SUPPORTING INFORMATION

Identification of Biomarkers of exposure to FTOHs and PAPs in Humans using a targeted and non-targeted analysis approach

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Contents

Experimental

Chemicals

Potassium perfluorobutane sulfonate (PFBS), Potassium perfluorohexane sulfonate (PFHxS) and potassium salt (PFOS), Perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), PFOA, perfluorononanoic acid (C9), and perfluorodecanoic acid (C10), perfluoroundecanoic acid (C11), perfluorododecanoic acid (C12), perfluoro (C13), perfluoro (C14), 8:2 fluorotelomer alcohol (8:2 FTOH), 6:2, 8:2, 10:2 fluorotelomer acid (6:2, 8:2 and 10:2 FTCA), 5:3 and 7:3 unsaturated acids (5:3 Acid, 7:3 Acid), 6:2 and 8:2 mono- polyfluoroalkyl phosphate esther (6:2 and 8:2 mono-PAP), 6:2, 6:2 di-polyfluoroalkyl phosphate esther (6:2, 6:2 diPAP), were purchased from Wellington laboratories (Ontario, Canada). 8:2, 8:2 di-polyfluoroalkyl phosphate esther (8:2, 8:2 diPAP) was purchased from Synquest Laboratories. 8:2 FTOH-Sulfate was custom synthesized and purchased by Toronto Research Chemicals. Five stable, isotopically labeled internal standards (ISs) were used to quantitate PFASs. Isotopically labeled PFBA (13C4HF7O2), PFHxA (13C212C4HF11O2), PFOA (13C212C6HF15O2), PFNA (13C512C4HF17O2), PFDA (13C212C8HF19O2), PFUnA (13C212C9HF21O2), 8:2 FTOH (13C212C82H2H3F17O), 8:2-8:2 diPAP (13C412C16H8F34PO4Na), 8:2 FTCA (13C212C8H3F17O2), Oxygen-labeled ammonium PFOS (C8F17S18O216ONa), , oxygen labeled sodium PFHxS (C6F13S18O216ONa), were purchased from Wellington Laboratories (Ontario, Canada). GIBCO newborn calf serum was purchased from Invitrogen (Carlsbad, CA). Deionized (DI) water was obtained from a Barnstead EASYpure ultraviolet/ultrafiltration (UV/UF) compact reagent-grade water system (Dubuque, IA). Methanol was purchased from Fisher Scientific (Fairlawn, NJ). Ammonium acetate and formate were obtained from Sigma-Aldrich Chemical (St Louis, MO).

Animal treatment

All procedures involving the use of laboratory animals were conducted in accordance to the guidelines set forth by the U.S. EPA ORD/NHEERL Institutional Animal Care and Use Committee. Animal care procedures and facilities (AAALAC accredited) were consistent with the recommendations provided by the 1996 National Research Council's "Guide for the Care and Use of Laboratory Animals", the Animal Welfare Act, and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals. Animal facility was set to maintain a mean temperature of 20–24°C, relative humidity of 40–60%, and kept under a 12-h light–dark cycle (lights off 7:00 p.m.–7:00 a.m.). Sprague–Dawley rats (10–12 weeks old, 200–250 g) were purchased from Charles River Laboratory (Raleigh, NC). Upon arrival, animals were housed

individually in polycarbonate, metabolic cages. Food (Harlan Teklad Diet (Madison, WI) and tap water were provided *ad libitum*. All animals were allowed one week to acclimate to their environment prior to the beginning of the study. Non-fasted male rats (n = 3 per dose group and n=1 for control, total n=13) were given a single dose by oral gavage of 8:2 FTOH or 8:2 di-PAP at 5 or 50 mg/kg body weight, at a volume of 1ml/kg. Blood samples (approximately 500µL) were collected from the tail vein at 8, 24, 48, 72, 96, 120 hours after dosing. Serum samples were prepared after blood clotting and centrifugation at 2000 x g for 30 min, and stored at−70 ◦C until analysis. Urine and feces were collected at the same time points and stored at -70 ◦C until analysis.

Dosed animals serum, urine and feces

For quantitation, dosed animals serum and urine were prepared using a simplified preparation technique from a previously described method used for rodents [1]. Briefly, serum and urine samples were thawed and vortexed, 50 µL or 100 µL subsample (for serum and urine respectively) were placed in 2 mL polypropylene micro centrifuge tube. A volume of 100 µL of ice cold acetonitrile containing each isotopically labeled IS at 25 pg/µL or 100 pg/µL was added (giving a final IS concentration of 50 ng/mL in serum or urine). Samples were vortexed and centrifuged for 10 minutes at 1800 g to separate the precipitated proteins, and the supernatants were transferred to autosampler vials and adjusted to a final concentration of 50% acetonitrile extract and 50 % 10mM ammonium acetate aqueous buffer to approximate initial LC conditions. All analytes were spiked into newborn calf serum or previously tested PFASs free blank human urine and prepared as described above to make a 9 to 12 points calibration curve ranging from 5 ng/mL to 2000 ng/mL for serum and 5 ng/mL to 1000 ng/mL for urine. Analytes were quantified by regressing the relative response of the unknown over the most appropriate IS against concentration. Feces were weighted, and an aliquot of 0.1 to 0.5 g was used for extraction. Feces were dissolved in 2mL of acetonitrile containing isotopically labeled IS at 25 pg/µL. The dissolved extract was then sonicated for 30min and subsequently centrifuged at 1800g for 5 minutes. A 500 uL aliquot of the supernatant was filtered with a 0.45 µm filtration vial, the filtrate was then stored for analysis. All analytes were spiked into control rat feces previously tested as PFASs free and prepared as described above to make a 9 to 12 points calibration curve ranging from 5 ng/g to 3000 ng/g of feces. Limits of quantitation and recoveries for rat's serum, urine and feces are available on SI Table S.3.

For the analysis of unknown metabolites, dosed animals serum and urine were prepared by solid phase extraction. An aliquot of 25 µL of each time point (serum or urine) was taken and pooled

together for each animal, resulting in a 150 µL aliquot of serum/animal. Sample was diluted in 1 mL of DI water and spiked with internal standard solution (7.5 ng/sample). SPE was carried on an OASIS HLB 3cc, conditioned with 2mL of methanol with 0.1 % ammonium hydroxyde, 2mL of methanol and 2 mL of DI water prior to loading samples. Cartridges were then washed with 2mL of DI water and 2 mL of a DI water and methanol mixture (50:50) and eluted with 2mL of methanol with 0.1 % ammonium hydroxyde. Eluates were blown down to 100 µL under a gentle stream of nitrogen and stored for analysis.

