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Analytical Report

Definitive Study: Quantitative Screening of Commercial Lots of Human Serum Obtained in 2004 for Endogenous Fluorochemicals Using Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry

Laboratory Request Number: E05-0442

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Analytical Report

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1 Introduction / Summary

The following analytical report presents data from commercially available normal pooled human serum that were purchased by the 3M Environmental Laboratory and subsequently screened for endogenous fluorochemicals.

Thirty-six separate lots of pooled normal human serum were extracted using a solid phase extraction technique and screened for sixteen different fluorochemicals using liquid chromatography/tandem mass spectrometry (LC/MS/MS). Serum samples were received from various suppliers between 9/9/04 and 12/2/04. Samples were extracted on 5/11/2005 and analyzed 5/23/2005.

Heptafluorobutyric acid (PFBA) was included in the original list of sixteen target analytes. However, due to instability in the methanol extract, analytical data for PFBA will not be included in this report. Instead, a separate study will be conducted including the re-extraction of the thirty-six lots of commercial serum with acetonitrile and analysis using a newly developed method that provides isomer resolution. Those results will be reported in a separate report.

Sample results are summarized in Tables 1 and 2. The average values reported represent samples in which there was detectable analyte. Sample values that were less than the limit of quantitation were not included in the average value calculations. Please refer to Section 5 for individual sample results.

Table 1. Summary of Perfluorinated Acid Results

Analyte	NFPA (ng/mL)	PFHA (ng/mL)	TDHA (ng/mL)	PFOA (ng/mL)	C9 Acid (ng/mL)	C10 ACID (ng/mL)	C11 ACID (ng/mL)	C12 ACID (ng/mL)
Mean	1.04	0.0111	0.151[1]	2.98	0.813	0.282	0.185	0.0246
Median	1.05	0.00838	0.118	3.21	0.737	0.281	0.134	0.0197
High	2.11	0.0323	0.474	5.72	1.97	0.599	0.734	0.0833
Low	0.327	0.00633	0.0232	0.359	0.0950	0.0274	0.0152	0.0108
Standard Deviation	0.378	0.00822	0.111	1.27	0.445	0.137	0.176	0.0149
Number of Samples with Detectable Analyte	36/36	9/36	33/36	36/36	36/36	36/36	36/36	30/36

Table 2. Summary of Other Perfluorinated and Highly Fluorinated Analyte Results

Analyte	FBSA (ng/mL)	FOSA (ng/mL)	PFBS (ng/mL)	PFHS (ng/mL)	PFOS (ng/mL)	THPFOS (ng/mL)	THPFDS (ng/mL)
Mean	NA[1]	0.149	0.0165	2.55	14.9	NA[2]	0.0442
Median	NA[1]	0.124	0.0101	2.85	15.5	NA[2]	0.0366
High	0.00542	0.446	0.0840	4.18	27.0	NA[2]	0.126
Low	0.00542	0.0378	0.00545	0.606	2.03	NA[2]	0.00772
Standard Deviation	NA[1]	0.0876	0.0169	1.07	5.94	NA[2]	0.0227
Number of Samples with Detectable Analyte	1/36	36/36	33/36	36/36	36/36	0/36	36/36

NA[1] = Not Applicable because this analyte was only detected in one of the samples.

NA[2] = Not Applicable because this analyte was not detected in any of the samples.

2 Reference Substances

Table 3. Reference Substances

Name	Synonym or Acronym	Standard Source Material Vendor	Traceability Number	Purity *	Additional Correction Factor (K^+ salt or H^+)
Nonafluoropentanoic acid	NFPA (C5 Acid)	Aldrich	TCR-333 (00017-045)	96.5%	0.996
Perfluorohexanoic acid	PFHA (C6 Acid)	Oakwood Products	TCR-673	97.7%	0.997
Tridecafluoroheptanoic acid	TDHA (C7 Acid)	Aldrich	TCR-267	98.2%	0.997
Perfluorooctanoic acid	PFOA (C8 Acid)	3M	TCR-123 (99030-030)	95.0%	0.958
Heptadecafluorononanoic acid	C9 Acid	Oakwood Products	TCR-618	98.0%	0.998
Nonadecafluorodecanoic acid	C10 Acid	Oakwood Products	TCR-36	98.01%	0.998
Perfluoroundecanoic acid	C11 Acid	Oakwood Products	TCR-619	96.4%	0.998
Perfluorododecanoic acid	C12 Acid	Oakwood Products	TCR-37 (SD037)	99.65%	0.998
Perfluorobutanesulfonamide	FBSA	3M	TCR-314 (00017-026)	unknown	None
Perfluorooctanesulfonamide	FOSA	3M	TCR-280 (99131-038)	98.94%	None
Perfluorobutane sulfonate	PFBS	3M	TCR-116 (99030-023)	96.7%	0.8844
Perfluorohexane sulfonate	PFHS	3M	TCR-83 (SE036)	98.6%	0.911
Perfluorooctane sulfonate	PFOS	3M	TCR-696	86.4%	0.927
1H, 1H, 2H, 2H, perfluorooctanesulfonic acid	THPFOS	SynQuest Labs	TCR-343 (00017-055)	93.5%	0.998
1H, 1H, 2H, 2H, perfluorodecane sulfonate	THPFDS	Pace/3M	TCR-627	94.7%	0.998
Perfluorooctanoic acid, isotopically labeled	PFOA [1,2-^{13}C]	Perkin Elmer	TCR-744	>97%	None
Perfluorooctane sulfonate, ammonium salt, isotopically labeled	PFOS [$^{18}O_2$]	RTI International	TCR-756	100 ug/mL Standard	None

