-31313	0 ppm a.i. 9592 F Liver	1.0390g			
E01-1367-31319	0 ppm a.i. 9598 F Liver	1.1204g			
E01-1367-31325	0 ppm a.i. 9603 F Liver	1.1550g			
E01-1367-31331	0 ppm a.i. 9606 M Liver	1.1314g			
E01-1367-31337	0 ppm a.i. 9610 F Liver	1.0217g			
E01-1367-31343	0 ppm a.i. 9573 F Liver	1.0122g			
E01-1367-31349	0 ppm a.i. 9578 M Liver	1.0413g			
E01-1367-31356	10 ppm a.i. 9625 M Liver	1.0166g			
E01-1367-31362	10 ppm a.i. 9629 F Liver	1.1676g			
E01-1367-31368	10 ppm a.i. 9633 F Liver	1.0510g			
E01-1367-31374	10 ppm a.i. 9634 F Liver	1.1638g			
E01-1367-31380	10 ppm a.i. 9645 F Liver	1.1610g			
E01-1367-31386	10 ppm a.i. 9648 F Liver	1.1125g			
E01-1367-31392	10 ppm a.i. 9636 M Liver	1.1367g			
E01-1367-31398	10 ppm a.i. 9642 M Liver	1.1252g			
E01-1367-31404	10 ppm a.i. 9654 F Liver	1.0770g			
E01-1367-31410	10 ppm a.i. 9659 F Liver	1.0581g			

Prep Date: 3/4/2002

Analysts initials: OK/HOJ

OK/HOJ
3402

Method Revision: ETS-8-231

Study Number: E01-1256

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
E01-1326-36778	Blank H2O-day 1	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-8009	NA
E01-1326-36780	Blank mallard liver-day 1	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-8009	
E01-1326-36781	Mallard Liver MS-day 1	40+6	Waters, 1g, 6ml, lot W2045B2	50ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36782	Mallard Liver MSD-day 1	40+6	Waters, 1g, 6ml, lot W2045B2	50ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36783	Curve point-0.2 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	0.4ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36784	Curve point-0.5 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	1ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36785	Curve point-1 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	2ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36786	Curve point-2.5 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	5ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36787	Curve point-5 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	10ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36788	Curve point-10 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	20ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36789	Curve point-25 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	50ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36790	Curve point-50 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	100ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36791	Curve point-75 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	150ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36792	Curve point-100 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	200ul of 02001-66	2ml of MeOH TN-A-8009	
E01-1326-36793	Curve point-100 ppb-2 in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	4ul of 02001-53	2ml of MeOH TN-A-8009	
E01-1326-36794	Curve point-250 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	10ul of 02001-53	2ml of MeOH TN-A-8009	
E01-1326-36795	Curve point-500 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	20ul of 02001-53	2ml of MeOH TN-A-8009	
E01-1326-36796	Curve point-750 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	30ul of 02001-53	2ml of MeOH TN-A-8009	
E01-1326-36797	Curve point-1000 ppb in liver extract	40+6	Waters, 1g, 6ml, lot W2045B2	40ul of 02001-53	2ml of MeOH TN-A-8009	
E01-1326-36798	QC- 25ppb -1	40+6	Waters, 1g, 6ml, lot W2045B2	1ul of 02001-53	2ml of MeOH TN-A-8009	
E01-1326-36799	QC- 25ppb -2	40+6	Waters, 1g, 6ml, lot W2045B2	1ul of 02001-53	2ml of MeOH TN-A-8009	

Blank Liver TN-A-6023; Amount of liver: 5.0697 g

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

Aliquot 1 ml of liver 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 VWR 31N 041694)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge #1769613)

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-6009)

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-6009) into appropriate 15 ml centrifuge tube

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

Transfer sample into appropriately marked autovial

(AC) Homogenizing was done by HOJ, & everything else by OK. 8-28-02

Prep Date: 3/4/2002
Analysts initials: OK/HOJ3.4.02
OK/HOJ

Method Revision: ETS-8-231

Study Number: E01-1256 (2-4)
Matrix: Liver 1256 3.4.02

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
E01-1326-36800	QC- 25ppb -3	40+6	Waters, 1g, 6ml, lot W2045B2	1ul of 02001-53	2ml of MeOH TN-A-6009	NA
E01-1326-36801	QC- 250ppb -1	40+6	Waters, 1g, 6ml, lot W2045B2	10ul of 02001-53	2ml of MeOH TN-A-6009	
E01-1326-36802	QC- 250ppb -2	40+6	Waters, 1g, 6ml, lot W2045B2	10ul of 02001-53	2ml of MeOH TN-A-6009	
E01-1326-36803	QC- 250ppb -3	40+6	Waters, 1g, 6ml, lot W2045B2	10ul of 02001-53	2ml of MeOH TN-A-6009	
E01-1326-36804	QC- 4.0ppm -1	40+6	Waters, 1g, 6ml, lot W2045B2	160ul of 02001-53	2ml of MeOH TN-A-6009	
E01-1326-36805	QC- 4.0ppm -2	40+6	Waters, 1g, 6ml, lot W2045B2	160ul of 02001-53	2ml of MeOH TN-A-6009	
E01-1326-36806	QC- 4.0ppm -3	40+6	Waters, 1g, 6ml, lot W2045B2	160ul of 02001-53	2ml of MeOH TN-A-6009	
E01-1326-29473	0 ppm a.i. 3217 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29482	0 ppm a.i. 3218 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29574	0 ppm a.i. 3219 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29583	0 ppm a.i. 3220 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29592	0 ppm a.i. 3221 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29601	0 ppm a.i. 3222 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29610	0 ppm a.i. 3223 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29619	0 ppm a.i. 3224 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29628	0 ppm a.i. 3225 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29637	0 ppm a.i. 3226 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29646	0 ppm a.i. 3227 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29655	0 ppm a.i. 3228 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29664	0 ppm a.i. 3229 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29673	0 ppm a.i. 3230 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	✓

OK 4.9.02

Blank Liver TN-A-6023; Amount of liver: 5.0597 g

Homogenize liver with Kandiyohi water; Amount of water added 45 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 51N 041094)

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-6009)

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-6009) into appropriate 15 ml centrifuge tube

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

Transfer sample into appropriately marked autovial

AC Homogenizing was done by HOJ & everything else by OK 8.28.02 OK

SPE Standard Curves--Solids

Prep Date(s): 03/04/02, 03/05/02, 03/06/02, 03/07/02
 Analyst(s): OK/HOJ
 Sample Matrix: Mallard Liver
 Method/Revision: ETS-8-231.1
 Target Analyte(s): PFOS

Study Number: E01-1256
 Equipment Number: NA
 Final Solvent & TN Number: MeOH/TN-A-6009
 Blank Tissue/Identifier: Mallard Liver/TN-A-6023

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

Initial homogenate: 5.0597g/50.0597g+mL = 0.1011 g/mL

Blank liver initial homogenate (g/mL) for curves: 0.1011
 Final solvent volume (mL) for curves: 2.000

Actual Concentrations of Standards in the FC Mix

Standard Number	PFOS Std Conc. ug/mL	PFOS Am't Spiked mL	PFDA Std Conc. ug/mL	All Am't Spiked mL	All Initial Homogenate g/mL	All Final Vol. mL
E01-1326-36783, 0.2 ppb	1.004	0.0004	100.7	0.002	0.1011	2.000
E01-1326-36784, 0.5 ppb	1.004	0.0010	100.7	0.002	0.1011	2.000
E01-1326-36785, 1.0 ppb	1.004	0.0020	100.7	0.002	0.1011	2.000
E01-1326-36786, 2.5 ppb	1.004	0.0050	100.7	0.002	0.1011	2.000
E01-1326-36787, 5.0 ppb	1.004	0.0100	100.7	0.002	0.1011	2.000
E01-1326-36788, 10 ppb	1.004	0.0200	100.7	0.002	0.1011	2.000
E01-1326-36789, 25 ppb	1.004	0.0500	100.7	0.002	0.1011	2.000
E01-1326-36790, 50 ppb	1.004	0.1000	100.7	0.002	0.1011	2.000
E01-1326-36791, 75 ppb	1.004	0.1500	100.7	0.002	0.1011	2.000
E01-1326-36792, 100 ppb	1.004	0.2000	100.7	0.002	0.1011	2.000
E01-1326-36793, 100 ppb-2	50.20	0.0040	100.7	0.002	0.1011	2.000
E01-1326-36794, 250 ppb	50.20	0.0100	100.7	0.002	0.1011	2.000
E01-1326-36795, 500 ppb	50.20	0.0200	100.7	0.002	0.1011	2.000
E01-1326-36796, 750 ppb	50.20	0.0300	100.7	0.002	0.1011	2.000
E01-1326-36797, 1000 ppb	50.20	0.0400	100.7	0.002	0.1011	2.000
QC-25 ppb	50.20	0.0010	100.7	0.002	0.1011	2.000
QC-250 ppb	50.20	0.0100	100.7	0.002	0.1011	2.000
QC-4 ppm	50.20	0.1600	100.7	0.002	0.1011	2.000
Mallard Liver-MS/MSD-Day 1	1.004	0.0500	100.7	0.002	0.1011	2.000
Mallard Liver-MS/MSD-Day 2	1.004	0.0500	100.7	0.002	0.1011	2.000
Mallard Liver-MS/MSD-Day 3	1.004	0.0500	100.7	0.002	0.1011	2.000
Mallard Liver-MS/MSD-Day 4	1.004	0.0500	100.7	0.002	0.1011	2.000

Calculated Concentrations of Standards in the sample matrix

Standard Number	PFOS Std Conc. ng/g	PFDA Std Conc. ng/g
E01-1326-36783, 0.2 ppb	3.97	1993
E01-1326-36784, 0.5 ppb	9.93	1993
E01-1326-36785, 1.0 ppb	19.9	1993
E01-1326-36786, 2.5 ppb	49.7	1993
E01-1326-36787, 5.0 ppb	99.3	1993
E01-1326-36788, 10 ppb	199	1993
E01-1326-36789, 25 ppb	497	1993
E01-1326-36790, 50 ppb	993	1993
E01-1326-36791, 75 ppb	1490	1993
E01-1326-36792, 100 ppb	1987	1993
E01-1326-36793, 100 ppb-2	1987	1993
E01-1326-36794, 250 ppb	4967	1993
E01-1326-36795, 500 ppb	9933	1993
E01-1326-36796, 750 ppb	14900	1993
E01-1326-36797, 1000 ppb	19867	1993
QC-25 ppb	497	1993
QC-250 ppb	4967	1993
QC-4 ppm	79467	1993
Mallard Liver-MS/MSD-Day 1	497	1993
Mallard Liver-MS/MSD-Day 2	497	1993
Mallard Liver-MS/MSD-Day 3	497	1993
Mallard Liver-MS/MSD-Day 4	497	1993