Human serum and urine

Human serum and urine were prepared by solid phase extraction using an OASIS WAX, Waters cartridge. A volume of 500 µL of serum or 15mL of urine was mixed with 200 µL of formic acid at 0.1mM containing each isotopically labeled IS at 62.5 pg/µL for serum and 0.2 pg/uL for urine (giving a final IS concentration of 50 ng/mL in serum and 10 ng/mL in urine). A volume of 2.5 mL of acetonitrile was added to the serum for protein precipitation. Serum and urine were centrifuged for 10 minutes at 1800 g, and supernatant was transferred to a new vial. Serum supernatant was diluted up to 15 mL with DI Water. Solid phase extraction was conducted on an OASIS wax cartridge 3cc for serum and an OASIS wax plus 6cc for urine. Cartridges were conditioned subsequentially with a mixture of methanol and ammonium hydroxyde at 0.1%, methanol and DI water. Samples were loaded to the cartridge; cartridges were then dried under vacuum for 5 minutes. Elution was performed with methanol and ammonium hydroxide at 0.1% and extract were evaporated under a gentle stream of nitrogen down to a volume of 150 µL. Extracts were transferred to an autosampler vials and adjusted to a final concentration of 50% acetonitrile extract and 50 % 10mM ammonium acetate aqueous buffer to approximate initial LC conditions. All analytes were spiked into newborn calf serum or previously tested PFASs free blank human urine and prepared as described above to make an 8 or 5 point calibration curve ranging from 0.01 ng/mL to 50 ng/mL for serum and 0.1 ng/L to 50 ng/L for urine. Analytes were quantified by regressing the relative response of the unknown over the most appropriate IS against concentration. Limits of quantitation and recoveries for human serum and urine are available on SI Table S.4.

Quality assurance and control

Analysis were separated in batches, for each batch method and matrix blanks were analyzed for contamination or background levels for the studied compounds. The limit of quantitation (LOQ) was determined as the concentration of the lowest working standard which back-predicted within 30% of a theoretical value. 10% of randomly selected samples were replicated in each quantitative experiment to ensure consistency within the dataset. Quality control at high and low concentration (QC) containing the mixture of measured compounds described in materials and methods were run with each batch of human serum to ensure analytical precision and accuracy.

Table S.1. Database of known metabolites and transformation products of the 8:2 FTOH and 8:2 diPAP.
(This table is based on the following work [1-4]). Lines highlighted in grey indicate compounds with available standards for confirmation.

Compound	Elemental composition	Abbreviation	Monoisotopic Mass
PFOA	C8HO2F15	Perfluorooctanoic acid	413.9737
PFNA	C9HO2F17	Perfluorononanoic acid	463.9705
8:2 FTOH	C10H5F17O	8:2 Fluorotelomer alcohol	464.0068
8:2 monoPAP	C10H6O4PF17	8:2 mono phosphate ester	543.9732
8:2-8:2-diPAP	C20H9O4PF34	8:2-8:2 di phosphate ester	989.9696
8:2 FTUCA	C10H2O2F16	8:2 fluorotelomer unsaturated acid	457.9799
8:2 FTAL	C10H3F17O	8:2 Fluorotelomer aldehyde	461.9912
GLUT-FTUAL	C20H18F15N3O7S	8:2 Fluorotelomer unsaturated aldehyde Glutathione conjugate	729.0626
GLUTAHTIONE-FTUCA	C20H18F15N3O8S	8:2 Fluorotelomer unsaturated acid Glutathione conjugate	745.0575
DHPFCA	C10H3F15O2	Dihydroperfluoroalkyl carboxylate	493.9894
THPFCA	C10H5F15O2	Tetrahydroperfluoroalkyl carboxylate	442.005
8:2 FTCA	C10H3O2F17	8:2 fluorotelomer saturated acid	477.9862
7-3 UA	C10H3F15O2	7:3 Fluorotelomer Unsaturared acid	439.9893
7-3 Acid	C10H5F15O2	7:3 Fluorotelomer acid	442.005
7-3 Acid T-A	C12H10F15NO4S	7:3 Acid taurine amide	549.009
7-3 UAL	C10H3F15O	7:3 Unsaturated acid	423.9944
7-3 AL	C10H5F15O	7:3 Aldehyde	426.0101
7-3 BETA-OH UAL	C10H3F15O2	7:3 ß-hydroxy unsaturated aldehyde	439.9893
7-3 BETA-OH UA	C10H3F15O3	7:3 ß-hydroxy unsaturated acid	455.9842
7-3 BETA-KETO ACID	C10H3F15O3	7:3 ß keto acid	455.9842
7-32KETONE	C10H3F15O2	Perfluoroheptyl methyl ketone	439.9893
7-2 sFTOH-Gluc	C15H13F15O7	7:2 Secondary Fluorotelomer alcohol glucoronide conjugate	590.0421
7-2 sFTOH	C10H5F15O	7:2 Secondary Fluorotelomer alcohol	414.01
F(CF2)7-C(GSH)=CH-CH2OH	C19H1F15N3O7S	8:2 Fluorotelomer unsaturated alcohol glutathione	717.0625
F(CF2)7-C(GSH)=CH-CO2H	C19H16F15N3O8S	8:2 Fluorotelomer unsaturated acid glutathione	731.0418
F(CF2)7-C(SCysGly)=CH-CH2OH	C14H10F15NO4S	8:2 Fluorotelomer unsaturated alcohol cysteinylglycine	573.009
F(CF2)7-C(SCysGly)=CH-CO2H	C14H8F15NO5S	8:2 Fluorotelomer unsaturated acid cysteinylglycine	586.9883
F(CF2)7-C(SCys)=CH-CH2OH	C13H10F15NO3S	8:2 Fluorotelomer unsaturated alcohol cysteine	545.0141
F(CF2)7-C(SCys)=CH-CO2H	C13H8F15NO4S	8:2 Fluorotelomer unsaturated acid cysteine	558.9934
F(CF2)7-C(SCyNAcetyl)=CH-CH2OH	C15H12F15NO4S	8:2 Fluorotelomer unsaturated alcohol N-acetylcysteine	587.0247
F(CF2)7-C(SCyNAcetyl)=CH-CO2H	C15H10F15NO5S	8:2 Fluorotelomer unsaturated acid N-acetylcysteine	601.004
F(CF2)7-C(SH)=CH-CH2OH	C10H5F15OS	8:2 Fluorotelomer unsaturated alcohol 3-thiol	457.9821
F(CF2)7-C(SH)=CH-CO2H	C10H3F15O2S	8:2 Fluorotelomer unsaturated acid 3-thiol	471.9614
8:2 FTOH Glucoronide	C16H13F17O7	8:2 Fluorotelomer alcohol glucoronide conjugate	640.0389
8:2 FTOH Sulfate	C10H5F17O4S	8:2 Fluorotelomer alcohol sulfate conjugate	543.9637

Table S.2. Descriptive demographics of studied population

a. Gender distribution

General population (%)		Office workers (%)	
Female	**Male**	**Female**	**Males**
70	30	86	13

b. Age distribution

GENERAL POPULATION

Group of age (years)	%
12-19	2
20-39	38
40-59	47
60 and older	13

OFFICE WORKERS

Group of age (years)	%
12-19	0
20-39	40
40-59	40
60 and older	20

Table S.3. (A, B and C). Summary of instrumental parameters for LC-MS-TOF

A. HPLC Parameters

Agilent LC-1100 parameters	
Column	Agilent Eclipse Plus C18 column (2.1 mmx 50 m, 3.5 µm
Flow Rate	200 uL/min
Column Temperature	25 °C
Injection Volume	40 µL
Solvents	A : 0.4 mM ammonium formate, 95:5 DI water :Methanol B: 0.4 mM ammonium formate, 95:5 Methanol :DI water