* Reference substance purities have not been corrected for impurities due to shorter chain homologues which cannot be distinguished by NMR analysis.

NMR results indicate that the PFOS reference substance consists of 30.8% branched isomer, and the PFOA reference substance consists of 22.1% branched isomer. These values were used for the determination of the isomer ratios in the serum samples (presented in Table 10).

3 Sample Preparation

The samples for this study were prepared as follows:

1. The serum samples were allowed to thaw and warm to room temperature.
2. Four milliliters of serum (or water in the case of method blanks) were added to a 15 mL screw-capped polypropylene centrifuge tube.
3. Matrix spikes and method blanks were prepared by spiking the serum (or water) with the analytes of interest. In addition, ^{13}C PFOA and $^{18}O_2$ PFOS were added as surrogates to all serum samples, calibration standards, matrix blanks, and method blanks at concentrations of 1.04 and 0.950 ng/mL serum respectively to evaluate extraction method performance.
4. Ten milliliters of acetonitrile were added to the serum and the centrifuge tubes were capped and vortex mixed, allowing the serum proteins to precipitate.
5. The tubes were centrifuged at approximately 3500 rpm for 20 minutes to pellet the precipitate.
6. After removal from the centrifuge, the supernatant was transferred to a bottle containing 70 mL of water.
7. The samples were passed through a preconditioned SPE cartridge and eluted with 2 mL of methanol.

Quantitation was accomplished using high performance liquid chromatography tandem mass spectrometry. The following parameters were used:

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column: Keystone® Betasil™ C_{18} 2.1x100mm, 5µm particle size
Column temperature: 30 °C
Stop Time: 20.0 minutes
Flow rate: 300 µL/min
Injection volume: 5 µL
Mobile phase components:
Solvent A: 2.0 mM ammonium acetate in ASTM Type I water
Solvent B: HPLC Grade Methanol
Solvent Gradient:

Time	%B
0.00	20%
1.00	20%
14.50	90%
15.50	90%
16.50	20%
20.00	20%

Mass Spectrometer: MDX Sciex® API 4000 Q-Trap Mass Spectrometer Detector
Software: Analyst™ Version 1.4
Ionization Mode: Electrospray Negative
Temperature: 450 °C
GS1: 35
GS2: 45
Entrance Potential: -10
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 4. Acquisition Parameters

Compound	Q1 Mass (amu)	Q3 Mass (amu)	Declustering Potential
NFPA (C_5 Acid)	262.9	219.0	-30
PFHA (C_6 Acid)	313.0	268.7, 118.9	-45
TDHA (C_7 Acid)	362.9	318.7, 168.8, 118.9	-35
PFOA (C_8 Acid)	413.0	368.9, 219.0, 169.0	-45
C_9 Acid	463.0	418.7, 168.9, 218.9	-45
C_{10} Acid	512.9	468.8, 218.9, 269.1	-35
C_{11} Acid	563.0	518.7, 268.9, 218.8	-45
C_{12} Acid	613.0	568.7, 168.9, 318.7	-50
FBSA	297.9	78.0	-55
FOSA	497.9	77.9	-90
PFBS	298.9	98.9, 79.9	-65
PFHS	398.9	98.9, 80.0	-95
PFOS	498.9	80.0, 98.9, 130.0	-120
THPFOS	426.9	406.8, 80.9	-70
THPFDS	526.9	506.7, 81.0	-70
PFOA [1,2-^{13}C]	414.9	369.8	-40
PFOS [$^{18}O_2$]	503.0	103.0, 84.0	-100

When multiple transitions were monitored, the total ion current (TIC) was used to quantify each analyte, thereby providing greater sensitivity than individual Q3 ion measurements.

4 Analysis

4.1 Calibration

4.1.1 Calibration Curve

An extracted calibration curve was formed by spiking rabbit serum at prescribed levels and extracting the curve in the same manner as the samples. Stock solutions were individually prepared from neat fluorochemicals and diluted in methanol. Samples were quantified versus a 15-point extracted calibration curve.