Calculated Concentrations of Standards in the final solvent

Standard Number	PFOS Std Conc. ng/mL	PFDA Std Conc. ng/mL
E01-1326-36783, 0.2 ppb	0.201	101
E01-1326-36784, 0.5 ppb	0.502	101
E01-1326-36785, 1.0 ppb	1.00	101
E01-1326-36786, 2.5 ppb	2.51	101
E01-1326-36787, 5.0 ppb	5.02	101
E01-1326-36788, 10 ppb	10.0	101
E01-1326-36789, 25 ppb	25.1	101
E01-1326-36790, 50 ppb	50.2	101
E01-1326-36791, 75 ppb	75.3	101
E01-1326-36792, 100 ppb	100	101
E01-1326-36793, 100 ppb-2	100	101
E01-1326-36794, 250 ppb	251	101
E01-1326-36795, 500 ppb	502	101
E01-1326-36796, 750 ppb	753	101
E01-1326-36797, 1000 ppb	1004	101
QC-25 ppb	25.1	101
QC-250 ppb	251	101
QC-4 ppm	4016	101
Mallard Liver-MS/MSD-Day 1	25.1	101
Mallard Liver-MS/MSD-Day 2	25.1	101
Mallard Liver-MS/MSD-Day 3	25.1	101
Mallard Liver-MS/MSD-Day 4	25.1	101

Calculation sheet
added for clarity

LAS
11/26/02

Verified by: LAS 11/26/02

Bottle-Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR, 5mL	
Date:	3/4/02	<i>OK</i> <i>34.02</i>
Initials:	ok	
Dispenser ID:	799	
Balance ID:	900	
Volume (mL):	5	
Solvent:	Acetonitrile	
ID #:	tn-a-4145	

	Mass of solvent (g)	Density ¹ (g/ml)	Volume of Solvent
1	3.9613	0.7857	5.0417
2	3.9484	0.7857	5.0253
3	4.0278	0.7857	5.1264
4	3.9249	0.7857	4.9954
5	3.9858	0.7857	5.0729
Average Volume:			5.0524
% Accuracy:			101.05
Std. Dev.:			0.04997
% C.V.:			0.99
Pass/Fail:			PASS

PASS

PASS

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

Corrective Actions: None Needed! OK 34.02

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

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

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

Acetone $d_0^{25} = 0.788$

Methanol $d_4^{25} = 0.7866$

Methylene Chloride $d_4^{20} = 1.3255$

Acetonitrile $d_4^{20} = 0.7857$

Prep Date: 3.5.02
Analysts initials: OK

Method Revision: ETS-8-231

Study Number: E01-1326-20

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
E01-1326-36807	Blank H2O-day 2	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	NA
E01-1326-36809	Blank mallard liver-day 2	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-36810	Mallard Liver MS-day 2	40+6	Waters, 1g, 6ml, lot W2045B2	50ul of 02001-66	2ml of MeOH TN-A-6009	
E01-1326-36811	Mallard Liver MSD-day 2	40+6	Waters, 1g, 6ml, lot W2045B2	50ul of 02001-66	2ml of MeOH TN-A-6009	
E01-1326-29682	0 ppm a.i. 3231 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29691	0 ppm a.i. 3232 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29700	0 ppm a.i. 3233 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29709	0 ppm a.i. 3234 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29718	0 ppm a.i. 3235 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29727	0 ppm a.i. 3236 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29736	0 ppm a.i. 3237 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29745	0 ppm a.i. 3238 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29754	0 ppm a.i. 3239 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29763	0 ppm a.i. 3240 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29772	0 ppm a.i. 3241 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29781	0 ppm a.i. 3242 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29790	0 ppm a.i. 3243 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29799	0 ppm a.i. 3244 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29808	0 ppm a.i. 3245 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29817	0 ppm a.i. 3246 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29826	0 ppm a.i. 3247 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	

Blank Liver TN-A-6023; Amount of liver: 5.0597g
 Homogenize liver with Kandiyohi water; Amount of water added 45 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 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-6009)
 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-6009) 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

OK
3.5.02

Method Revision: ETS-8-231
Study Number: E01-1326-02-04
Matrix: Liver 1256 35.02

Blank Liver TN-A-6023; Amount of liver: 5. 0597g
Homogenize liver with Kandiyohi water; Amount of water added 45 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 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-6009)
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-6009) into
Spike samples with internal standard PFDA standard number 02001-4

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Bottle-Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR, 5mL	
Date:	3/5/02	<i>3.5.02</i> <i>OK</i>
Initials:	ok	
Dispenser ID:	799	
Balance ID:	900	
Volume (mL):	5	
Solvent:	Acetonitrile	
ID #:	tn-a-4145	

	Mass of solvent (g)	Density ¹ (g/ml)	Volume of Solvent
1	3.9429	0.7857	5.0183
2	3.9904	0.7857	5.0788
3	3.959	0.7857	5.0388
4	3.967	0.7857	5.0490
5	3.9712	0.7857	5.0543
Average Volume:			5.0479
% Accuracy:			100.96
Std. Dev.:			0.02210
% C.V.:			0.44
Pass/Fail:			PASS

PASS

PASS

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

Corrective Actions: None Needed! <i>3.5.02</i> <i>OK</i>

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

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

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

Acetone $d_0^{25} = 0.788$

Methanol $d_4^{25} = 0.7866$

Methylene Chloride $d_4^{20} = 1.3255$

Acetonitrile $d_4^{20} = 0.7857$

(AC)

Mallard Liver

US 07/12/02

Page 1 of 2

Prep Date: 3-6-02
Analysts initials: OK

Method Revision: ETS-8-231

Study Number: E01-1326 (R) 3602

Matrix: Liver 1256

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
E01-1326-36812	Blank H2O-day 3	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	NA
E01-1326-36814	Blank mallard liver-day 3	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-36815	Mallard Liver MS-day 3	40+6	Waters, 1g, 6ml, lot W2045B2	50ul of 02001-66	2ml of MeOH TN-A-6009	
E01-1326-36816	Mallard Liver MSD-day 3	40+6	Waters, 1g, 6ml, lot W2045B2	50ul of 02001-66	2ml of MeOH TN-A-6009	
E01-1326-29980	10 ppm a.i. 3264 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29989	10 ppm a.i. 3265 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-29998	10 ppm a.i. 3266 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30007	10 ppm a.i. 3267 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30016	10 ppm a.i. 3268 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30025	10 ppm a.i. 3269 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30034	10 ppm a.i. 3270 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30043	10 ppm a.i. 3271 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30052	10 ppm a.i. 3272 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30061	10 ppm a.i. 3273 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30070	10 ppm a.i. 3274 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30079	10 ppm a.i. 3275 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30088	10 ppm a.i. 3276 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30097	10 ppm a.i. 3277 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30106	10 ppm a.i. 3278 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30115	10 ppm a.i. 3279 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30124	10 ppm a.i. 3280 F Liver	40+7	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-30133	10 ppm a.i. 3281 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	

Blank Liver TN-A-6023; Amount of liver: 5.0597g

Homogenize liver with Kandiyohi water; Amount of water added 45 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 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-6009)

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-6009) 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

Prep Date: 36.02
Analysts initials: OK

Method Revision: ETS-8-231
Study Number: E01-1228-26 3.6-2
Matrix: Liver 1256

[illegible]

Blank Liver TN-A-6023; Amount of liver: 5. 0597g

Homogenize liver with Kandiyoji water; Amount of water added 45 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 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-6009)

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-6009) into appropriate 15 ml centrifuge tube

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

Bottle-Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR, 5mL	
Date:	3/6/02	<i>3.6.02</i> <i>OK</i>
Initials:	ok	
Dispenser ID:	799	
Balance ID:	900	
Volume (mL):	5	
Solvent:	Acetonitrile	
ID #:	tn-a-4145	

	Mass of solvent (g)	Density ¹ (g/ml)	Volume of Solvent
1	3.9506	0.7857	5.0281
2	3.9857	0.7857	5.0728
3	3.9874	0.7857	5.0750
4	3.9772	0.7857	5.0620
5	3.983	0.7857	5.0694
Average Volume:			5.0614
% Accuracy:			101.23
Std. Dev.:			0.01927
% C.V.:			0.38
Pass/Fail:			PASS

PASS

PASS

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

Corrective Actions: <i>None taken!</i> <i>3.6.02</i> <i>OK</i>

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

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

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

Acetone $d_0^{25} = 0.788$

Methanol $d_4^{25} = 0.7866$

Methylene Chloride $d_4^{20} = 1.3255$

Acetonitrile $d_4^{20} = 0.7857$

Prep Date: OK

Analysts initials: 3.7.02

Method Revision: ETS-8-231

Study Number: E01-1326 (Ad) 3.7.02

Matrix: Liver 1256

Lims Assigned sample number	Sample Number description	Volume of sample filtered (ml)	Type of column used and lot	Amount and spike mix used	Elution solvent and volume	Comments
E01-1326-36817	Blank H2O-day 4	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	NA
E01-1326-36819	Blank mallard liver-day 4	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1326-36820	Mallard Liver MS-day 4	40+6	Waters, 1g, 6ml, lot W2045B2	50ul of 02001-66	2ml of MeOH TN-A-6009	
E01-1326-36821	Mallard Liver MSD-day 4	40+6	Waters, 1g, 6ml, lot W2045B2	50ul of 02001-66	2ml of MeOH TN-A-6009	
E01-1367-31295	0 ppm a.i. 9583 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31301	0 ppm a.i. 9591 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31307	0 ppm a.i. 9589 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31313	0 ppm a.i. 9592 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31319	0 ppm a.i. 9598 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31325	0 ppm a.i. 9603 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31331	0 ppm a.i. 9606 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31337	0 ppm a.i. 9610 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31343	0 ppm a.i. 9573 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31349	0 ppm a.i. 9578 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31356	10 ppm a.i. 9625 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31362	10 ppm a.i. 9629 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31368	10 ppm a.i. 9633 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31374	10 ppm a.i. 9634 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31380	10 ppm a.i. 9645 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31386	10 ppm a.i. 9648 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31392	10 ppm a.i. 9636 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31398	10 ppm a.i. 9642 M Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31404	10 ppm a.i. 9654 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	
E01-1367-31410	10 ppm a.i. 9659 F Liver	40+6	Waters, 1g, 6ml, lot W2045B2	NA	2ml of MeOH TN-A-6009	↓

Blank Liver TN-A-6023; Amount of liver: 5.0597g

Homogenize liver with Kandiyohi water; Amount of water added 45 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 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-6009)

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-6009) 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

Bottle-Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR , 5mL	
Date:	3/7/02	<i>3.7.02</i> <i>OK</i>
Initials:	ok	
Dispenser ID:	799	
Balance ID:	900	
Volume (mL):	5	
Solvent:	Acetonitrile	
ID #:	tn-a-4145	

	Mass of solvent (g)	Density ¹ (g/ml)	Volume of Solvent
1	3.9564	0.7857	5.0355
2	3.9674	0.7857	5.0495
3	3.982	0.7857	5.0681
4	3.9753	0.7857	5.0596
5	3.9713	0.7857	5.0545
Average Volume:			5.0534
%Accuracy:			101.07
Std. Dev.:			0.01215
%C.V.:			0.24
Pass/Fail:			PASS

PASS

PASS

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

Corrective Actions: NONE NEEDED! 3.7.02 OK
--

¹Density values taken from the Merck Index, 12th 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 $d^{20}_4 = 1.3255$

Acetonitrile $d^{20}_4 = 0.7857$

Attachment D: Dilutions Summary Worksheet

Study: E01-1326/1367

Dilution Date/Analyst: ①

Box Number: E01-1326

E01-1326 (AC) 4/11/02

Solvent/TN Number: MeOH/5457

Extraction Date/Analyst: 3.4.02/3.5.02/3.6.02

Matrix/Timepoint: HALLARD LIVER

Sample Number or Description	Dilutions						Comments	Verified By:
	1/10	1/100	1/	1/	1/	1/		
E01-1326-29961	x	NA						6/11/02
E01-1326-30034	x							
E01-1326-30070	x							
E01-1326-30088	x							
E01-1326-30178	x							
E01-1326-30196	x							
E01-1326-30214	x							
E01-1326-29916	x							
E01-1326-29934	x						1AS	
E01-1326-29952	x						08/21/02	
E01-1326-29970	NA	x						
E01-1326-29989	x	NA						
E01-1326-30007	x							
E01-1326-30025	x							
E01-1326-30043	x							
E01-1326-30061	x							
E01-1326-30079	x							

Notes:

1/10 dilution = 100µl of sample + 900µl of solvent

1/100 dilution = 10µl of sample + 990µl of solvent.