B. Liquid chromatography gradient methods:

Method 1:

Time	A %	B%
0	50	50
5	0	100
10	0	100
11	50	50
20	50	50

Method 2:

Time	A %	B%
0	50	50
20	0	100
30	0	100
35	50	50
36	50	50

C. MS-TOF Parameters

Agilent 6200 MS-TOF Instrument parameters	
Gas temperature	350 °C
Drying gas flow ratw	10 L/min
Nebulizer pressure	30 psi
Capillary	3500 V
Fragmentor	80 V
Skimmer	65 V
Octopole radio frequency	250 V

Table S3 Limits of quantification (LOQ) and recoveries (±SD) of analytes in rat serum and urine

Analyte	LOQ in rat serum ng/mL	LOQ in rat urine ng/L	LOQ in rat urine ng/g	Recoveries in rat serum	Recoveries in rat urine	Recoveries in rat feces
PFOA	0.1	0.1	50	108% (±24%)	70 % (±6%)	102% (±13%)
PFNA	0.1	0.1	50	102% (±17%)	94% (±8%)	103% (±11%)
8:2 FTOH	5	10	50	107% (±25%)	74% (±13%)	120% (±15%)
8:2PAP	0.1	1	250	115% (±25%)	93% (±13%)	112% (±6%)
8:2-8:2-diPAP	0.1	1	50	92% (±25%)	102% (±37%)	89% (±15%)
8:2 FTCA	1	5	250	108% (±19%)	89% (±15%)	95% (±10%)
8:2 FTOH-Sulfate	0.5	0.1	250	103% (±12%)	76% (±10%)	112% (±6%)

Table S4 Limits of quantification (LOQ) and recoveries % (±SD) of analytes in human serum and urine

Analyte	LOQ in human serum ng/mL	LOQ in human urine ng/L	Recoveries in human serum	Recoveries in human urine
PFBA	1	na	77 % (±4%)	73 % (±35%)
PFPeA	0.1	5	87% (±3%)	98% (±17%)
PFHxA	0.05	5	85% (±8%)	101% (±22%)
PFHpA	0.01	5	80% (±9%)	109% (±23%)
PFOA	0.1	1	85% (±9%)	99% (±21%)
PFNA	0.5	5	86% (±11%)	108% (30±%)
PFDA	0.05	10	91% (±17%)	105% (±28%)
PFUnA	0.05	10	93% (±21%)	89% (±19%)
PFDoA	0.1	10	95% (±19%)	69% (±7%)
PFTriA	0.05	10	95% (±17%)	40% (±3%)
PFTetA	0.05	10	89% (±16%)	30% (±7%)
PFBS	0.01	5	97% (±6%)	68% (±18%)
PFHxS	0.05	5	90% (±6%)	100% (±21%)
PFOS	0.1	1	92% (±9%)	70% (±58%)
8:2 FTOH	5	nd	65%(±10%)	nd
6:2PAP	1	nd	86% (±9%)	20% (±5%)
8:2PAP	0.5	nd	92% (±10%)	88% (±23%)
6:2-6:2-diPAP	0.01	50	96% (±14%)	40% (±10%)
8:2-8:2-diPAP	0.01	50	50% (±3%)	40% (±15%)
6:2 FTCA	0.5	nd	60% (±4%)	nd
8:2 FTCA	0.5	nd	60% (±5%)	nd
10:2 FTCA	0.5	nd	77% (±14%)	nd
7-3 FTCA	0.1	nd	45% (±6%)	nd
5-3 FTCA	0.5	nd	55% (±10%)	nd
8:2 FTOH-Sulfate	0.05	5	92% (±10%)	89% (±24%)

nd: not detected or not analyzed

Table S.5: Results of p-values for Shapiro-Wilk normality test. A *p*-value of 0.05 was chosen for statistical significance such as if $p<0.05$, the null hypothesis H0= data follow a normal distribution, isrejected, and the data are unlikely to be normally distributed. If the test statistic is above 0.05 ($p>0.05$), the Shapiro-Wilk test can only conclude there is no evidence of non-normality (nd= not detected).

Analyte	Sample group	Data without log transformation	Log transformed data
PFBA	General population	nd	nd
	Office workers	nd	nd
PFPeA	General population	<0.0001	<0.0001
	Office workers	<0.0001	<0.0001
PFHxA	General population	<0.0001	<0.0001
	Office workers	<0.0001	0.0018
PFHpA	General population	<0.0001	<0.0001
	Office workers	<0.0001	<0.0001
PFOA	General population	<0.0001	<0.0001
	Office workers	0.0557	<0.0001
PFNA	General population	<0.0001	0.4717
	Office workers	0.1481	0.0088
PFDA	General population	<0.0001	0.0187
	Office workers	<0.0001	0.0073
PFUnA	General population	<0.0001	0.1969
	Office workers	<0.0001	0.1766
PFDoA	General population	<0.0001	<0.0001
	Office workers	<0.0001	<0.0001
PFTriA	General population	<0.0001	<0.0001
	Office workers	<0.0001	<0.0001
PFTetA	General population	<0.0001	<0.0001
	Office workers	<0.0001	<0.0001
PFBS	General population	<0.0001	<0.0001
	Office workers	<0.0001	0.0002
PFHxS	General population	<0.0001	0.0264
	Office workers	<0.0001	0.0057
PFOS	General population	<0.0001	0.0171
	Office workers	<0.0001	0.1260
4:2 FTOH	General population	nd	nd
	Office workers	nd	nd
6:2 FTOH	General population	nd	nd
	Office workers	nd	nd
8:2 FTOH	General population	nd	nd
	Office workers	nd	nd
10:2 FTOH	General population	nd	nd
	Office workers	nd	nd
6:2PAP	General population	nd	nd
	Office workers	nd	nd
8:2PAP	General population	<0.0001	<0.0001
	Office workers	<0.0001	<0.0001
6:2-6:2-diPAP	General population	<0.0001	<0.0001
	Office workers	<0.0001	<0.0001
8:2-8:2-diPAP	General population	<0.0001	<0.0001
	Office workers	<0.0001	<0.0001
6:2 FTCA	General population	nd	nd
	Office workers	nd	nd
8:2 FTCA	General population	nd	nd
	Office workers	nd	nd
10:2 FTCA	General population	nd	nd
	Office workers	nd	nd
7-3 FTCA	General population	nd	nd
	Office workers	nd	nd
5-3 FTCA	General population	nd	nd
	Office workers	nd	nd
8:2 FTOH-Sulfate	General population	<0.0001	<0.0001
	Office workers	<0.0001	<0.0001