The regression analysis was performed using a quadratic curve fit weighted 1/x. Calibration standards were within ± 25% (±30% for the Lower Limit of Quantitation (LLOQ)) with a coefficient of determination >0.990. Low-level calibration standards which were not within ±30% were not included in the calibration curve. Calibration standards that were not greater than 2x the method blanks were not included in the calibration curve, except where noted.

4.1.2 Continuing Calibration Verification (CCV)

A Continuing Calibration Verification (CCV) was run at least once every 10 samples, bracketing the samples with the calibration curve. The CCV recovery was within ±25% for all analytes.

4.1.3 System Suitability

Five system suitability standards were analyzed before the initial calibration curve and after the last CCV. These samples had area counts with a %RSD of ≤5% and a retention time %RSD of ≤2% when evaluated independently except for the following instances:

The system suitability samples for each of the perfluorinated acids and FOSA failed. In each case the %RSD of the retention times was well within tolerance limits, but the area counts varied by as much as 20.3%. The failure of every perfluorinated acid tested suggests the presence of a systematic effect on that class of analytes. This should be kept in mind when evaluating the data presented in this report.

4.1.4 Lower Limit of Quantitation (LLOQ)

The LLOQ for each analyte was equal to the nominal concentration of the lowest standard in the calibration curve that met accuracy requirements of 70% -130% and that had at least twice as many area counts as the method blanks.

4.2 Blanks

4.2.1 Method Blanks

Method blanks consisted of aliquots of Milli-Q water extracted in the same manner as the samples. All of the method blanks in this study had area counts < 50% of the LLOQ for their respective analytes with the following exceptions:

- PFHA: Two out of three method blanks was greater than 50% of the LLOQ; none were above the LLOQ.
- C7 Acid: One of three method blanks had area counts greater than 50% of the LLOQ; none were above the LLOQ.
- C11 Acid: One of three method blanks had area counts greater than 50% of the LLOQ; none were above the LLOQ
- FOSA: Two out of three method blanks were greater than 50% of the LLOQ; none were above the LLOQ.

- PFBS: Two method blanks had area counts greater than 50% of the LLOQ; one showed anomalous contamination and was above the LLOQ.

4.2.2 Matrix Blanks

Matrix blanks consisted of aliquots of blank rabbit serum extracted in the same manner as the samples. Three matrix blanks were prepared and analyzed with the samples. Matrix blanks containing endogenous levels of analyte that were above the LLOQ are detailed below.

Table 5. Endogenous Analyte in Rabbit Serum

Sample ID	TDHA	C11 ACID	PFBS	THPFOS	THPFDS
LLOQ (ng/mL)	0.00504	0.00992	0.00506	0.00520	0.00503
MxBlk_050511-1	0.0065	0.0196	0.00941	0.0260*	0.0146
MxBlk_050511-2	0.00252*	0.0138	0.00729	0.0260*	0.0131
MxBlk_050511-3	0.0102	0.0169	0.00774	0.0249	0.0137
Average	0.00641	0.0168	0.00815	0.0256	0.0138

*Please refer to the note to file included in the raw data describing the calculation of the endogenous levels.

The average endogenous values were used to correct the matrix spike response.

4.2.3 Solvent Blanks

Methanol blanks were analyzed throughout the analytical run. Analyte response was less than ½ the LLOQ in all cases.

Surrogate

PFOA [1,2-^{13}C] and PFOS [$^{18}O_2$] were added as surrogates to all serum samples, calibration standards, matrix blanks, and method blanks to evaluate the extraction method performance. All PFOA [1,2-^{13}C] surrogate recoveries were within ±30%, but two of the PFOS [$^{18}O_2$] recoveries were outside ±30%, extending the surrogate recovery range to +/-34%. Specific surrogate recovery data is presented below.

Table 6. Surrogate Recovery

Sample ID	PFOA [1,2-^{13}C] Recovery, %	PFOS [$^{18}O_2$] Recovery, %		Sample ID	PFOA [1,2-^{13}C] Recovery, %	PFOS [$^{18}O_2$] Recovery, %
MxBlk_050511-1	110%	111%		TN-A-6992	94%	83%
MxBlk_050511-2	102%	98%		TN-A-6994	87%	77%
MdBlk_050511-1	80%	112%		TN-A-6995	83%	74%
MdBlk_050511-2	99%	103%		TN-A-6997	84%	75%
LS_0505115-1	114%	103%		TN-A-7000	78%	72%
LS_0505115-2	109%	94%		TN-A-7006	91%	84%
LS_0505115-3	98%	98%		TN-A-6938	92%	76%
HS_0505115-1	85%	95%		TN-A-6939	78%	88%
HS_0505115-2	120%	98%		TN-A-6941	88%	82%
HS_0505115-3	90%	88%		TN-A-6950	96%	85%
TN-A-6840	80%	87%		TN-A-6953	78%	81%
TN-A-6843	93%	82%		TN-A-6955	85%	81%
TN-A-6847	78%	79%		TN-A-6962	84%	85%
TN-A-6934	102%	72%		TN-A-6964	89%	86%
TN-A-6935	89%	82%		TN-A-6966	83%	75%
TN-A-6944	80%	79%		TN-A-6970	84%	84%
TN-A-6959	85%	80%		TN-A-6972	73%	75%
TN-A-6960	78%	66%		TN-A-6978	74%	80%
TN-A-6967	91%	85%		TN-A-6979	92%	78%
TN-A-6973	76%	76%		TN-A-6982	113%	87%
TN-A-6975	84%	82%		TN-A-6984	86%	86%
TN-A-6977	82%	73%		TN-A-6986	81%	83%
TN-A-6981	83%	67%		TN-A-6988	85%	83%