① Dilutions were made on the same day of extraction
or the day after analysis. (AC) OK 4.8.02

WRE OK 8.28.02

Form Completion Verified By: AS 4.11.02

(AC) Page 1 of 2 1AS 08/21/02

Attachment D: Dilutions Summary Worksheet

Study: ~~E01-1256~~ E01-1326/1367 E01-1256 ^{AC 08/21/02}
 Solvent/TN Number: MeOH/5457
 Dilution Date/Analyst:
 Extraction Date/Analyst:
 Box Number: E01-1326 Matrix/Timepoint: MALLARD LIVER

Sample Number or Description	Dilutions							Comments	Verified By:
	1/10	1/	1/	1/	1/	1/	1/		
E01-1326-30097	x								08/21/02 LAS
E01-1326-30115	x								
E01-1326-30133	x								
E01-1326-30151	x								
E01-1326-30169	x								
E01-1326-30187	x								
E01-1326-30205	x								
E01-1326-30223	x								
E01-1326-30241	x								
E01-1326-30259	x								
E01-1326-36804	x								
E01-1326-36805	x								
E01-1326-36806	x								
—	—								
—	—								
—	—								
—	—								

Notes:

1/10 dilution = 100 μ l of sample + 900 μ l of solvent

Form Completion Verified By:

08/21/02
LAS

(AC) Page 2 of 2
LAS 08/21/02

MS 2.19.02

(AC) Mallard Sera 3 LAS 08/21/02
Page 1 of 2

sample number	description	Amount of sera aliquoted(ml)	aliquoted by/date	Diluted with water to (ml)	diluted by/date
E01-1256-28662	454-109-3217, 0 PPM, Male, Adult	0.50	MS 10/22/02	10	MS 2.19.02
E01-1256-28663	454-109-3218, 0 PPM, Female, Adult	0.50	MS 12.19.02	10	
E01-1256-28664	454-109-3219, 0 PPM, Male, Adult	0.50		10	
E01-1256-28665	454-109-3220, 0 PPM, Female, Adult	0.50		10	
E01-1256-28666	454-109-3221, 0 PPM, Male, Adult	0.50		10	
E01-1256-28667	454-109-3222, 0 PPM, Female, Adult	0.50		10	
E01-1256-28668	454-109-3223, 0 PPM, Male, Adult	0.50		10	
E01-1256-28669	454-109-3224, 0 PPM, Female, Adult	0.50		10	
E01-1256-28670	454-109-3226, 0 PPM, Female, Adult	0.50		10	
E01-1256-28671	454-109-3227, 0 PPM, Male, Adult	0.50		10	
E01-1256-28672	454-109-3228, 0 PPM, Female, Adult	0.50		10	
E01-1256-28673	454-109-3229, 0 PPM, Male, Adult	0.50		10	
E01-1256-28674	454-109-3230, 0 PPM, Female, Adult	0.50		10	
E01-1256-28675	454-109-3231, 0 PPM, Male, Adult	0.50		10	
E01-1256-28676	454-109-3232, 0 PPM, Female, Adult	0.50		10	
E01-1256-28677	454-109-3233, 0 PPM, Male, Adult	0.50		10	
E01-1256-28678	454-109-3234, 0 PPM, Female, Adult	0.50		10	
E01-1256-28679	454-109-3236, 0 PPM, Female, Adult	0.50		10	MS 2.19.02 (A)
E01-1256-28680	454-109-3237, 0 PPM, Male, Adult	0.3		5	
E01-1256-28681	454-109-3238, 0 PPM, Female, Adult	0.5		10	
E01-1256-28682	454-109-3239, 0 PPM, Male, Adult	0.5		10	
E01-1256-28683	454-109-3240, 0 PPM, Female, Adult	0.5		10	
E01-1256-28684	454-109-3241, 0 PPM, Male, Adult	0.5		10	
E01-1256-28685	454-109-3242, 0 PPM, Female, Adult	0.5		10	
E01-1256-28686	454-109-3243, 0 PPM, Male, Adult	0.5		10	
E01-1256-28687	454-109-3244, 0 PPM, Female, Adult	0.5		10	
E01-1256-28688	454-109-3245, 0 PPM, Male, Adult	0.5		10	
E01-1256-28689	454-109-3246, 0 PPM, Female, Adult	0.5		10	
E01-1256-28690	454-109-3247, 0 PPM, Male, Adult	0.5		10	
E01-1256-28691	454-109-3248, 0 PPM, Female, Adult	0.5		10	
E01-1256-28692	454-109-3249, 0 PPM, Male, Adult	0.5		10	(A)
E01-1256-28693	454-109-3250, 0 PPM, Female, Adult	0.5		10	
E01-1256-28694	454-109-3251, 0 PPM, Male, Adult	0.5		10	(A)
E01-1256-28695	454-109-3252, 0 PPM, Female, Adult	0.5		10	
E01-1256-28696	454-109-3253, 0 PPM, Male, Adult	0.1		2	
E01-1256-28697	454-109-3254, 0 PPM, Female, Adult	0.5		10	
E01-1256-28698	454-109-3255, 0 PPM, Male, Adult	0.5		10	(A)
E01-1256-28699	454-109-3256, 0 PPM, Female, Adult	0.5		10	
E01-1256-28700	454-109-3257, 10 PPM, Male, Adult	0.5		10	
E01-1256-28701	454-109-3258, 10 PPM, Female, Adult	0.5		10	
E01-1256-28702	454-109-3259, 10 PPM, Male, Adult	0.2		4	
E01-1256-28703	454-109-3260, 10 PPM, Female, Adult	0.5		10	
E01-1256-28704	454-109-3261, 10 PPM, Male, Adult	0.5		10	
E01-1256-28705	454-109-3262, 10 PPM, Female, Adult	0.5		10	(A)
E01-1256-28706	454-109-3263, 10 PPM, Male, Adult	0.06		1.2	
E01-1256-28707	454-109-3264, 10 PPM, Female, Adult	0.5		10	
E01-1256-28708	454-109-3265, 10 PPM, Male, Adult	0.2		4	(A)
E01-1256-28709	454-109-3266, 10 PPM, Female, Adult	0.5		10	
E01-1256-28710	454-109-3267, 10 PPM, Male, Adult	0.5		10	

(A) Diluted samples had blood clot type debris in them. MS 2.19.02

sample number	description	Amount of sera aliquoted(ml)	aliquoted by/date	Diluted with water to (ml)	diluted by/date
E01-1256-28711	454-109-3268, 10 PPM, Female, Adult	0.5	OK 2/19/02	10	HS 2/19/02
E01-1256-28712	454-109-3269, 10 PPM, Male, Adult	0.5		10	
E01-1256-28713	454-109-3270, 10 PPM, Female, Adult	0.5		10	
E01-1256-28714	454-109-3271, 10 PPM, Male, Adult	0.5		10	
E01-1256-28715	454-109-3272, 10 PPM, Female, Adult	0.5		10	
E01-1256-28716	454-109-3273, 10 PPM, Male, Adult	0.5		10	
E01-1256-28717	454-109-3274, 10 PPM, Female, Adult	0.5		10	(A)
E01-1256-28718	454-109-3275, 10 PPM, Male, Adult	0.5		10	
E01-1256-28719	454-109-3276, 10 PPM, Female, Adult	0.5		10	
E01-1256-28720	454-109-3277, 10 PPM, Male, Adult	0.5		10	
E01-1256-28721	454-109-3278, 10 PPM, Female, Adult	0.5		10	(A)
E01-1256-28722	454-109-3279, 10 PPM, Male, Adult	0.5		10	
E01-1256-28723	454-109-3280, 10 PPM, Female, Adult	0.2		4.0	(A)
E01-1256-28724	454-109-3281, 10 PPM, Male, Adult	0.5		10	
E01-1256-28725	454-109-3282, 10 PPM, Female, Adult	0.5		10	
E01-1256-28726	454-109-3283, 10 PPM, Male, Adult	0.5		10	
E01-1256-28727	454-109-3284, 10 PPM, Female, Adult	0.5		10	
E01-1256-28728	454-109-3285, 10 PPM, Male, Adult	0.5		10	
E01-1256-28729	454-109-3286, 10 PPM, Female, Adult	0.5		10	
E01-1256-28730	454-109-3287, 10 PPM, Male, Adult	0.3		6.0	
E01-1256-28731	454-109-3288, 10 PPM, Female, Adult	0.5		10	
E01-1256-28732	454-109-3289, 10 PPM, Male, Adult	0.3		6.0	
E01-1256-28733	454-109-3290, 10 PPM, Female, Adult	0.5		10	
E01-1256-28734	454-109-3291, 10 PPM, Male, Adult	0.3		6.0	(A)
E01-1256-28735	454-109-3292, 10 PPM, Female, Adult	0.5		10	
E01-1256-28736	454-109-3293, 10 PPM, Male, Adult	0.5		10	
E01-1256-28737	454-109-3294, 10 PPM, Female, Adult	0.5		10	
E01-1256-28738	454-109-3295, 10 PPM, Male, Adult	0.5		10	
E01-1256-28739	454-109-3296, 10 PPM, Female, Adult	0.5		10	
E01-1256-28740	454-109-9583, 0 PPM, Female, Offspring	0.15		3.0	(A)
E01-1256-28741	454-109-9591, 0 PPM, Male, Offspring	0.10		2.0	
E01-1256-28742	454-109-9589, 0 PPM, Female, Offspring	0.2		4.0	(A)
E01-1256-28743	454-109-9592, 0 PPM, Female, Offspring	0.2		4.0	(A)
E01-1256-28744	454-109-9598, 0 PPM, Female, Offspring	0.1		2.0	(A)
E01-1256-28745	454-109-9603, 0 PPM, Female, Offspring	0.2		4.0	(A)
E01-1256-28746	454-109-9606, 0 PPM, Male, Offspring	0.2		4.0	(A)
E01-1256-28747	454-109-9610, 0 PPM, Female, Offspring	0.2		4.0	(A)
E01-1256-28748	454-109-9573, 0 PPM, Female, Offspring	0.05		1.0	
E01-1256-28749	454-109-9578, 0 PPM, Male, Offspring	0.2		4.0	(A)
E01-1256-28750	454-109-9625, 10 PPM, Male, Offspring	0.2		4.0	(A)
E01-1256-28751	454-109-9629, 10 PPM, Female, Offspring	0.1		2.0	
E01-1256-28752	454-109-9633, 10 PPM, Female, Offspring	0.1		2.0	(A)
E01-1256-28753	454-109-9634, 10 PPM, Female, Offspring	0.5		10	
E01-1256-28754	454-109-9645, 10 PPM, Female, Offspring	0.1		2.0	(A)
E01-1256-28755	454-109-9648, 10 PPM, Female, Offspring	0.2		4.0	(A)
E01-1256-28756	454-109-9636, 10 PPM, Male, Offspring	0.1		2.0	(A)
E01-1256-28757	454-109-9642, 10 PPM, Male, Offspring	0.2		4.0	
E01-1256-28758	454-109-9654, 10 PPM, Female, Offspring	0.1		2.0	(A)
E01-1256-28759	454-109-9659, 10 PPM, Female, Offspring	0.1		2.0	(A)

