SUPPORTING INFORMATION

A Pilot Survey of Legacy and Current Commercial Fluorinated Chemicals in Human Sera from United States Donors in 2009

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EXPERIMENTAL

Chemicals. Perfluorobutanoic acid (PFBA, >99%), perfluoropentanoic acid (PFPeA, >99%), perfluorohexanoic acid (PFHxA, >99%), perfluoroheptanoic acid (PFHpA, >99%), perfluorooctanoic acid (PFOA, >99%), perfluorononanoic acid (PFNA, >99%), perfluorodecanoic acid (PFDA, >99%), perfluoroundecanoic acid (PFUnA, >99%), perfluorododecanoic acid (PFDoA, >99%), perfluorotridecanoic acid (PFTrA, >99%), perfluorotetradecanoic acid (PFTeA, >99%), perfluorobutanesulfonate (PFBS, >99%), perfluorohexanesulfonate (PFHxS, >99%), perfluorooctanesulfonate (PFOS, >99%), perfluorodecanesulfonate (PFDS, >99%), perfluorooctanesulfonamidoacetate (FOSAA, >99%), N-methylperfluorooctanesulfonamidoacetate (N-MeFOSAA, >99%), N-ethylperfluorooctanesulfonamidoacetate (N-EtFOSAA, >99%), 4:2, 6:2, and 8:2 fluorotelomer sulfonates (4:2, 6:2, 8:2 FTS, <99%), C6 perfluorohexylphosphonate (C6 PFPA, >99%), C8 perfluorooctylphosphonate (C8 PFPA, >99%), C10 perfluorodecylphosphonate (C10 PFPA, >99%), C6/C6 bis(perfluorohexyl)phosphinate (C6/C6 PFPiA, >98%), C6/C8 perfluorohexylperfluorooctylphosphinate (C6/C8 PFPiA, >98%), and C8/C8 bis(perfluorooctyl)phosphinate (C8/C8 PFPiA, >98%) were obtained from Wellington Laboratories Inc. (Guelph, ON). Mass-labeled internal standards were donated from Wellington Laboratories and they included: $^{13}C_4$-PFBA (>99%), $^{13}C_2$-PFHxA (>99%), $^{13}C_4$-PFOA (>99%), $^{13}C_5$-PFNA (>99%), $^{13}C_2$-PFDA (>99%), $^{13}C_2$-PFUnA (>99%), $^{13}C_2$-PFDoA (>99%), $^{18}O_2$-PFHxS (>99%), and $^{13}C_4$-PFOS (>99%), d_3-N-MeFOSAA (>99%) and d_3-N-EtFOSAA (>99%).

Due to a lack of authentic standards at the time of analysis, the Masurf® FS-780 technical product was purchased from Mason Chemical Co. (Arlington Heights, IL) to be used as a standard for the following chemicals: C6/C6, C6/C8, C8/C8, C6/C10, C8/C10,

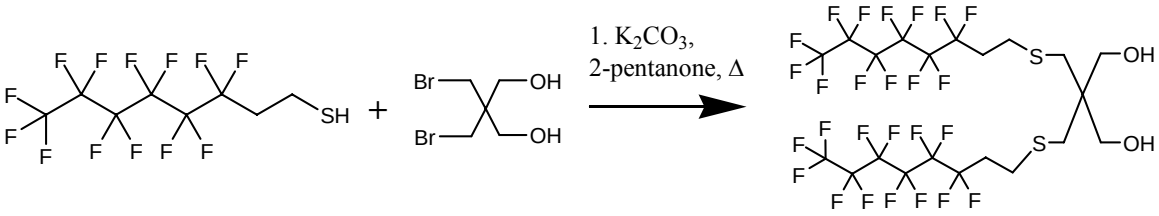
and C6/C12 perfluorophosphinates (PFPiA, no purity information available). The recently released authentic standards of C6/C6 PFPiA, C6/C8 PFPiA, and C8/C8 PFPiA (Wellington Laboratories, Guelph, ON) were used to determine the percent composition of these three PFPiAs in the Masurf® 780 technical product, as 36.9±0.1% C6/C6 PFPiA, 33±6% C6/C8 PFPiA, and 27±3% C8/C8 PFPiA. The concentrations of these three PFPiAs reported in human sera here, as determined by using the Masurf® as the standard, were corrected for based on this percent composition. The C6/C10, C8/C10, and C6/C12 PFPiAs were also detected in the Masurf®, but the lack of authentic standards precluded the determination of their percent composition in the product. As such, the concentrations of C6/C10, C8/C10, and C6/C12 PFPiAs, as determined by using the Masurf® as the standard, were reported as is in the Supporting Information here and should be treated as relative concentrations. All concentrations of the PFPiAs, whether corrected or not, were used in the statistical tests, as described below.

Potassium chlorate (K_2CO_3, 99%) was purchased from Caledon Laboratory Ltd. (Georgetown, ON). Dibromoneopentyl glycol ($HOCH_2C(CH_2Br)_2CH_2OH$, 98%), 2-pentanone ($CH_3COCH_2CH_2CH_3$, >99%), phosphorus (V) oxychloride ($POCl_3$, 99%), and tetrabutylammonium hydrogen sulfate (TBAS, $(CH_3CH_2CH_2CH_2)_4N(HSO_4)$, 99%) were purchased from Sigma Aldrich (Oakville, ON; St. Louis, MO). Dichloromethane (CH_2Cl_2, >99%) was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Toluene ($C_6H_5CH_3$, >99%), acetone (CH_3COCH_3, >99%), and *m*-xylene ($C_6H_4(CH_3)_2$) were purchased from Fisher Scientific (Fairlawn, NJ). Methanol (Omnisolv, >99%), water (Omnisolv, >99%), methyl-*tert*-butyl ether (MTBE, Omnisolv, >99%), and ammonia (NH_3, 30%) were purchased from EMD Chemicals, Inc. (Mississauga, ON).