Table S.6: P-Values from Mann-Whitney U test to compare concentration between General population and Office workers and for gender differences. A *p*-value of 0.05 was chosen for statistical significance such as if *p*>0.05, the null hypothesis H0=there is a significant difference between the two groups, is rejected. If *p*>0.05, Ho is rejected, there is no significant difference between the two groups of data. (nd= not detected)

Analyte	Comparison	
	General Population Vs Office workers	Males Vs Females
PFBA	nd	nd
PFPeA	0.6088	0.8370
PFHxA	<0.0001	0.780
PFHpA	0.8396	0.1849
PFOA	0.1228	0.0073
PFNA	0.0416	0.1724
PFDA	0.0002	0.9840
PFUnA	0.019	0.3012
PFDoA	0.6067	0.5227
PFTriA	0.0191	0.7998
PFTetA	0.9160	0.8338
PFBS	<0.0001	0.1577
PFHxS	0.4188	<0.0001
PFOS	0.2273	0.0018
4:2 FTOH	nd	nd
6:2 FTOH	nd	nd
8:2 FTOH	nd	nd
10:2 FTOH	nd	nd
6:2PAP	nd	nd
8:2PAP	nd	nd
6:2-6:2-diPAP	0.3269	0.2615
8:2-8:2-diPAP	0.8114	0.3216
6:2 FTCA	nd	nd
8:2 FTCA	nd	nd
10:2 FTCA	nd	nd
7-3 FTCA	nd	nd
5-3 FTCA	nd	nd
8:2 FTOH-Sulfate	0.8112	0.6910

Figure S.1 : Expected biotransformation pathway of 8:2,8:2 diPAP and 8:2 FTOH showing combined suggestion by Martin et al. (2005), Fasano et al. (2006 and 2009), and D'eon and Mabury (2011)

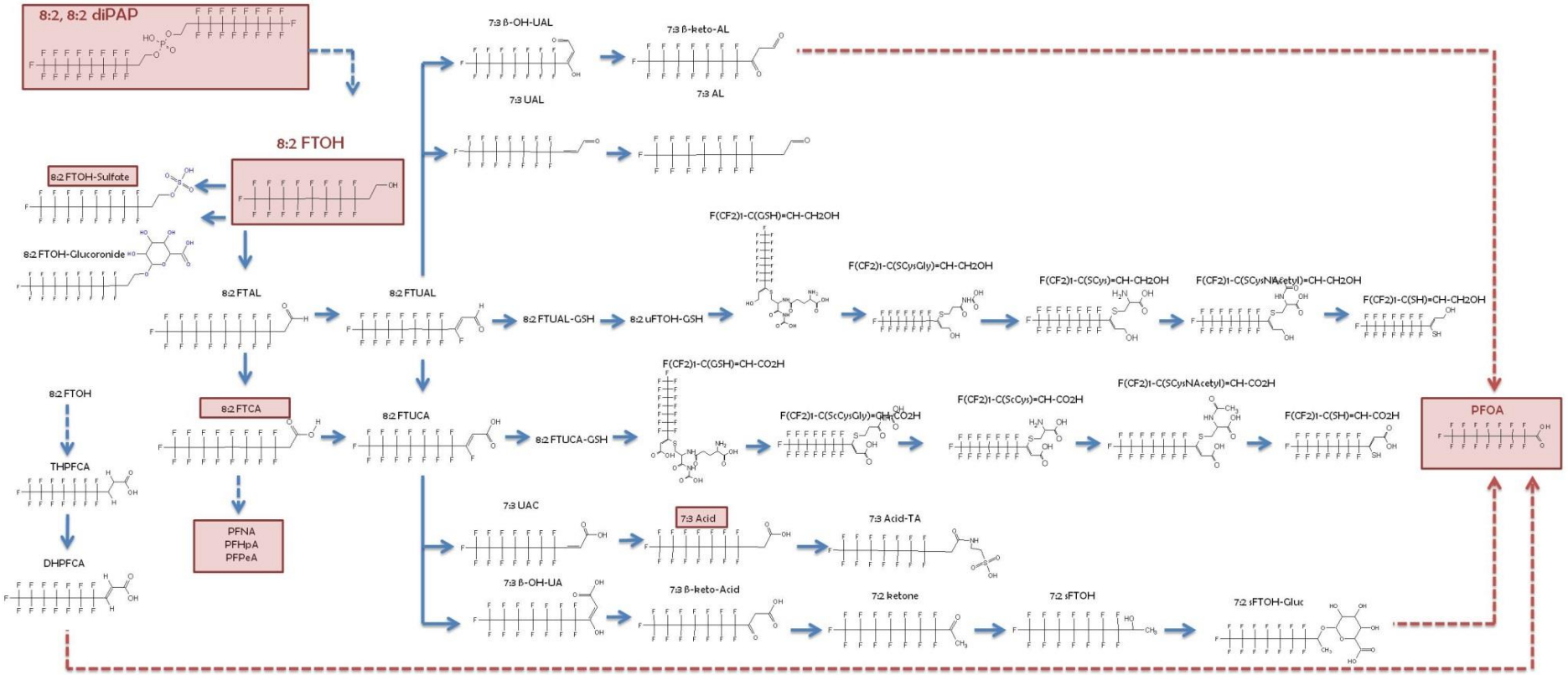
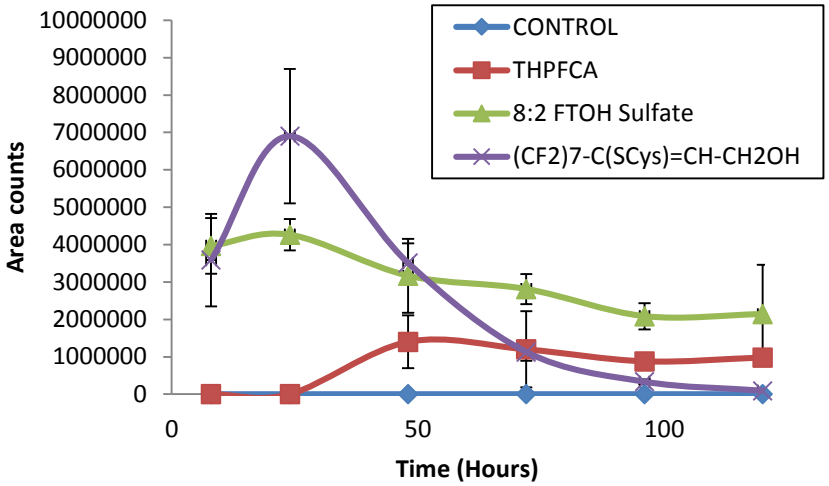
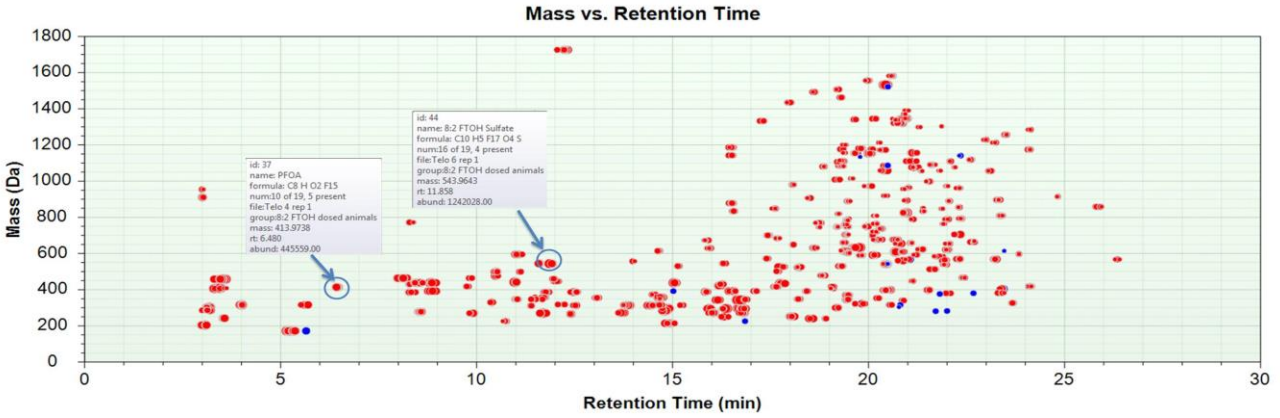