4.4 Laboratory Control Spikes (LCS)

Six Laboratory Control Spikes (LCS) were prepared in rabbit serum and analyzed with the samples. Results were corrected for endogenous levels of analyte in the matrix (where appropriate), and are presented below.

Table 7. Matrix Spike Recoveries

Analyte	Sample ID	Nominal Conc., ng/mL	Observed Conc., ng/mL	Recovery, %	% RSD
NFPA	LS_0505115-1	0.306	0.517	169%	23%
	LS_0505115-2	0.306	0.373	122%	
	LS_0505115-3	0.309	0.336	109%	
	HS_0505115-1	3.06	2.89	94%	11%
	HS_0505115-2	3.06	3.48	114%	
	HS_0505115-3	3.06	3.55	116%	
PFHA	LS_0505115-1	0.303	0.699	231%	65%
	LS_0505115-2	0.303	0.281	93%	
	LS_0505115-3	0.303	0.224	74%	
	HS_0505115-1	3.03	3.43	113%	10%
	HS_0505115-2	3.03	2.79	92%	
	HS_0505115-3	3.03	3.09	102%	

Table 7. Matrix Spike Recoveries (continued)

PFOA	LS_0505115-1	0.297	0.235	79%	38%
	LS_0505115-2	0.297	0.129	43%	
	LS_0505115-3	0.297	0.136	46%	
	HS_0505115-1	2.97	2.46	83%	6.9%
	HS_0505115-2	2.97	2.82	95%	
	HS_0505115-3	2.97	2.7	91%	
C9 Acid	LS_0505115-1	0.300	0.285	95%	13%
	LS_0505115-2	0.300	0.237	79%	
	LS_0505115-3	0.300	0.31	103%	
	HS_0505115-1	3.00	2.14	71%	19%
	HS_0505115-2	3.00	3.03	101%	
	HS_0505115-3	3.00	3.04	101%	
C10 ACID	LS_0505115-1	0.311	0.291	94%	5.9%
	LS_0505115-2	0.311	0.323	104%	
	LS_0505115-3	0.311	0.323	104%	
	HS_0505115-1	3.11	3.30	106%	3.8%
	HS_0505115-2	3.11	3.16	102%	
	HS_0505115-3	3.11	3.06	98%	
C12 ACID	LS_0505115-1	0.30	0.323	108%	7.0%
	LS_0505115-2	0.30	0.371	124%	
	LS_0505115-3	0.30	0.341	114%	
	HS_0505115-1	2.99	2.91	97%	12%
	HS_0505115-2	2.99	3.65	122%	
	HS_0505115-3	2.99	3.56	119%	
FBSA	LS_0505115-1	0.300	0.305	102%	7.8%
	LS_0505115-2	0.300	0.354	118%	
	LS_0505115-3	0.300	0.318	106%	
	HS_0505115-1	3.00	3.00	100%	7.8%
	HS_0505115-2	3.00	3.25	108%	
	HS_0505115-3	3.00	3.51	117%	

Table 7. Matrix Spike Recoveries (continued)

Analyte	Sample ID	Nominal Conc., ng/mL	Observed Conc., ng/mL	Recovery, %	% RSD
FOSA	LS_0505115-1	0.299	0.370	124%	21%
	LS_0505115-2	0.299	0.274	92%	
	LS_0505115-3	0.299	0.251	84%	
	HS_0505115-1	2.99	2.79	93%	19%
	HS_0505115-2	2.99	3.45	115%	
	HS_0505115-3	2.99	2.36	79%	
PFHS	LS_0505115-1	0.315	0.305	97%	2.6%
	LS_0505115-2	0.315	0.302	96%	
	LS_0505115-3	0.315	0.317	101%	
	HS_0505115-1	3.15	3.12	99%	4.9%
	HS_0505115-2	3.15	2.85	90%	
	HS_0505115-3	3.15	2.90	92%	
PFOS	QC050202-001	0.308	0.195	63%	18%
	QC050202-002	0.308	0.160	52%	
	QC050202-003	0.308	0.230	75%	
	QC050202-004	3.08	3.07	100%	11%
	QC050202-005	3.08	3.08	100%	
	QC050202-006	3.08	2.52	82%	