Prep Date: 2/25/2002

Analysts initials: OK/HOJ

Method Revision: ETS-8-231.0

Study Number: E01-1256

Matrix: Sera

DRE 2-25-02

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
e01-1256-36231	Blank H2O-day 1	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	NA
e01-1256-36232	Blank mallard sera-day 1	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
e01-1256-36233	Mallard Sera MS-day 1	40+6	Waters, 1g, 6ml, lot 1290B2	50ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36234	Mallard Sera MSD-day 1	40+6	Waters, 1g, 6ml, lot 1290B2	50ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36235	Curve point-0.2 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	0.4ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36236	Curve point-0.5 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	1ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36237	Curve point-1 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	2ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36238	Curve point-2.5 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	5ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36239	Curve point-5 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	10ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36240	Curve point-10 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	20ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36241	Curve point-25 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	50ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36242	Curve point-50 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	100ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36243	Curve point-75 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	150ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36244	Curve point-100 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	200ul of 02001-66	2ml of MeOH TN-A-5457	
e01-1256-36245	Curve point-100 ppb-2 in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	4ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36246	Curve point-250 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	10ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36247	Curve point-500 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	20ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36248	Curve point-750 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	30ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36249	Curve point-1000 ppb in sera extract	40+6	Waters, 1g, 6ml, lot 1290B2	40ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36250	QC 25pb-1	40+6	Waters, 1g, 6ml, lot 1290B2	1ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36251	QC 25pb-2	40+6	Waters, 1g, 6ml, lot 1290B2	1ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36252	QC 25pb-3	40+6	Waters, 1g, 6ml, lot 1290B2	1ul of 02001-53	2ml of MeOH TN-A-5457	

E02-0241-36238 OK 2-25-02

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

Dilute Sera with Kandiyohi water; Amount of water added 36 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 SN 041694)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge SN#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-6012)

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 into appropriate 15 ml centrifuge tube

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

Transfer sample into appropriately marked autovial

8.19.02

SERA USED FOR BLANKS, MS, MSD, AND CAL. CURVES
 AMERAS TO NOT HAVE BEEN SEPARATED WELL FROM
 THE BLOOD. EVEN DILUTED SERA IS VERY RED IN COLOR,
 IN COMPARISON TO THE STANDARDS FROM THE STUDY
 2-25-02

AC Mallard Sera } LAS 08/31/02
Page 2 of 2

Prep Date: 225-02
Analysts initials: OK/HOJ

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

00225-02 oik

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
e01-1256-36253	QC-250ppb -1	40+6	Waters, 1g, 6ml, lot 1290B2 w 2022	10ul of 02001-53	2ml of MeOH TN-A-5457 6012	NA
e01-1256-36254	QC-250ppb -2	40+6	Waters, 1g, 6ml, lot 1290B2	10ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36255	QC-250ppb -3	40+6	Waters, 1g, 6ml, lot 1290B2	10ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36256	QC-4ppm -1	40+6	Waters, 1g, 6ml, lot 1290B2	160ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36257	QC-4ppm -2	40+6	Waters, 1g, 6ml, lot 1290B2	160ul of 02001-53	2ml of MeOH TN-A-5457	
e01-1256-36258	QC-4ppm -3	40+6	Waters, 1g, 6ml, lot 1290B2	160ul of 02001-53	2ml of MeOH TN-A-5457	
E01-1256-28662	454-109-3217, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28663	454-109-3218, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28664	454-109-3219, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28665	454-109-3220, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28666	454-109-3221, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28667	454-109-3222, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28668	454-109-3223, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28669	454-109-3224, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28670	454-109-3226, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28671	454-109-3227, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28672	454-109-3228, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28673	454-109-3229, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28674	454-109-3230, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28675	454-109-3231, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	
E01-1256-28676	454-109-3232, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot 1290B2	NA	2ml of MeOH TN-A-5457	

Blank Sera TN-A- 6022; Amount of sera aliquoted: 4 ml

Dilute Sera with Kandiyohi water; Amount of water added 36 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 SW 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- 6012)

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 into appropriate 15 ml centrifuge tube

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

Transfer sample into appropriately marked autovial

SPE Standard Curves-Fluids

Prep Date(s): 02/25/02, 02/26/02, 02/27/02, 02/28/02
 Analyst(s): OK/HOJ
 Sample Matrix: Mallard Sera
 Method/Revision: ETS-8-231.1
 Target Analyte(s): PFOS

Study Number: E01-1256
 Equipment Number: NA
 Final Solvent & TN Number: MeOH/TN-A-05457
 Blank Tissue/Identifier: Mallard Sera-E02-0241-36386

PFOS Std: 1.00 ppm: 02001-66
 PFOS Std: 50.2 ppm: 02001-53
 Surr.Std Approx. 100.7 ppm: 02001-45 PFDA

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

Actual Concentrations of Standards in the FC Mix

PFOS Std Conc. ug/mL	PFOS Am't Spiked mL	PFDA Std Conc. ug/mL	PFDA Am't Spiked mL	All Initial Dilution mL/mL	All Final Vol. mL
1.004	0.0004	100.7	0.002	0.0500	2.000
1.004	0.0010	100.7	0.002	0.0500	2.000
1.004	0.0020	100.7	0.002	0.0500	2.000
1.004	0.0050	100.7	0.002	0.0500	2.000
1.004	0.0100	100.7	0.002	0.0500	2.000
1.004	0.0200	100.7	0.002	0.0500	2.000
1.004	0.0500	100.7	0.002	0.0500	2.000
1.004	0.1000	100.7	0.002	0.0500	2.000
1.004	0.1500	100.7	0.002	0.0500	2.000
1.004	0.2000	100.7	0.002	0.0500	2.000
50.20	0.0040	100.7	0.002	0.0500	2.000
50.20	0.0100	100.7	0.002	0.0500	2.000
50.20	0.0200	100.7	0.002	0.0500	2.000
50.20	0.0300	100.7	0.002	0.0500	2.000
50.20	0.0400	100.7	0.002	0.0500	2.000
50.20	0.0010	100.7	0.002	0.0500	2.000
50.20	0.0100	100.7	0.002	0.0500	2.000
50.20	0.1600	100.7	0.002	0.0500	2.000
1.004	0.0500	100.7	0.002	0.0500	2.000
1.004	0.0500	100.7	0.002	0.0500	2.000
1.004	0.0500	100.7	0.002	0.0500	2.000
1.004	0.0500	100.7	0.002	0.0500	2.000

Calculation sheet
 added for clarity.

US 08/22/02

Calculated Concentrations of Standards in the sample matrix

Standard Number	PFOS Std Conc. ng/mL	PFDA Std Conc. ng/mL
E01-1256-36235, 0.2 ppb	8.03	4028
E01-1256-36236, 0.5 ppb	20.1	4028
E01-1256-36237, 1.0 ppb	40.2	4028
E01-1256-36238, 2.5 ppb	100	4028
E01-1256-36239, 5.0 ppb	201	4028
E01-1256-36240, 10 ppb	402	4028
E01-1256-36241, 25 ppb	1004	4028
E01-1256-36242, 50 ppb	2008	4028
E01-1256-36243, 75 ppb	3012	4028
E01-1256-36244, 100 ppb	4016	4028
E01-1256-36245, 100-2 ppb	4016	4028
E01-1256-36246, 250 ppb	10040	4028
E01-1256-36247, 500 ppb	20080	4028
E01-1256-36248, 750 ppb	30120	4028
E01-1256-36249, 1000 ppb	40160	4028
QC-25 ppb	1004	4028
QC-250 ppb	10040	4028
QC-4 ppm	160640	4028
Mallard Sera MS/MSD-Day 1	1004	4028
Mallard Sera MS/MSD-Day 2	1004	4028
Mallard Sera MS/MSD-Day 3	1004	4028
Mallard Sera MS/MSD-Day 4	1004	4028

Calculated Concentrations of Standards in the final solvent

Standard Number	PFOS Std Conc. ng/mL	PFDA Std Conc. ng/mL
E01-1256-36235, 0.2 ppb	0.201	101
E01-1256-36236, 0.5 ppb	0.502	101
E01-1256-36237, 1.0 ppb	1.00	101
E01-1256-36238, 2.5 ppb	2.51	101
E01-1256-36239, 5.0 ppb	5.02	101
E01-1256-36240, 10 ppb	10.0	101
E01-1256-36241, 25 ppb	25.1	101
E01-1256-36242, 50 ppb	50.2	101
E01-1256-36243, 75 ppb	75.3	101
E01-1256-36244, 100 ppb	100	101
E01-1256-36245, 100-2 ppb	100	101
E01-1256-36246, 250 ppb	251	101
E01-1256-36247, 500 ppb	502	101
E01-1256-36248, 750 ppb	753	101
E01-1256-36249, 1000 ppb	1004	101
QC-25 ppb	25.1	101
QC-250 ppb	251	101
QC-4 ppm	4016	101
Mallard Sera MS/MSD-Day 1	25.1	101
Mallard Sera MS/MSD-Day 2	25.1	101
Mallard Sera MS/MSD-Day 3	25.1	101
Mallard Sera MS/MSD-Day 4	25.1	101

Verified by:

[Signature] 8-2008

Bottle-Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR , 5mL
Date:	25 February 2002
Initials:	hoj/ok <i>OK 2-25-02</i>
Dispenser ID:	799
Balance ID:	901 <i>OK 2-26-02</i>
Volume (mL):	5
Solvent:	Acetonitrile
ID #:	tn-a-4145

	Mass of solvent (g)	Density ¹ (g/ml)	Volume of Solvent
1	3.9739	0.7857	5.0578
2	3.9683	0.7857	5.0507
3	3.9559	0.7857	5.0349
4	3.9767	0.7857	5.0613
5	3.9718	0.7857	5.0551
Average Volume:			5.0520
% Accuracy:			101.04
Std. Dev.:			0.01031
% C.V.:			0.20
Pass/Fail:			PASS

PASS

PASS

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

Corrective Actions:
<i>NONE NEEDED OK 2-25-02</i>

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

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

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

Acetone $d_0^{25} = 0.788$

Methanol $d_4^{25} = 0.7866$

Methylene Chloride $d_4^{20} = 1.3255$

Acetonitrile $d_4^{20} = 0.7857$

SPE Columns Extraction Worksheet

AC Mallard Sera } US 08/21/02
Page 1 of 2

Prep Date: 2/26/2002

Analysts initials: OK/HOJ

Method Revision: ETS-8-231

Study Number: E01-1256

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
E01-1256-36259	Blank H2O-day 2	40+6	Waters, 1g, 6ml, lot W2036B2	NA	2ml of MeOH TN-A-6012	NA
E01-1256-36260	Blank mallard sera-day 2	40+6	Waters, 1g, 6ml, lot W2036B2	↓	2ml of MeOH TN-A-6012	
E01-1256-36261	Mallard Sera MS-day 2	40+6	Waters, 1g, 6ml, lot W2036B2	50ul of 02001-66	2ml of MeOH TN-A-6012	
E01-1256-36262	Mallard Sera MSD-day 2	40+6	Waters, 1g, 6ml, lot W2036B2	50ul of 02001-66	2ml of MeOH TN-A-6012	
E01-1256-28677	454-109-3233, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2	NA	2ml of MeOH TN-A-6012	
E01-1256-28678	454-109-3234, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28679	454-109-3236, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28680	454-109-3237, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28681	454-109-3238, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28682	454-109-3239, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28683	454-109-3240, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28684	454-109-3241, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28685	454-109-3242, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28686	454-109-3243, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28687	454-109-3244, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28688	454-109-3245, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28689	454-109-3246, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28690	454-109-3247, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28691	454-109-3248, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28692	454-109-3249, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28693	454-109-3250, 0 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-5457	
E01-1256-28694	454-109-3251, 0 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2	↓	2ml of MeOH TN-A-5457	4

Blank Sera TN-A-6022; Amount of sera aliquoted: 4 ml
 Dilute Sera with Kandiyohi water; Amount of water added 36 ml

Aliquot 1 ml of sera into 15 ml polypropylene tube

Spike samples accordingly

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

Shake sample for 20 min @300 rpm (Shaker VWR 0416964)

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-6012)

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 into appropriate 15 ml centrifuge tube

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

Transfer sample into appropriately marked autovial

AC 02001-45
OK 4.9.02

① Mallard Sera } LAS 08/21/02

Prep Date: 2/26/2002
Analysts initials: OK/HOJ

Method Revision: ETS-8-231
Study Number: E01-1256
Matrix: Sera

[illegible]

Blank Sera TN-A- 6022 ; Amount of sera aliquoted: 4 ml
Dilute Sera with Kandiyoji water; Amount of water added 30 ml

Aliquot 1 ml 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 UWR5N | 941694)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge 34 76 9613)

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-60R)

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 into appropriate 15 ml centrifuge tube

Spike samples with internal standard PFDA standard number

PDA

conc. 100.7 μ M amount added

2nd

Bottle-Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR , 5mL	
Date:	26 Febuary 2002	
Initials:	ok	22602 OK
Dispenser ID:	799	
Balance ID:	900	
Volume (mL):	5	
Solvent:	Acetonitrile	
ID #:	tn-a-4145	

	Mass of solvent (g)	Density ¹ (g/ml)	Volume of Solvent
1	3.9525	0.7857	5.0305
2	3.9576	0.7857	5.0370
3	3.9768	0.7857	5.0615
4	3.9686	0.7857	5.0510
5	3.9994	0.7857	5.0902
Average Volume:			5.0541
% Accuracy:			101.08
Std. Dev.:			0.02354
% C.V.:			0.47
Pass/Fail:			PASS

PASS

PASS

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

Corrective Actions: NONE NEEDED! OK 22602

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

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

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

Acetone $d_0^{25} = 0.788$

Methanol $d_4^{25} = 0.7866$

Methylene Chloride $d_4^{20} = 1.3255$

Acetonitrile $d_4^{20} = 0.7857$

Prep Date: 227.02
Analysts initials: OK

Method Revision: ETS-8-231.0

Study Number: E01-1256

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
E01-1256-36263	Blank H2O-day 3	40+6	Waters, 1g, 6ml, lot W2036B2	NA	2ml of MeOH TN-A-6012	NA
E01-1256-36264	Blank mallard sera-day 3	40+6	Waters, 1g, 6ml, lot W2036B2	U	2ml of MeOH TN-A-6012	
E01-1256-36265	Mallard Sera MS-day 3	40+6	Waters, 1g, 6ml, lot W2036B2	50ul of 02001-66	2ml of MeOH TN-A-6012	
E01-1256-36266	Mallard Sera MSD-day 3	40+6	Waters, 1g, 6ml, lot W2036B2	50ul of 02001-66	2ml of MeOH TN-A-6012	
E01-1256-28710	454-109-3267, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2	NA	2ml of MeOH TN-A-6012	
E01-1256-28711	454-109-3268, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28712	454-109-3269, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28713	454-109-3270, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28714	454-109-3271, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28715	454-109-3272, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28716	454-109-3273, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28717	454-109-3274, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28718	454-109-3275, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28719	454-109-3276, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28720	454-109-3277, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28721	454-109-3278, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28722	454-109-3279, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28723	454-109-3280, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28724	454-109-3281, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28725	454-109-3282, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28726	454-109-3283, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28727	454-109-3284, 10 PPM, Female, Adult	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6012	
E01-1256-28728	454-109-3285, 10 PPM, Male, Adult	40+6	Waters, 1g, 6ml, lot W2036B2	U	2ml of MeOH TN-A-6012	

Blank Sera TN-A-6022; Amount of sera aliquoted: 4 ml

Dilute Sera with Kandiyohi water; Amount of water added 36 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 WWR 41 041694)

Centrifuge sample for 10 min @ 2000 rpm (Centrifuge WWR 41 041694) 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-6012)

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 into appropriate 15 ml centrifuge tube

Spike samples with internal standard PFDA standard number PFDA, conc. 100 ppm, amount added 2ul

Transfer sample into appropriately marked vial

3M Environmental Laboratory

Prep Date: 22702
Analysts initials: OK

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

[illegible]

Blank Sera TN-A-6022; Amount of sera aliquoted: 4 ml
Dilute Sera with Kandiyohi water; Amount of water added 36 ml

~ Aliquot 1 ml 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 VWR 3/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-6012)

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 into appropriate 15 ml centrifuge tube

1. Spike samples with internal standard PFDA standard number PFDA, conc. 100.7921, amount added 2.0

3M Environmental Laboratory

Bottle-Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR, 5mL	
Date:	27 Febuary 2002	
Initials:	ok	
Dispenser ID:	799	<i>OK</i> <i>227-02</i>
Balance ID:	900	
Volume (mL):	5	
Solvent:	Acetonitrile	
ID #:	tn-a-4145	

	Mass of solvent (g)	Density ¹ (g/ml)	Volume of Solvent
1	3.9579	0.7857	5.0374
2	3.9883	0.7857	5.0761
3	3.9754	0.7857	5.0597
4	3.9804	0.7857	5.0661
5	3.9907	0.7857	5.0792
Average Volume:			5.0637
% Accuracy:			101.27
Std. Dev.:			0.01662
% C.V.:			0.33
Pass/Fail:			PASS

PASS

PASS

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

Corrective Actions: None needed! OK 2.27.02
--

¹Density values taken from the Merck Index, 12th 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 $d^{20}_4 = 1.3255$

Acetonitrile $d^{20}_4 = 0.7857$

Prep Date: 2/28/02

Analysts initials: OK

Method Revision: ETS-8-231

Study Number: E01-1256

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
E01-1256-36267	Blank H2O-day 4	40+6	Waters, 1g, 6ml, lot W2036B2	NA	2ml of MeOH TN-A-6009	NA
E01-1256-36268	Blank mallard sera-day 4	40+6	Waters, 1g, 6ml, lot W2036B2	↓	2ml of MeOH TN-A-6009	
E01-1256-36269	Mallard Sera MS-day 4	40+6	Waters, 1g, 6ml, lot W2036B2	50ul of 02001-66	2ml of MeOH TN-A-6009	
E01-1256-36270	Mallard Sera MSD-day 4	40+6	Waters, 1g, 6ml, lot W2036B2	50ul of 02001-66	2ml of MeOH TN-A-6009	
E01-1256-28740	454-109-9583, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2	NA	2ml of MeOH TN-A-6009	
E01-1256-28741	454-109-9591, 0 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28742	454-109-9589, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28743	454-109-9592, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28744	454-109-9598, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28745	454-109-9603, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28746	454-109-9606, 0 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28747	454-109-9610, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28748	454-109-9573, 0 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28749	454-109-9578, 0 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28750	454-109-9625, 10 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28751	454-109-9629, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28752	454-109-9633, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28753	454-109-9634, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28754	454-109-9645, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28755	454-109-9648, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28756	454-109-9636, 10 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28757	454-109-9642, 10 PPM, Male, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28758	454-109-9654, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2		2ml of MeOH TN-A-6009	
E01-1256-28759	454-109-9659, 10 PPM, Female, Offspring	40+6	Waters, 1g, 6ml, lot W2036B2	↓	2ml of MeOH TN-A-6009	↓

Blank Sera TN-A- 6022; Amount of sera aliquoted: 4 ml

Dilute Sera with Kandiyohi water; Amount of water added 36 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 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- 6009)

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 into appropriate 15 ml centrifuge tube

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

Bottle-Top Dispenser Daily Calibration Check

Manufacturer and Model:	VWR, 5mL
Date:	28 February 2002
Initials:	ok <i>OK</i>
Dispenser ID:	799 <i>22802</i>
Balance ID:	900
Volume (mL):	5
Solvent:	Acetonitrile
ID #:	tn-a-4145

	Mass of solvent (g)	Density ¹ (g/ml)	Volume of Solvent
1	3.9524	0.7857	5.0304
2	3.9923	0.7857	5.0812
3	3.9673	0.7857	5.0494
4	3.9812	0.7857	5.0671
5	3.9989	0.7857	5.0896
Average Volume:			5.0635
%Accuracy:			101.27
Std. Dev.:			0.02398
%C.V.:			0.47
Pass/Fail:			PASS

PASS

PASS

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

Corrective Actions:
<i>NONE NEEDED!</i> <i>22802 OK</i>

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

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

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

Acetone $d_0^{25} = 0.788$

Methanol $d_4^{25} = 0.7866$

Methylene Chloride $d_4^{20} = 1.3255$

Acetonitrile $d_4^{20} = 0.7857$

Attachment D: Dilutions Summary Worksheet

Study: E01-1256

Dilution Date/Analyst: ①

Box Number: E01-1256

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

Extraction Date/Analyst: 225-02; 227-02;

Matrix/Timepoint: MALLARD SERA

Sample Number or Description	Dilutions							Comments	Verified By:
	1/10	1/100	1/	1/	1/	1/	1/		
E01-1256-28713	x	NA							AJ 4.11.02
E01-1256-28719	x								
E01-1256-28723	x								
E01-1256-28729	x								
E01-1256-28731	x								
E01-1256-28700	x								
E01-1256-28702	x								
E01-1256-28704	x								
E01-1256-28706	—	x						IAS 08/21/02	
E01-1256-28708	x	NA							
E01-1256-28710	x								
E01-1256-28712	x								
E01-1256-28714	x								
E01-1256-28716	x								
E01-1256-28718	x								
E01-1256-28720	x								
E01-1256-28722	x								

Notes:

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 = $\frac{10\mu\text{l}}{1000\mu\text{l}}$ of sample + $\frac{990\mu\text{l}}{1000\mu\text{l}}$ of solvent

① DILUTIONS WERE MADE ON THE SAME DAY OF
 EXTRACTION OR THE DAY AFTER ANALYSIS.