Figure S.2 Metabolites of 8:2 FTOH in serum



Mean area counts and standard deviation from serum samples of 8:2 FTOH dosed rat for the potential metabolites THPFCA, 8:2 FTOH sulfate and FTOH cysteine ((CF2)7-C(SCys)=CH-CH2OH)).

Figure S.3. Mass profiler of 8:2 FTOH dosed animal serum

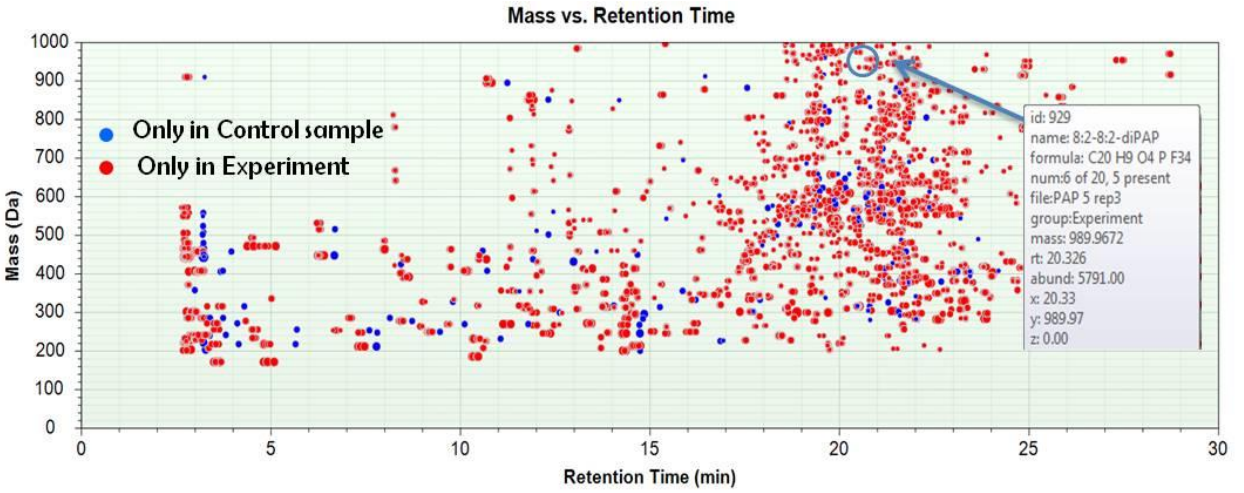


Expression: Both Up Down **276 features**

	Feature summary											8:2 FTOH dosed animals(18)					Control animals(1)				
	ID	Name	Formula	RT	SD	Mass	SD	Abundance	RSD	Freq.	Mark	RT	Mass	Abundance	RSD	Freq.	RT	Mass	Abundance	RSD	Freq.
1	25	THPFCA	C10 H5 F15 O2	10.992	0.021	442.0053	0.0004	214270	1.74	6		10.992	442.0053	226174	1.68	6			0		0
2	37	PFOA	C8 H O2 F15	6.444	0.026	413.9740	0.0002	142920	1.75	5		6.444	413.9740	150860	1.69	5			0		0
3	47	F(CF2)7-C(SCys...	C13 H10 F15 N...	11.599	0.013	545.0130	0.0007	108201	1.54	6		11.599	545.0130	114212	1.48	6			0		0
4	44	8:2 FTOH Sulfate	C10 H5 F17 O4 S	11.912	0.037	543.9638	0.0004	113379	2.62	4		11.912	543.9638	119677	2.55	4			0		0

Plots comparing lists of compounds present in 8:2 FTOH dosed serum samples, and Control samples. Figure is obtained with Mass Profiler software. Blue dots represent compounds that are present in Control only, red dots compounds that are present in Dosed serum only. Size of dots is proportional to abundance of the compound. The table below summarizes only a small portion in the scroll bar of the list of all features proposed formulae as well as score and database identification.

Figure S.4. Mass profiler of 8:2 diPAP dosed animal serum



Expression: Both Up Down 1049 features

	Feature summary											Experiment(19)					Control(1)				
	ID	Name	Formula	RT	SD	Mass	SD	Abundance	RSD	Freq.	Mark	RT	Mass	Abundance	RSD	Freq.	RT	Mass	Abundance	RSD	Freq.
2	929	8:2-8:2-diPAP	C20 H9 O4 P F34	20.296	0.063	989.9694	0.0019	1674	1.84	5		20.296	989.9694	1762	1.78	5			0		0
3	3			23.008	0.004	404.3147	0.0001	4174825	2.72	10		23.008	404.3147	4394553	2.64	10			0		0
4	4			21.795	0.095	569.3698	0.0001	2491993	3.85	4		21.795	569.3698	2623151	3.75	4			0		0

Plots comparing features present in 8:2 diPAP dosed serum samples, and Control samples. Figure is obtained with Mass Profiler software. Blue dots represent compounds that are present in Control only, red dots compounds that are present in Dosed serum only. Size of dots is proportional to abundance of the compound. Table below summarizes the list all features as well as their matching to score to the database identification.

Figure S.5. Feces analysis

Upper panel: Arithmetic mean ± SD for the concentration of the 8:2 FTOH and its expected biotransformation products in serum, urine and feces of the animals dosed with 5 mg/kg of 8:2 FTOH. Lower panel:: Arithmetic mean ± SD for the concentration of the 8:2 diPAP and its expected biotransformation products in serum, urine and feces of the animals dosed with 5 mg/kg of 8:2 diPAP.