Table 7. Matrix Spike Recoveries (continued and corrected for endogenous target analytes)

Analyte	Sample ID	Nominal Conc., ng/mL	Observed Conc., ng/mL	Endogenous Level in Matrix, ng/mL	Observed Conc., Corrected for Endogenous Level, ng/mL	Recovery, %	% RSD
TDHA	LS_0505115-1	0.302	0.392	0.00641	0.39	128%	8%
	LS_0505115-2	0.302	0.354	0.00641	0.35	115%	
	LS_0505115-3	0.302	0.334	0.00641	0.33	108%	
	HS_0505115-1	3.02	2.93	0.00641	2.9	97%	3%
	HS_0505115-2	3.02	2.84	0.00641	2.8	94%	
	HS_0505115-3	3.02	2.74	0.00641	2.7	91%	
C11 ACID	LS_0505115-1	0.297	0.355	0.0168	0.338	114%	11%
	LS_0505115-2	0.297	0.292	0.0168	0.275	93%	
	LS_0505115-3	0.297	0.310	0.0168	0.293	99%	
	HS_0505115-1	2.97	2.89	0.0168	2.87	97%	3.6%
	HS_0505115-2	2.97	2.82	0.0168	2.80	94%	
	HS_0505115-3	2.97	2.69	0.0168	2.67	90%	
PFBS	LS_0505115-1	0.304	10.8	0.00815	10.8*	3550%*	6.2%
	LS_0505115-2	0.304	0.314	0.00815	0.306	101%	
	LS_0505115-3	0.304	0.342	0.00815	0.334	110%	
	HS_0505115-1	3.04	2.97	0.00815	2.96	97%	2.9%
	HS_0505115-2	3.04	3.10	0.00815	3.09	102%	
	HS_0505115-3	3.04	3.14	0.00815	3.13	103%	
THPFOS	LS_0505115-1	0.312	NR	0.0256	NR	NR	NR
	LS_0505115-2	0.312	NR	0.0256	NR	NR	
	LS_0505115-3	0.312	0.589	0.0256	0.56	181%	
	HS_0505115-1	3.12	2.94	0.0256	2.9	93%	12%
	HS_0505115-2	3.12	3.06	0.0256	3.0	97%	
	HS_0505115-3	3.12	2.44	0.0256	2.4	77%	
THPFDS	LS_0505115-1	0.302	0.330	0.0138	0.316	105%	5.1%
	LS_0505115-2	0.302	0.306	0.0138	0.292	97%	
	LS_0505115-3	0.302	0.302	0.0138	0.288	95%	
	HS_0505115-1	3.02	2.94	0.0138	2.93	97%	1.8%
	HS_0505115-2	3.02	2.85	0.0138	2.84	94%	
	HS_0505115-3	3.02	2.94	0.0138	2.93	97%	

*This value can be eliminated as an outlier at the 99% confidence level and will be excluded in all further calculations.

NR = Not Reported because measurable amounts of target analyte were not observed.

4.5 Accuracy

The accuracy for each analyte reported was calculated by taking the average of the matrix spike recoveries for that analyte. The results are presented in Table 8.

Table 8. Method Accuracy for Individual Analytes

Analyte	% Accuracy		Analyte	% Accuracy
NFPA	± 21%		FBSA	± 8%
PFHA	± 17%		FOSA	± 2%
TDHA	± 5%		PFBS	± 3%
PFOA	± 27%		PFHS	± 4%
C9 Acid	± 8%		PFOS	± 21%
C10 Acid	± 1%		THPFOS	± 12%
C11 Acid	± 2%		THPFDS	± 3%
C12 Acid	± 14%			

4.6 Sample Related Comments

The extraction and analysis of the samples was performed utilizing a non-validated method. The matrix spike results, PFOA [1,2-^{13}C] and PFOS [$^{18}O_2$] surrogate recoveries, and the calibration curve should be considered when evaluating the accuracy and precision of the sample results.

5 Individual Sample Data

Tables 9 and 10 summarize individual sample data.