OK 34.8.02

①

AC

① ② OK 8.28.02

Form Completion Verified By: AJ 4.11.02

Attachment D: Dilutions Summary Worksheet

Study: E01-1256

Dilution Date/Analyst: ①

Box Number: E01-1256

Solvent/TN Number: MeOH/5457

W/ESTDA

Extraction Date/Analyst: 2-25-02/22702

Matrix/Timepoint:

MALLARD SERA

Sample Number or Description	Dilutions							Comments	Verified By:
	1/10	1/100	1/	1/	1/	1/	1/		
E01-1256-28724	x	NA							KJ 4-11-02
E01-1256-28726	x								
E01-1256-28728	x								
E01-1256-28730	x								
E01-1256-28732	x								
E01-1256-28734	x								
E01-1256-28736	x							1AS 08/21/02	
E01-1256-28738	x								
E01-1256-36256	x								
E01-1256-36257	x								
E01-1256-36258	x	↓							PREP BY OK; ENTER OK 4-11-02
E01-1256-28706	-	X							
E01-1256-28717	λ	NA							

Notes:

1/10 dilution = $\frac{10\mu\text{l}}{100\mu\text{l}}$ of sample + $\frac{90\mu\text{l}}{100\mu\text{l}}$ of solvent1/100 dilution = $\frac{10\mu\text{l}}{1000\mu\text{l}}$ of sample + $\frac{990\mu\text{l}}{1000\mu\text{l}}$ of solvent.

Form Completion Verified By: KJ 4-11-02

MOH TN-A-5457

Solvent/TN Number: HOONW/IS
Extraction Date/Analyst:
Matrix/Timepoint: HALLARD SERA

Sample Number or Description	Dilutions							Comments	Verified By:
	1/100	1/	1/	1/	1/	1/	1/		
E01-1256-28706	x								4.15.02
LAS 08/21/02									

1/10 dilution = 1 mL of sample + 9 mL of solvent

1/10 dilution = 10 μ l of sample + 90 μ l of solvent
1/100 dilution = 10 μ l of sample + 90 μ l of solvent. (AC) OK 4/502

Form Completion Verified By: AS 4.15.02

(AL) Page 1 of 1 LAS 08/21/02

② Directors Practised ^{11 (15) Nov 4.15.02} 4.8.02, Street filled out on 4.15.02 from two samples taken 11.15.02

**ATTACHMENT E: STUDY PROTOCOL, PROTOCOL AMENDMENTS, AND
DEVIATIONS**

WILDLIFE INTERNATIONAL, LTD

**PROJECT NO.: 454-109,
E01-1256**

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE MALLARD

PROTOCOL NO.: 454/120700/MR/SUB454

AMENDMENT NO.: 9

SPONSOR: 3M Corporation

PROJECT NO.: 454-109, E01-1256

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 E01-1256.

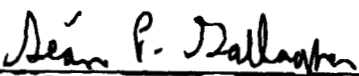
REASON:

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

JAN. 23. 2002 5:25PM

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NO. 5293 P. 3

WILDLIFE INTERNATIONAL, LTDPROJECT NO.: 454-109,
E01-1256
Sean P. Gallagher, Study Director

Date

1/24/02


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

Date

1/24/02


Harold O. Johnson, Principal Analytical Investigator

Date

4.4.02


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

Date

04/04/02


Rochelle B. Robideau, Sponsor's Representative

Date

1/25/02

WILDLIFE INTERNATIONAL, LTD**PROJECT NO.: 454-109,
E01-1256**

Sean P. Gallagher, Study Director

Date

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

Date

Harold O. Johnson, Principal Analytical Investigator

Date

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

Date

Rochelle R. Robideau, Sponsor's Representative

Date



Protocol Amendment # 9 - Attachment A

Wildlife International Ltd. # 454-109

STUDY TITLE

PFOS: A Reproduction Study with the Mallard

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: E01-1256
Wildlife International Ltd.: 454-109**

Study Identification**PFOS: A Reproduction Study with the Mallard****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, mallards 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 21-week period. The purpose of this analytical phase of the study is to quantify PFOS in the liver and sera of both control and dosed mallards. 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 454-109.

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 (C₈F₁₇SO₃K). Note: The molecular weight of the anion (PFOS) is 499 (C₈F₁₇SO₃).

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 mallard (*Anas platyrhynchos*). 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. Mallard 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

Mallard duck 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 454-109) 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

- 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 mallard sera and liver was performed at Centre Analytical Laboratories, Inc. in study 023-044. 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. 454-109, "PFOS: A Reproduction Study with the Mallard".
- 18.2** 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 Mallard Serum and Liver."

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

19. *PROTOCOL AMENDMENTS AND DEVIATIONS*

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

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-109,
E01-1256

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE MALLARD

PROTOCOL NO.: 454/120700/MR/SUB454

AMENDMENT NO.: 10

SPONSOR: 3M Corporation

PROJECT NO.: 454-109, E01-1256

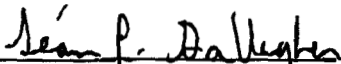
EFFECTIVE DATE: January 30, 2002

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:

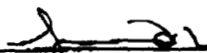
The amendment 9 attachment A was updated to include a different extraction and analytical method, to update the data quality objectives, and to change the liver and sera extraction location.



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 K. Robideau, Sponsor's Representative

01/31/02

Date

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-109,
E01-1256**AMENDMENT TO STUDY PROTOCOL**

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE MALLARD

PROTOCOL NO.: 454/120700/MR/SUB454

AMENDMENT NO.: 10

SPONSOR: 3M Corporation

PROJECT NO.: 454-109, E01-1256

EFFECTIVE DATE: January 30, 2002

AMENDMENT:

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

REASON:

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

Sean P. Gallagher, Study Director_____
Date_____
Joann B. Beavers, Wildlife International, LTD,
Laboratory Management_____
Date_____
Harold O. Johnson, Principal Analytical Investigator_____
Date_____
William K. Reagen, 3M Environmental Laboratory,
Laboratory Management_____
Date_____
Rochelle R. Robideau, Sponsor's Representative_____
Date



Protocol Amendment # 10 - Attachment A

Wildlife International Ltd. # 454-109

STUDY TITLE

PFOS: A Reproduction Study with the Mallard

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: E01-1256
Wildlife International Ltd.: 454-109

Study Identification

PFOS: A Reproduction Study with the Mallard

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, mallards 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 21-week period. The purpose of this analytical phase of the study is to quantify PFOS in the liver and sera of both control and dosed mallards. 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 454-109.

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 (C₈F₁₇SO₃K). Note: The molecular weight of the anion (PFOS) is 499 (C₈F₁₇SO₃⁻).

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 mallard (*Anas platyrhynchos*). 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. Mallard 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

Mallard duck 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 454-109) 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 Mallard control matrix preclude a cross validation, however, the E01-1277 study methods will be applied to sample analyses of Mallard 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. 454-109, "PFOS: A Reproduction Study with the Mallard".
- 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.: 454-109,
E01-1256****AMENDMENT TO STUDY PROTOCOL****STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE MALLARD****PROTOCOL NO.: 454/120700/QR/SUB454****AMENDMENT NO.: 11****SPONSOR: 3M Corporation****PROJECT NO.: 454-109, E01-1256****EFFECTIVE DATE: March 27, 2002**

AMENDMENT: Section 11.1, Sentence 4

Change: The peak area precision must be equal to or less than 2.0% RSD, the precision of the retention time must be equal to or less than 2.5% RSD.

To: The peak area precision must be equal to or less than 5.0% RSD, the precision of the retention time must be equal to or less than 2.5% RSD.

REASON: Information received from our instrument supplier indicates that a 2.0 % RSD for the area count is common to UV detector specifications. Mass spectrometers, which we used for this study, are more sensitive, in general and the fact that analyte ionization can be suppressed by other species present in the sample matrix and mobile phase make area reproducibility more challenging to maintain. Sample and source cleanliness are the determining factors, but $\leq 2.0\%$ is still a bit aggressive for mass spec. The application chemists at Micromass, the instrument vendor, suggest a value of $\leq 5.0\%$.

AMENDMENT: Section 11.3, Sentence 5.

Change: Any level outside 75% - 125% of nominal value must be deactivated, and regression recalculated, except the LLOQ which must be within 30% of nominal.

To: In some cases the lowest curve point, although outside the 30% nominal requirement, may be included in the linear range to influence the lower end of the curve. Only one point, the lowest level point, can be left in for this purpose. It will not be used to determine the limit of quantitation. The limit of quantitation will be the next point on the curve meeting the 30% nominal requirement.

REASON: The removal of the lowest calibration curve point in some cases causes the next lowest calibration point to deviate by greater than 30%. Removal of this point in turn causes the next calibration point to deviate by greater than 30%. This continued deletion of calibration points in some cases could continue until there are very few points

remaining in the curve. The inclusion of a curve point with a deviation greater than 30% is used to influence the lower end of the curve and is not used for determination of the LOQ.

AMENDMENT: Section 11.3, the last sentence.

Change: A maximum of four (4) levels may be deactivated in any one set, or the set will be reanalyzed.