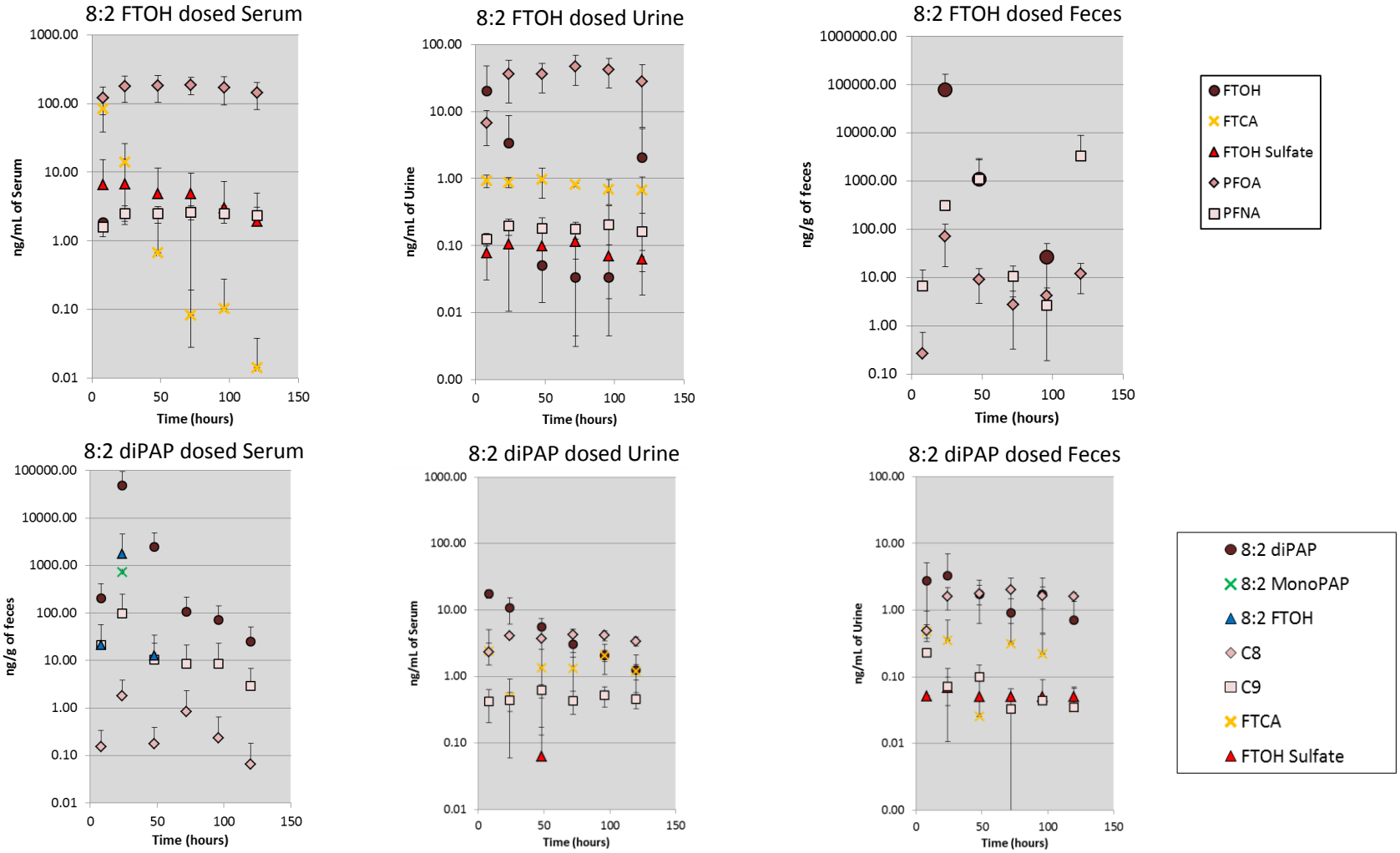
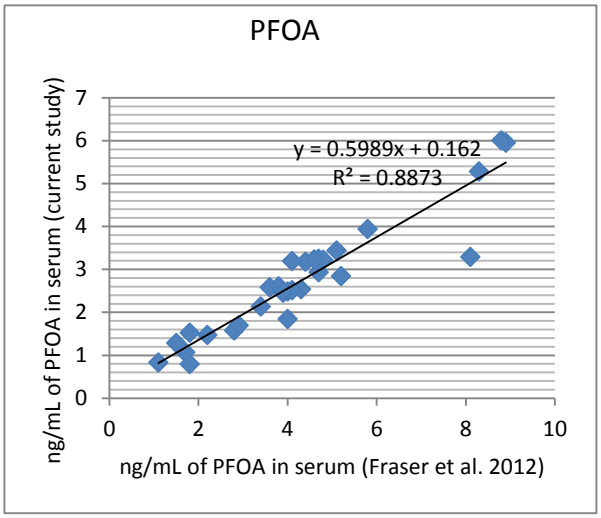
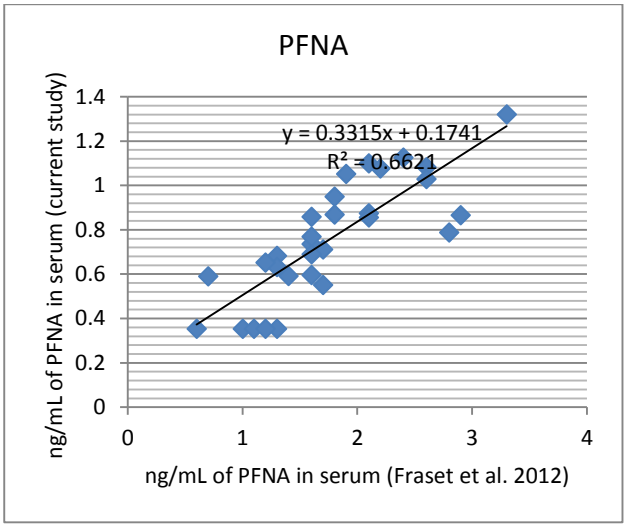
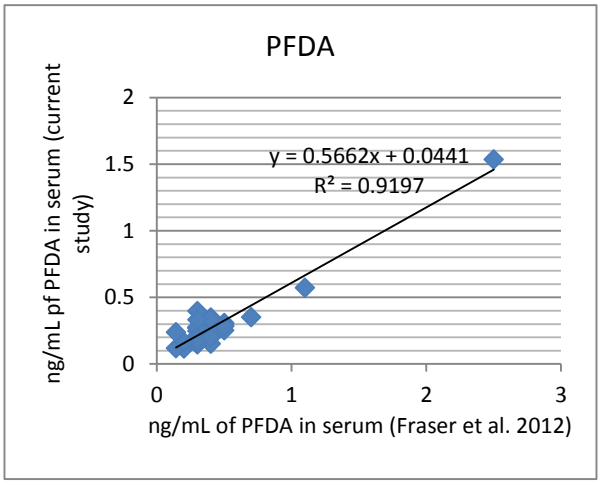
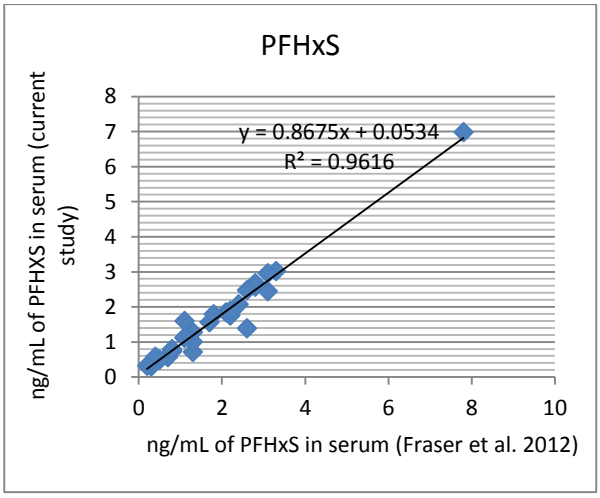


Figure S.6. Plots for Spearman correlation of analysis of selected compounds in the office workers from the present study and the analysis from previous work [5].