Table 9. Perfluorinated Acid Results

Sample ID	NFPA (ng/mL)	PFHA (ng/mL)	TDHA (ng/mL)	PFOA (ng/mL)	C9 Acid (ng/mL)	C10 ACID (ng/mL)	C11 ACID (ng/mL)	C12 ACID (ng/mL)
LLOQ (ng/mL)	0.102	0.00505	0.00504	0.0991	0.0150	0.0259	0.00992	0.00997
TNA 6840	0.445	< LLOQ	< LLOQ	3.89	0.656	0.198	0.0968	0.0282
TNA 6843	1.51	< LLOQ	0.0752	0.509	0.127	0.0768	0.0259	0.0212
TNA 6847	1.56	0.0133	0.118	3.55	0.756	0.309	0.141	0.0207
TNA 6934	0.866	0.00633	0.0232	5.72	1.19	0.450	0.188	0.0264
TNA 6935	1.04	0.00838	0.0964	3.48	1.17	0.551	0.384	0.0833
TNA 6944	1.43	< LLOQ	0.290	5.67	1.18	0.584	0.196	0.0465
TNA 6959	0.795	0.00964	0.195	2.52	1.96	0.193	0.734	0.0370
TNA 6960	0.932	0.00889	0.210	2.91	0.611	0.300	0.121	0.0190
TNA 6967	0.450	< LLOQ	0.270	3.66	0.531	0.209	0.109	0.0198
TNA 6973	0.680	0.00652	0.352	3.60	0.940	0.391	0.124	0.0328
TNA 6975	0.682	0.00779	0.398	4.02	0.906	0.402	0.149	0.0295

The uncertainty of these results (based on isotopically labeled surrogate recovery) is +/- 34%.

Table 9. Perfluorinated Acid Results (continued)

Sample ID	NFPA (ng/mL)	PFHA (ng/mL)	TDHA (ng/mL)	PFOA (ng/mL)	C9 Acid (ng/mL)	C10 ACID (ng/mL)	C11 ACID (ng/mL)	C12 ACID (ng/mL)
LLOQ (ng/mL)	0.102	0.00505	0.00504	0.0991	0.0150	0.0259	0.00992	0.00997
TNA 6977*	1.23	< LLOQ	0.0442	0.406	0.0950	0.0374	0.0152	< LLOQ
TNA 6981	0.327	< LLOQ	0.0555	2.26	0.423	0.147	0.0708	< LLOQ
TNA 6992	1.09	< LLOQ	0.239	3.32	0.842	0.319	0.158	0.0124
TNA 6994	1.75	0.00694	0.0750	0.770	0.206	0.0830	0.142	0.0223
TNA 6995	1.06	< LLOQ	0.198	2.00	1.41	0.169	0.688	0.0147
TNA 6997	1.32	< LLOQ	0.179	2.33	1.97	0.202	0.637	0.0185
TNA 7000	1.08	< LLOQ	0.174	3.92	0.717	0.332	0.112	0.0240
TNA 7006	0.818	< LLOQ	0.166	3.32	0.686	0.306	0.0946	0.0128
TNA 6938	1.09	< LLOQ	0.474	4.36	1.20	0.421	0.165	0.0150
TNA 6939	0.615	0.0323	0.0828	2.01	1.09	0.344	0.28	0.0302
TNA 6941	1.02	< LLOQ	0.0608	4.07	1.61	0.599	0.424	0.0527
TNA 6950	2.11	< LLOQ	0.0925	3.20	0.792	0.229	0.116	0.0115
TNA 6953	1.27	< LLOQ	0.0442	3.08	0.785	0.249	0.0753	0.0150
TNA 6955	1.26	< LLOQ	0.111	2.20	0.603	0.341	0.196	0.0309
TNA 6962	1.09	< LLOQ	< LLOQ	3.51	0.690	0.311	0.111	0.0183
TNA 6964	1.28	< LLOQ	0.259	4.00	0.933	0.386	0.117	0.0176
TNA 6966	0.965	< LLOQ	< LLOQ	2.81	0.562	0.249	0.0916	0.0195
TNA 6970	0.781	< LLOQ	0.159	4.17	0.917	0.322	0.148	0.0119
TNA 6972	1.19	< LLOQ	0.123	3.79	0.761	0.257	0.126	0.0192
TNA 6978*	1.36	< LLOQ	0.0481	0.359	0.114	0.0274	0.0152	< LLOQ
TNA 6979	1.11	< LLOQ	0.126	3.21	0.568	0.304	0.156	0.0161
TNA 6982	0.751	< LLOQ	0.0660	2.16	0.653	0.173	0.0547	< LLOQ
TNA 6984	0.899	< LLOQ	0.0636	2.19	0.558	0.261	0.184	0.0108
TNA 6986	0.500	< LLOQ	0.0549	2.31	0.460	0.179	0.0394	< LLOQ
TNA 6988	0.916	< LLOQ	0.0443	1.93	0.610	0.239	0.164	< LLOQ
Mean	1.04	0.0111	0.151	2.98	0.813	0.282	0.185	0.0246
Median	1.05	0.00838	0.118	3.21	0.737	0.261	0.134	0.0197
High	2.11	0.0323	0.474	5.72	1.97	0.599	0.734	0.0833
Low	0.327	0.00633	0.0232	0.359	0.095	0.0274	0.0152	0.0108
Standard Deviation	0.378	0.00822	0.111	1.27	0.445	0.137	0.176	0.0149
# OF HITS	36	9	33	36	36	36	36	30

*TN-A-6977 and –6978 are from the same lot of pooled serum.
The uncertainty of these results (based on isotopically labeled surrogate recovery) is +/- 34%.