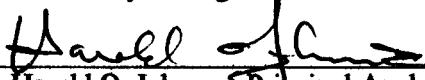
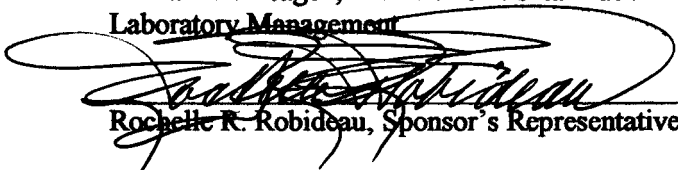
To: The calibration curve will contain a minimum of six (6) calibration curve points.

REASON: In most cases the calibration curves contain greater than nine (9) calibration points. The large number of points in the curve is used to select a range representative to the samples. Limiting the removal of only four (4) points in some cases could cause a calibration range not representative of the samples.

AMENDMENT: Section 11.3

Add: The coefficient of determination for the calibration curve should be ≥ 0.985 .

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

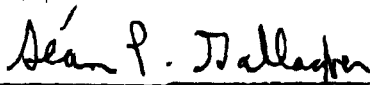
WILDLIFE INTERNATIONAL, LTDPROJECT NO.: 454-109,
E01-1256Sean P. Gallagher, Study DirectorDateJoann B. Beavers, Wildlife International, LTD
Laboratory ManagementDate
Harold O. Johnson, Principal Analytical Investigator4.2.02Date
William K. Reagen, 3M Environmental Laboratory,
Laboratory Management04/02/02Date
Rochelle R. Robideau, Sponsor's Representative4/1/02Date

04/01/02 18:29 ASD INF TECH 2-2E-01 → *84108228915

NO.905 P004

WILDLIFE INTERNATIONAL, LTD

**PROJECT NO.: 454-109,
E01-1256**


Sean P. Gallagher, Study Director

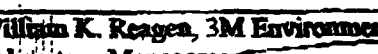
4/2/02
Date


Joann B. Beavers, Wildlife International, LTD
Laboratory Management

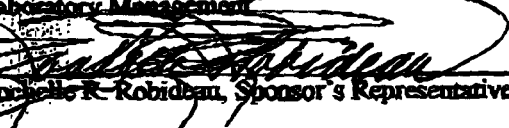
4/2/02
Date


Harold O. Johnson, Principal Analytical Investigator

Date


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

Date


Rochelle R. Robideau, Sponsor's Representative

4/1/02
Date

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-109,
E01-1256

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE MALLARD

PROTOCOL NO.: 454/120700/QR/SUB454

AMENDMENT NO.: 12

SPONSOR: 3M Corporation

PROJECT NO.: 454-109, E01-1256

EFFECTIVE DATE: August 16, 2002

AMENDMENT:

Change: The 3M Environmental Laboratory Project Identification Number (LIMS) to be included with the study is E01-1256.

To: The main 3M Environmental Laboratory Project Identification Number (LIMS) to be included with the study is E01-1256. Additional tracking numbers associated with this study are U2723, E01-1326, and E01-1367.

REASON:

Mallard 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, E01-1256, E01-1326, and E01-1367. Samples in each of these projects pertaining to this study were analyzed under one main study number E01-1256.

AMENDMENT:

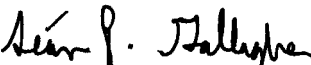
Change: Harold Johnson is the Principal Analytical Investigator.

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

REASON:

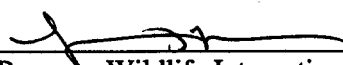
To change role of the Principal Analytical Investigator.

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-109,
E01-1256
Sean P. Gallagher, Study Director

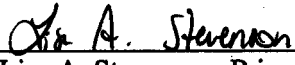
9/25/02

Date


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

9/25/02

Date


Lisa A. Stevenson, Principal Analytical Investigator

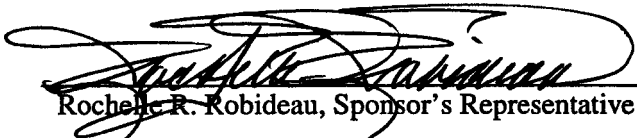
08/20/02

Date


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

09/20/02

Date


Rochelle R. Robideau, Sponsor's Representative

8/20/02

Date

WILDLIFE INTERNATIONAL, LTD

PROJECT NO.: 454-109,
E01-1256

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: PFOS: A REPRODUCTION STUDY WITH THE MALLARD

PROTOCOL NO.: 454/120700/QR/SUB454

AMENDMENT NO.: 13

SPONSOR: 3M Corporation

PROJECT NO.: 454-109, E01-1256

EFFECTIVE DATE: September 27, 2002

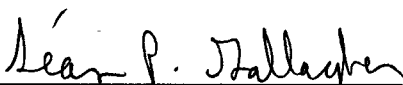
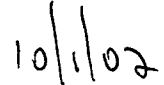
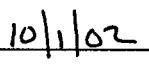
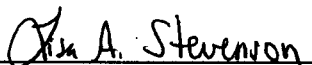
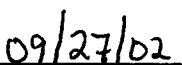
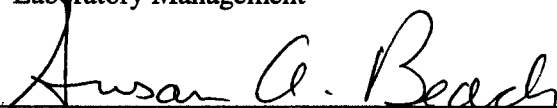
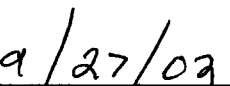
AMENDMENT:

Change: Rochelle R. Robideau is the Sponsor Representative.

To: Susan A. Beach is the Sponsor Representative.

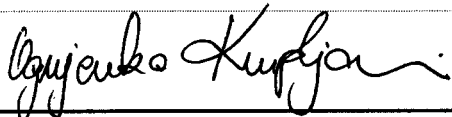
REASON:

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

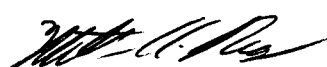
Record of Deviation

I. Identification	
Study / Project No. E01-1256	
Deviation type (Check one)	☒ SOP ☐ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:
Document number ETS-4-4.0	Date(s) of occurrence 2/19/02, 2/25/02, 2/26/02, 2/27/02, 2/28/02, 2/22/02, 3/4/02, 3/5/02, 3/6/02, 3/7/02
II. Description	
Required procedure/process: ETS-4-4.0 Internal chain of custody The internal chain of custody form to be used to track all movement of samples Within the lab. The form should be filed with study data or other relevant sample documents.	
Actual procedure/process: Internal chains of custodies were not used to track samples and sample extracts. Since receipt samples were Stored in freezer F1. After homogenizing liver and aliquating sera samples were stored in Freezer F8 Until extraction date. Samples remained frozen until extraction.	
III. Actions Taken (such as amendment issued, SOP revision, etc.)	
Deviation to SOP written.	
Recorded by 	Date 4902
Authorized by	Date

Study Director: Sean P. Gallagher
9/24/02Deviation No. 1

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

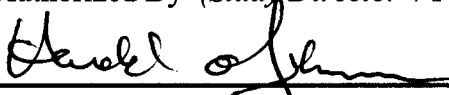
Laboratory Management:

 08/20/02

Record of Deviation

I. Identification	
Study / Project No.E01-1256	
Deviation Type (Check one)	☒ SOP ☐ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:
Document Number:ETS-8-231.1	Date(s) of occurrence2/25/02, 2/26/02, 2/27/02, 2/28/02, 3/1/02, 3/4/02, 3/5/02, 3/6/02, 3/7/02, 3/8/02,
II. Description:	
Required Procedure/process: Section 12.1, Calculations: If other calculations are used than those listed, they will be documented in the raw data.	
Actual Procedure/process: Standard E01-1342-36010, Prep sheet description "Curve Point – 10ppb in liver", was prepped by spiking 20uL of 02001-66 (1.00ug/mL) into 1 mL of liver homogenate. This homogenate consists of 5g liver plus 45mL water. Since 1mL out of 50mL homogenate is used to prep each extracted standard, 1/50 of the 5g (0.1g) of liver is "spiked". After this homogenate is spiked and prepared using SPE, the final eluate is 2.0mL of MeOH. THEREFORE... (20ul) (1.00ugPFOS⁻/mL MeOH) (1.0mL/1000uL) (1000ng/1.0ug) = 20ng PFOS⁻ onto SPE column 20ng PFOS⁻ / 2.0mL MeOH = 10ng PFOS⁻/mL MeOH. (Since this standard is recorded as 10ppb, the correct units are <u>10ngPFOS⁻/mL of MeOH.</u>) These are therefore the units being measured and reported on the chromatographic system. Similarly, the following statement is true. (20ng PFOS⁻ / 1.0mL homogenate) (1.0mL homogenate / 0.1g liver) = 200ng PFOS⁻ / g liver This relationship shows the factor of 20 used to "correct" the units when calculating the final result in ng/g from the chromatographic result that is in ng/mL. <u>Sample Calculation:</u> "E01-1342-30348, 0ppm a.i. 333 M liver" Chromatographic result is 10.76 ng/mL; reported value is 208.4 ng/g. Since the 1.0333g initial sample weight of liver is homogenized with 9.0mL water, and 1mL (10% of total liver weighed, since a liver density of 1.00 is assumed) is used for the entire SPE procedure, the calculation is as follows: (10.76ng PFOS⁻/ mL MeOH) (2mL MeOH / 0.1033 g liver) = 208.4 ng PFOS⁻ / g liver <u>Sample Calculation:</u> Sample "E01-1342-36017, QC@10ppb-1": This QC sample is prepared by adding 4uL of 02001-53 (5.020ug PFOS⁻ / mL MeOH) spiked into 1mL liver homogenate. ((4ul) (5.020ug/mL) (1mL/1000uL) (1000ng/ug)) / (0.1g liver) = 200.8ng PFOS⁻ / g liver Using the earlier calculation, the analytical result of 13.59ng/mL calculates to 271.8ng/g liver. Since the spike level is 200.8ng PFOS⁻ / g liver, the recovery is (271.8/200.8)(100)=135.4%.	