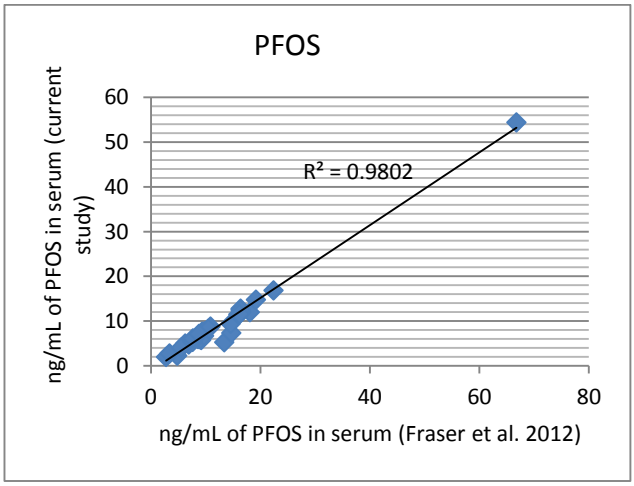


Figure S.7 8:2 FTOH sulfate in human serum

Upper panel, extracted Ion chromatogram (EIC) of spiked calf serum with the 8:2 FTOH sulfate standard (green) and the 8:2 FTOH sulfate measured in human serum sample (pink). Lower panel, spectra of selected compound in human serum after background subtractions. Table indicates results of identification software for the selected spectra, FTOH sulfate is highlighted in yellow and show a 47.37% probability of match.

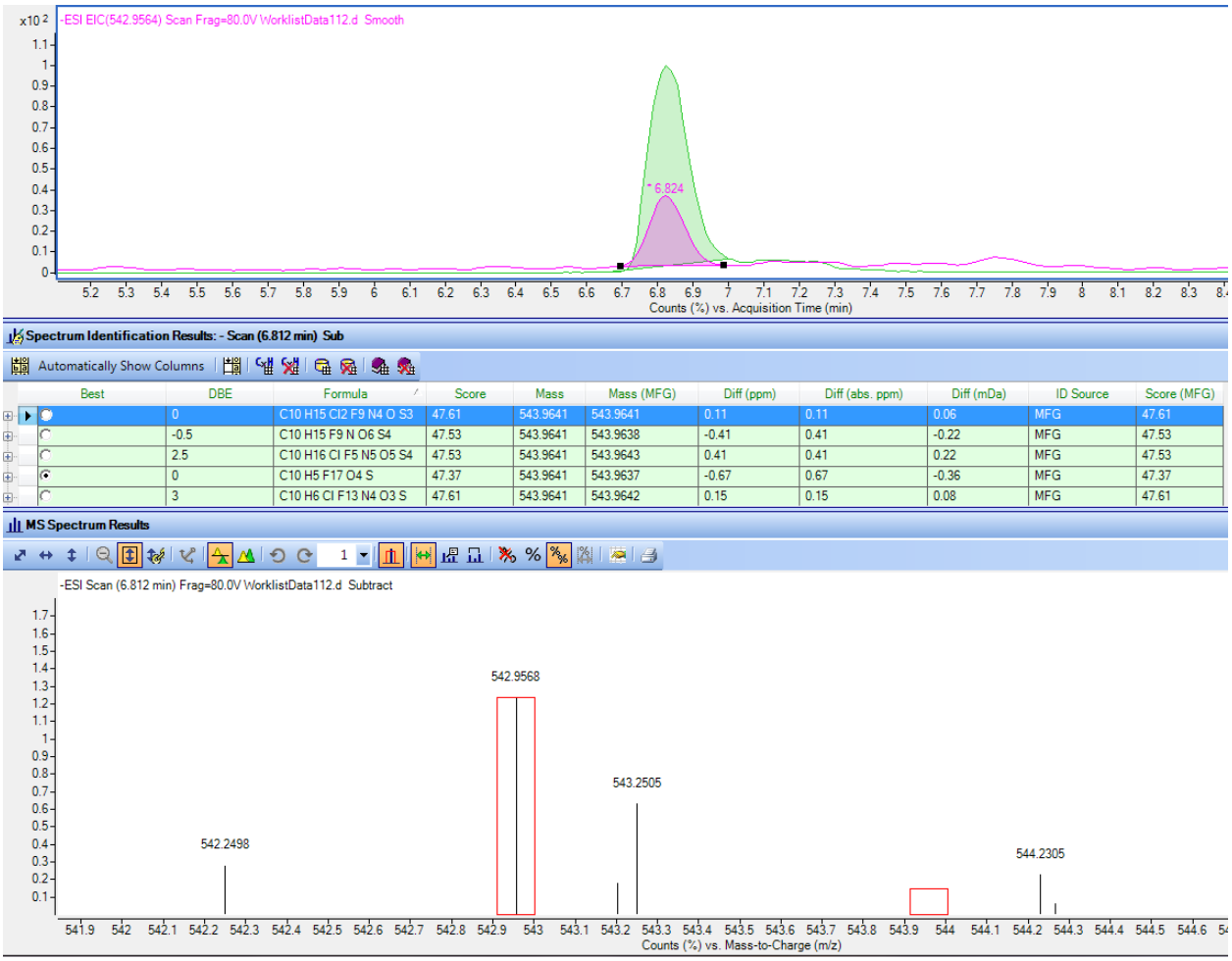
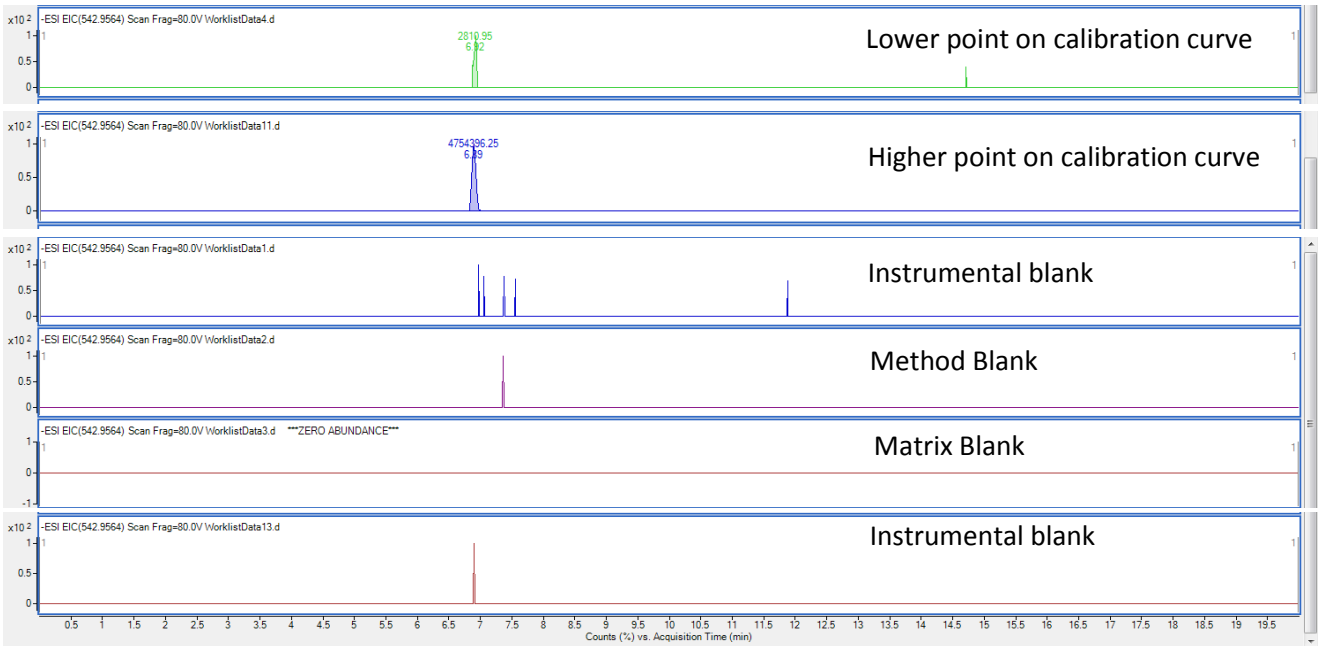