Table 9. Sample Results for Additional Perfluorinated and Highly Fluorinated Analytes

Sample ID	FBSA (ng/mL)	FOSA (ng/mL)	PFBS (ng/mL)	PFHS (ng/mL)	PFOS (ng/mL)	THPFOS (ng/mL)	THPFDS (ng/mL)
LLOQ (ng/mL)	0.00501	0.00498	0.00506	0.0158	0.103	0.00520	0.00503
TNA 6840	< LLOQ	0.356	0.00795	3.07	21.9	< LLOQ	0.0562
TNA 6843	< LLOQ	0.124	0.00854	0.606	2.78	< LLOQ	0.106
TNA 6847	< LLOQ	0.0694	0.00854	1.62	22.4	< LLOQ	0.126
TNA 6934	< LLOQ	0.314	0.0112	1.95	27.0	< LLOQ	0.00772
TNA 6935	< LLOQ	0.133	0.0101	1.75	14.5	< LLOQ	0.0457
TNA 6944	< LLOQ	0.0786	0.00969	3.66	16.9	< LLOQ	0.048
TNA 6959	< LLOQ	0.232	0.00979	2.85	12.4	< LLOQ	0.0311
TNA 6960	< LLOQ	0.166	0.0107	3.27	14.6	< LLOQ	0.0365
TNA 6967	0.00542	0.446	0.0238	3.04	19.7	< LLOQ	0.0425
TNA 6973	< LLOQ	0.156	0.0172	3.94	16.9	< LLOQ	0.0353
TNA 6975	< LLOQ	0.127	0.00858	3.97	18.0	< LLOQ	0.0366
TNA 6977*	< LLOQ	0.118	0.0840	0.662	2.03	< LLOQ	0.0413
TNA 6981	< LLOQ	0.122	< LLOQ	1.17	12.5	< LLOQ	0.0351
TNA 6992	< LLOQ	0.159	0.0103	3.66	16.6	< LLOQ	0.0417
TNA 6994	< LLOQ	0.116	0.0211	1.37	2.78	< LLOQ	0.0249
TNA 6995	< LLOQ	0.248	0.0160	2.88	11.6	< LLOQ	0.028
TNA 6997	< LLOQ	0.224	0.00942	2.92	12.0	< LLOQ	0.0273
TNA 7000	< LLOQ	0.0906	0.0148	3.97	17.5	< LLOQ	0.034
TNA 7006	< LLOQ	0.202	0.00989	3.43	19.2	< LLOQ	0.0358
TNA 6938	< LLOQ	0.0446	0.00711	2.38	16.3	< LLOQ	0.0259
TNA 6939	< LLOQ	0.0967	0.0202	1.65	6.43	< LLOQ	0.0349
TNA 6941	< LLOQ	0.130	0.00991	2.90	21.8	< LLOQ	0.0279
TNA 6950	< LLOQ	0.0378	0.00545	2.84	14.9	< LLOQ	0.0753
TNA 6953	< LLOQ	0.0459	0.00804	4.08	21.9	< LLOQ	0.0273
TNA 6955	< LLOQ	0.0981	0.00588	2.73	9.82	< LLOQ	0.0287
TNA 6962	< LLOQ	0.162	0.0122	3.64	17.8	< LLOQ	0.0345
TNA 6964	< LLOQ	0.187	0.0218	4.18	20.1	< LLOQ	0.0461
TNA 6966	< LLOQ	0.135	0.00802	3.10	15.2	< LLOQ	0.0281
TNA 6970	< LLOQ	0.238	0.00737	3.22	18.3	< LLOQ	0.0466
TNA 6972	< LLOQ	0.124	0.0557	2.82	14.4	< LLOQ	0.0319
TNA 6978*	< LLOQ	0.109	0.00577	0.640	2.21	< LLOQ	0.0485
TNA 6979	< LLOQ	0.121	0.0127	1.97	19.0	< LLOQ	0.0799
TNA 6982	< LLOQ	0.0903	0.0144	1.53	14.7	< LLOQ	0.0581
TNA 6984	< LLOQ	0.108	< LLOQ	1.32	14.2	< LLOQ	0.0529
TNA 6986	< LLOQ	0.0712	0.0567	1.57	14.1	< LLOQ	0.0459

The uncertainty of these results (based on isotopically labeled surrogate recovery) is +/- 34%.