Record of Deviation

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

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

Laboratory Management:  04/20/02

Record of Deviation

I. Identification	
Study / Project No. E01-1256	
Deviation Type (Check one)	☐ SOP ☐ Method ☐ Equipment Procedure ☒ Protocol ☐ Other:
Document Number: ETS-8-231.1	Date(s) of occurrence: Throughout the study
II. Description:	
Required Procedure/process:	
Section 11.3, Sentence 5 states: Any level outside 75% - 125% of nominal value must be deactivated, and regression recalculated...	
Actual Procedure/process:	
Many of the high calibration points in this study are kept in the curve despite being above the apex of the curve. When a calibration point lies above the apex, it is considered indeterminant (labeled with an I) and no concentration is calculated for the standard. If no concentration is calculated, no percent difference is calculated for evaluating whether or not that standard is within the stated criteria.	
Removal of these points in most cases causes the next calibration point to then be above the apex of the curve. This continued deletion of points could continue until there are no points remaining on the curve. Therefore, calibration points above the apex were included in the range of the curve with no evaluation of percent deviation criteria.	
III. Actions Taken:	
(such as amendment issued, SOP revision, etc.)	
This deviation was written, a note to file was written, and data points which were above the next highest calibration point were reanalyzed.	
Recorded By Lisa A. Stevenson <i>Lisa A. Stevenson</i>	Date 08/16/02 <i>08/16/02</i>
Authorized By (Study Director / Project Lead)	Date
Study Director: <i>Leann P. Gallagher</i> <i>9/24/02</i>	
Laboratory Management: <i>[Signature]</i> <i>08/20/02</i>	

Record of Deviation

I. Identification	
Study / Project No. E01-1256	
Deviation Type ☒ SOP ☐ Method ☐ Equipment Procedure (Check one) ☐ Protocol ☐ Other:	
Document Number: ETS-8-231.1	Date(s) of occurrence: Entire Study
II. Description:	
Required Procedure/process: Section 12.1, Calculations: If other calculations are used than those listed, they will be documented in the raw data.	
Actual Procedure/process: Standard E01-1256-36240, Prep sheet description "10 ppb sera curve point", was prepped by spiking 4uL of 02001-52 (5.020ug/mL) into 2 mL of pooled sera. This diluted sera consists of 2mL sera plus 38mL water. Since 1mL out of 40mL diluted sera is used to prepare each extracted standard, 1/40 of the 2mL of sera is "spiked". After 1.0 mL of diluted sera is spiked and prepared using SPE, the final eluate is 2.0mL of MeOH. THEREFORE... $(5.020\text{ug PFOS/mL MeOH}) * (4\text{ul}) * (1.0\text{mL}/1000\text{uL}) * (1000\text{ng}/1.0\text{ug}) = 20\text{ng PFOS} \text{ onto SPE column}$ $20\text{ng PFOS} / 2.0\text{mL MeOH} = 10\text{ng PFOS/mL MeOH.}$ (Since this standard is recorded as 10ppb, the correct units are <u>10ng PFOS/mL MeOH.</u>) These are therefore the units being measured and reported on the chromatographic system. Similarly, the following statement is true. $(20\text{ng PFOS} / 1.0\text{mL diluted sera}) * (40.0\text{mL diluted sera} / 2.0\text{mL initial sera}) = 400\text{ng PFOS} / \text{mL Sera}$ This relationship shows the factor of 40 (or 2.0mL MeOH/ 0.05mL Sera) used to "correct" the units when calculating the final result in ng PFOS/mL Sera from the chromatographic result that is in ng PFOS/mL MeOH. Sample Calculation: "E01-1256-28701, 454-109-3258, 10 ppm female, adult Chromatographic result is 340.3 ng/mL MeOH; reported value is 13612 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: $19.0, \text{ or } 1/20 \text{ dilution RE US 11/20/02}$ $(340.3\text{ng PFOS} / 1.0\text{mL MeOH}) * (2.0\text{mL MeOH} / 0.05\text{mL Sera}) = 13612\text{ng PFOS} / \text{mL Sera}$ Sample Calculation: Sample "E01-1256-36253, QC@250ppb-1": This QC sample is prepared by adding 10uL of 02001-53 (50.20ug PFOS / mL MeOH) spiked into 1mL diluted sera (or 0.05 mL of initial sera). $((50.20\text{ug/mL}) * (10\text{ul}) * (1\text{mL}/1000\text{uL}) * (1000\text{ng}/\text{ug})) / (0.05 \text{ mL Sera}) = 10040 \text{ ng PFOS} / \text{mL Sera}$ Using the earlier calculation, the analytical result of 264.06 ng/mL calculates to 10562.4 ng/mL Sera. Since the spike level is 10040ng PFOS/ mL Sera, the recovery is $(10562.4/10040)*(100)=105.2\%$.	
III. Actions Taken:	
(such as amendment issued, SOP revision, etc.)	
This deviation was written and a note to file was written.	
Recorded By Lisa A. Stevenson <i>Lisa A. Stevenson</i>	Date 08/16/02 08/16/02
Authorized By (Study Director / Project Lead) Study Director: <i>Sean P. Hallagher</i> 9/24/02	Date 11/29/02
Laboratory Management: <i>[Signature]</i> 08/20/02 3M Environmental Laboratory Form ETS-4-8.0	Deviation No. <u>4</u>

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

Record of Deviation

I. Identification									
Study / Project No. E01-1256									
Deviation Type ☐ SOP ☒ Method ☐ Equipment Procedure (Check one) ☐ Protocol ☐ Other:									
Document Number: ETS-8-231.1	Date(s) of occurrence: Throughout the study								
II. Description:									
Required Procedure/process: Section 13.3 of the method states: Two thirds of all quality control samples and 1/2 of each quality control sample at each level are expected to show an accuracy of 75-125%.									
Actual Procedure/process: All 25 ppb mallard sera quality control sample recoveries were >125%.									
III. Actions Taken: (such as amendment issued, SOP revision, etc.) This deviation was written, sera 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.									
<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 70%; padding: 5px;">Recorded By Lisa A. Stevenson <i>Lisa A. Stevenson</i></td> <td style="width: 30%; padding: 5px;">Date 08/26/02 8/26/02</td> </tr> <tr> <td style="padding: 5px;">Authorized By (Study Director / Project Lead)</td> <td style="padding: 5px;">Date</td> </tr> <tr> <td style="padding: 5px;">Study Director: <i>Sean P. Gallagher</i> 10/1/02</td> <td style="padding: 5px;"></td> </tr> <tr> <td colspan="2" style="padding: 5px;">Laboratory Management: <i>[Signature]</i> 08/26/02</td> </tr> </table>		Recorded By Lisa A. Stevenson <i>Lisa A. Stevenson</i>	Date 08/26/02 8/26/02	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	
Recorded By Lisa A. Stevenson <i>Lisa A. Stevenson</i>	Date 08/26/02 8/26/02								
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

I. Identification	
Study / Project No. E01-1256	
Deviation Type (Check one)	☒ SOP ☐ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:
Document Number: ETS-8-231.1	Date(s) of occurrence 2/25/02, 2/26/02, 2/27/02, 2/28/02, 3/1/02, 3/4/02, 3/5/02, 3/6/02, 3/7/02, 3/8/02,
II. Description:	
Required Procedure/process: Section 12.1, Calculations: If other calculations are used than those listed, they will be documented in the raw data.	
Actual Procedure/process: This deviation supersedes deviation #2 explaining the liver calculation: Standard E01-1326-36788, 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 5.0597g liver plus 45mL water. Since 1mL out of 50mL homogenate is used to prep each extracted standard, 1/50 of the 5.0597g (0.101073g = 5.0597 g / (45.0 mL water + 5.0597 g liver) is "spiked". After this homogenate is spiked and prepared using SPE, the final eluate is 2.0mL of MeOH. THEREFORE... $(20 \text{ uL}) * (1.00 \text{ ug PFOS} / \text{mL MeOH}) * (1.0 \text{ mL} / 1000 \text{ uL}) * (1000 \text{ ng} / 1.0 \text{ ug}) = 20.0 \text{ ng PFOS} \text{ onto SPE column}$ $20 \text{ ng PFOS} / 2.0 \text{ mL MeOH} = 10 \text{ ng PFOS} / \text{mL MeOH.}$ (Since this standard is recorded as 10ppb, the correct units are <u>10 ng PFOS/mL of MeOH.</u>) These are the units being measured and reported on the chromatographic system. Similarly, the following statement is true. $(20 \text{ ng PFOS} / 1.0 \text{ mL homogenate}) * (1.0 \text{ mL homogenate} / 0.101073 \text{ g liver}) = 198 \text{ ng PFOS} / \text{g liver}$ <u>Sample Calculation:</u> "E01-1326-29916, 10ppm a.i. 3257 M liver" Chromatographic result is 381.46 ng/mL; reported value is 72077 ng/g. Since the 1.0654 g initial sample weight of liver is homogenized with 9.0 mL water, and 1 mL is used for the entire SPE procedure, the calculation is as follows: $1.0654 \text{ g} / (9.0 \text{ mL water} + 1.0654 \text{ g liver}) = 0.105848 \text{ g of liver in } 1.0 \text{ mL homogenate removed for extraction.}$ $(381.46 \text{ ng PFOS} / \text{mL MeOH}) * (2 \text{ mL MeOH} / 0.105848 \text{ g liver}) * 10 = 72077 \text{ ng PFOS} / \text{g liver}$ <u>Sample Calculation:</u> Sample "E01-1326-36798, QC@25ppb-1": This QC sample is prepared by adding 1uL of 02001-53 (50.20ug PFOS / mL MeOH) spiked into 1mL liver homogenate. $((1 \text{ uL}) * (50.20 \text{ ug} / \text{mL}) * (1 \text{ mL} / 1000 \text{ uL}) * (1000 \text{ ng} / \text{ug})) / (0.101073 \text{ g liver}) = 497 \text{ ng PFOS} / \text{g liver}$ Using the earlier calculation, the analytical result of 38.87 ng/mL calculates to 571 ng/g liver. Since the spike level is 497 ng PFOS / g liver, the recovery is $(571 / 497) * (100) = 114.9\%$.	
III. Actions Taken:	
(such as amendment issued, SOP revision, etc.)	
Issuing of this SOP deviation.	
Recorded By Lisa A. Stevenson <i>Lisa A. Stevenson</i>	Date 12/02/02 <i>12/02/02</i>
Authorized By (Study Director / Project Lead) Study Director: Sean Gallagher <i>Sean P. Gallagher</i> Laboratory management: William Reagan <i>William Reagan</i> 3M Environmental Laboratory Form ETS-4-8.0	Date <i>12/3/02</i> Deviation No. <u>6</u> (assigned by Study Director or Project Lead at the end of study or project)

ATTACHMENT F: EXAMPLE CALCULATIONS

FORMULA USED FOR SERA ANALYSES IN STUDY E01-1256

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

Calculation Used for E01-1256-28701, 454-109-3258, 10 ppm Female adult (initial volume = 0.5 mL)

$$340.3 \text{ ng/mL MeOH} \times 1 \times 40 = 13600 \text{ ng/mL}$$

FORMULA USED FOR LIVER ANALYSES IN STUDY E01-1256

$$\text{AR (ng/mL MeOH)} \times \text{DF} \times \frac{\text{Final Volume (mL)}}{\text{amount of liver in diluted sample (g)}} = \text{ng/g Liver}$$

Calculation Used for E01-1326-29925, 10 ppm, a.i. 3258 F Liver (initial weight = 1.0034 g)

$$355.07 \text{ ng/mL MeOH} \times 1 \times \frac{2.0 \text{ mL MeOH}}{0.100306 \text{ g Liver}} = 7080 \text{ ng/g Liver}$$

AR = Analytical result from MassLynx summary

DF = Dilution factor

Dilution(diluted sera) = Final volume(mL)/amount of sera in diluted sample(mL)

Amount of sera in diluted sample(mL) = amount of sera used(mL)/total amount of sera + water(mL)

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

Examples:

Amount of sera in diluted sample(mL) = amount of sera used (0.5 mL)/total amount of sera + water (10 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 (1.0034 g)/amount of liver weighed (1.0034 g) + water added for homogenizing (9.00 mL) = 1.0034 g / 10.0034 g = 1.00306 g