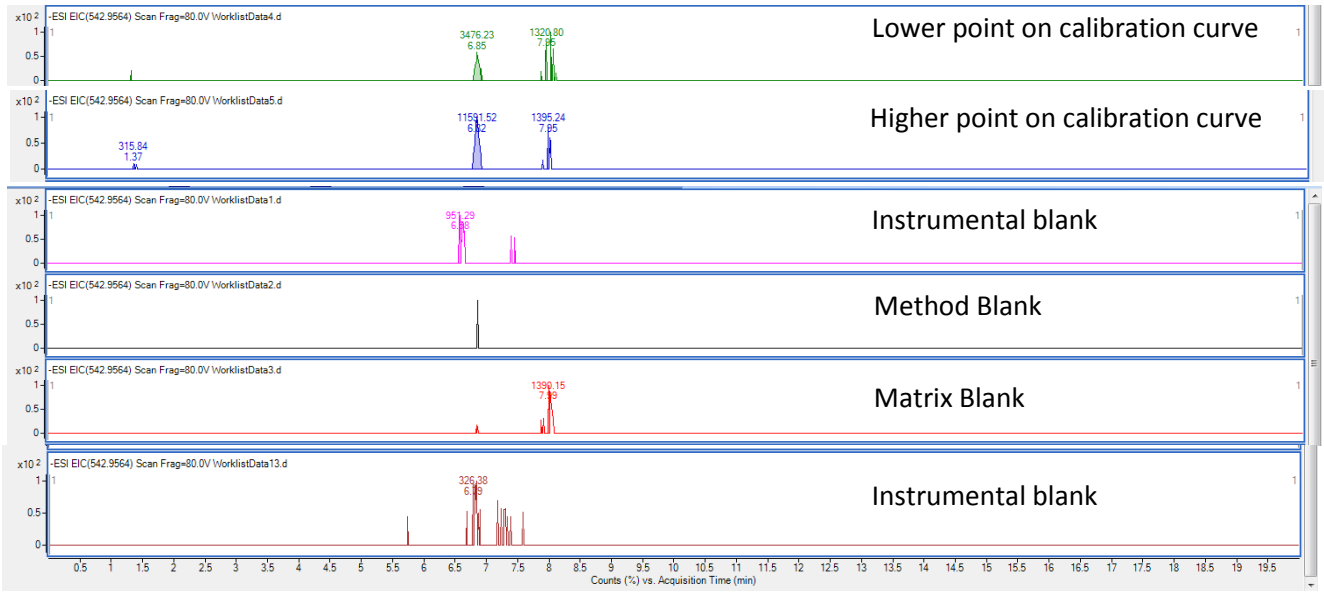
Figure S.8 Extracted Ion Chromatogram for FTOH Sulfate in blanks and standard curve points.

Sample analysis of human samples was divided in 2 batches, results of a selection of blanks and standard curve points for each batch are represented here. EIC of lower and higher calibration curve points are set as a visual reference. Method blank is obtained by extracting DI water with the same procedure as for the serum sample. Matrix blank is obtained by extracting blank calf serum with the same procedure as for the serum samples. Instrumental blanks are added to the run every 4 samples.

Batch 1



Batch 2



References

1. D'Eon J, C.; Mabury, S. A., Exploring indirect sources of human exposure to perfluoroalkyl carboxylates (PFCAs): Evaluating uptake, elimination, and biotransformation of polyfluoroalkyl phosphate esters (PAPs) in the rat. *Environ. Health Perspect.* **2011**, 119, (3), 344-50.

2. Fasano, W. J.; Carpenter, S. C.; Gannon, S. A.; Snow, T. A.; Stadler, J. C.; Kennedy, G. L.; Buck, R. C.; Korzeniowski, S. H.; Hinderliter, P. M.; Kemper, R. A., Absorption, distribution, metabolism, and elimination of 8-2 fluorotelomer alcohol in the rat. Toxico.l Sci. **2006**, 91, (2), 341-55.

3. Fasano, W. J.; Sweeney, L. M.; Mawn, M. P.; Nabb, D. L.; Szostek, B.; Buck, R. C.; Gargas, M. L., Kinetics of 8-2 fluorotelomer alcohol and its metabolites, and liver glutathione status following daily oral dosing for 45 days in male and female rats. *Chem. Biol. Interact.* 2009, 180, (2), 281-95.

4. Martin, M. T.; Brennan, R. J.; Hu, W.; Ayanoglu, E.; Lau, C.; Ren, H.; Wood, C. R.; Corton, J. C.; Kavlock, R. J.; Dix, D. J., Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. *Toxicol. Sci.* **2007**, 97, (2), 595-613.

5. Fraser, A. J.; Webster, T. F.; Watkins, D. J.; Nelson, J. W.; Stapleton, H. M.; Calafat, A. M.; Kato, K.; Shoeib, M.; Vieira, V. M.; McClean, M. D., Polyfluorinated compounds in serum linked to indoor air in office environments. *Environ. Sci. Technol.* **2012**, 46, (2), 1209-15.