Table 9. Sample Results for Additional Perfluorinated and Highly Fluorinated Analytes (continued)

Sample ID	FBSA (ng/mL)	FOSA (ng/mL)	PFBS (ng/mL)	PFHS (ng/mL)	PFOS (ng/mL)	THPFOS (ng/mL)	THPFDS (ng/mL)
LLOQ (ng/mL)	0.00501	0.00498	0.00506	0.0158	0.103	0.00520	0.00503
TNA 6988	< LLOQ	0.101	< LLOQ	1.45	15.7	< LLOQ	0.0586
Mean	NA	0.149	0.01645	2.55	14.9	NA	0.0442
Median	NA	0.124	0.0101	2.85	15.5	NA	0.0366
High	0.00542	0.446	0.084	4.18	27	NA	0.126
Low	0.00542	0.0378	0.0055	0.606	2.03	NA	0.00772
Standard Deviation	NA	0.0876	0.0169	1.07	5.94	NA	0.0227
# OF HITS	1	36	33	36	36	0	36

*TN-A-6977 and –6978 are from the same lot of pooled serum.
The uncertainty of these results (based on isotopically labeled surrogate recovery) is +/- 34%.

Table 10. Isomer Ratios for Individual Samples

Sample ID	Sample Origin	PFOA % Branched Isomer	PFOS % Branched Isomer
TNA 6840	California	<LLOQ*	37%
TNA 6843	Missouri	<LLOQ*	35%
TNA 6847	Pennsylvania	3.2%	42%
TNA 6934	Pennsylvania	1.3%	46%
TNA 6935	New York	6.8%	34%
TNA 6944	Michigan	2.7%	33%
TNA 6959	Virginia	3.9%	37%
TNA 6960	Virginia	2.3%	36%
TNA 6967	Maryland	<LLOQ*	40%
TNA 6973	Montreal, Quebec	2.3%	39%
TNA 6975	Montreal, Quebec	2.3%	40%
TNA 6977**	Massachusetts	<LLOQ*	35%
TNA 6981	Texas	<LLOQ*	40%
TNA 6992	California	<LLOQ*	36%
TNA 6994	California	3.1%	38%
TNA 6995	California	4.2%	39%
TNA 6997	California	3.9%	39%
TNA 7000	California	2.2%	40%
TNA 7006	California	<LLOQ*	39%
TNA 6938	New York	2.3%	40%
TNA 6939	New York	1.1%	33%
TNA 6941	New York	0.7%	35%
TNA 6950	Michigan	1.6%	37%
TNA 6953	Michigan	0.8%	39%

TNA 6955	Michigan	1.6%	34%
TNA 6962	Virginia	<LLOQ*	39%
TNA 6964	Virginia	<LLOQ*	39%
TNA 6966	Virginia	<LLOQ*	37%
TNA 6970	Maryland	<LLOQ*	38%
TNA 6972	Maryland	2.1%	36%
TNA 6978**	Massachusetts	<LLOQ*	36%
TNA 6979	Massachusetts	3.5%	30%
TNA 6982	Texas	0.8%	41%
TNA 6984	Texas	<LLOQ*	30%
TNA 6986	Texas	<LLOQ*	41%
TNA 6988	Texas	<LLOQ*	33%
	Average:	**2.5%*** **	**37%**
	Standard Deviation	**1.4%*** **	**3.3%**

*The branched isomer in the sample was < LLOQ
**TN-A-6977 and –6978 are from the same lot of pooled serum.
***The average and standard deviation represent only those samples with isomer values above the LLOQ.

6 Data/Sample Retention

All original raw data and the analytical report have been archived at the 3M Environmental Laboratory. The test materials and analytical reference standard reserve samples, as well as the samples pertaining to this project are archived at the 3M Environmental Laboratory.

Reserve samples, digital copies of original data and all original paper data will be retained in the archives of 3M Environmental Laboratory for a period of at least 10 years following report signing.

7 Conclusion

Under the conditions of the study, endogenous fluorochemicals were found to be present in all samples. NFPA, PFOA, C_9 Acid, C_{10} Acid, C_{11} Acid, FOSA, PFHS, PFOS and THPFDS were detected in every lot of serum. TDHA and PFBS were detected in all but three samples, and the C_{12} Acid was detected in 30 of 36 samples. Conversely, PFHA was only detected in 9 of 36 samples, and FBSA was only detected in 1 of 36 samples. THPFOS was not detected in any of the samples using the present methodology. PFOA [1,2-^{13}C] surrogate recoveries were all within ±30% while PFOS [$^{18}O_2$] surrogate recoveries were all within +/- 34%. Method accuracy for individual analytes ranged from +/- 1% to +/- 27% while overall method uncertainty (based on isotopically labeled surrogate recovery) was +/- 34%.

Mark Ellefson, Project Lead — 12/01/05 Date

William Reagen, Laboratory Manager — 12/02/05 Date

The 3M Environmental Laboratory's Quality Assurance Unit has audited the data and report for this project.

Quality Assurance Representative — 12-1-2005 Date