The 4:2, 6:2, 8:2, and 10:2 polyfluoroalkyl phosphate diesters (diPAPs, $y = x$ only) were synthesized to be used as standards, as described elsewhere *(1)*. Authentic standards for the diPAPs became available after the analysis of all samples (Chiron AS, Trondheim, Norway). The 6:2 (94%), 8:2 (98%), and 10:2 diPAPs (95%) were used to determine the purities of the synthesized 6:2, 8:2, and 10:2 diPAPs as 94±5%, 98±7%, and 39±5% respectively. The lack of an authentic standard for 4:2 diPAP at the time of analysis precluded purity determination of the synthesized 4:2 diPAP. The concentrations of the diPAPs reported in human sera here were not corrected for based on these purities.

Synthesis of 6:2 fluorotelomer mercaptoalkyl phosphate diester (6:2 FTMAP). The synthesis was performed as a bench-scale version of two patented processes*(2, 3)*. The reaction scheme of the two-step synthesis is shown below.

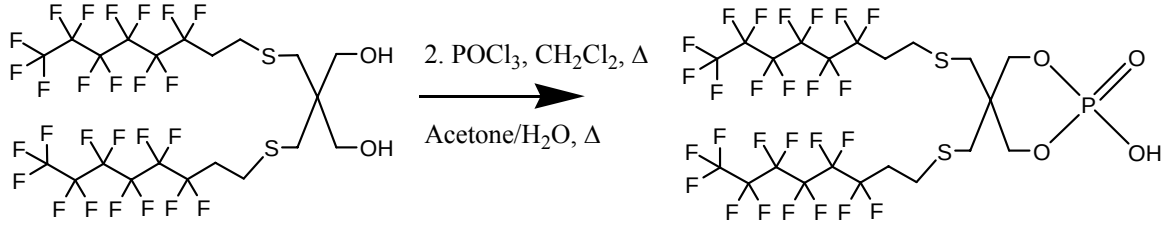
Step 1:



A mixture of 1H,1H,2H,2H-perfluorooctanethiol (CAS# 34451-26-8; 5.00 mmol, 2.00 eq.), dibromoneopentyl glycol (CAS# 3296-90-0; 2.50 mmol, 1.00 eq.), K_2CO_3 (CAS# 3811-04-9; 8.03 mmol, 3.21 eq.), and 2.50 mL of 2-pentanone (CAS# 107-87-9; solvent) was reacted under a nitrogen atmosphere at 105^{o}C for 16 hours. After cooling the mixture to 70^{o}C, 4.00 mL of H_2O was added and the entire mixture was transferred to a separatory funnel to separate the aqueous and organic phases. Evaporation of the organic phase and two rounds of recrystallization with toluene produced the white solid product of bis-(1H,1H,2H,2H-perfluorooctanethiolmethyl)-1,3-propanediol (1.70 mmol,

1.46 g, 68% pure). Product identification was confirmed by ^{1}H, ^{19}F, and ^{13}C NMR analysis: ^{1}H NMR (CD_3OD, 400 MHz): δ = 2.45-2.61 (m, 8H, CH_2), 2.80-2.87 (m, 8H, CH_2); ^{13}C NMR (CD_3OD, 101 MHz): δ = 25.0 (C), 30.7 (CH_2), 35.4 (CH_2), 46.4 (CH_2), 63.8 (CH_2); ^{19}F NMR (CD_3OD, 377 MHz): δ = 81.5 (t, 3F, CF_3), -114.4 (t, 2F, CF_2), -122.0 (m_c, 2F, CF_2), -123.0 (m_c, 2F, CF_2), -123.5 (m_c, 2F, CF_2), -126.5 (m_c, 2F, CF_2).

Step 2:



The bis-(1H,1H,2H,2H-perfluorooctanethiolmethyl)-1,3-propanediol (0.20 mmol, 1.0 eq.) was dissolved in 5.0 mL of anhydrous CH_2Cl_2 under a nitrogen atmosphere. Excess $POCl_3$ dissolved in 0.50 mL of dry CH_2Cl_2 was added dropwise to the above mixture. After refluxing for 21 hours, the reaction mixture was evaporated under vacuum, and the residue was redissolved in 5.0 mL of 90:10 mixture of acetone:H_2O and refluxed for another 24 hours. Any residual acetone was removed by a rotary evaporator. After recrystallization with *m*-xylene, a white solid product of 6:2 FTMAP was obtained (95% pure). Product identification was confirmed by ^{1}H, ^{19}F, and ^{31}P NMR analysis: ^{1}H NMR (CD_3OD, 400 MHz): δ = 2.45-2.63 (m, 4H, CH_2), 2.86-2.93 (m, 8H, CH_2), 4.34 (d, ^{2}J = 12.3 Hz, 4H, CH_2); ^{19}F NMR (CD_3OD, 377 MHz): δ = -81.5 (t, 3F, CF_3), -114.4 (t, 2F, CF_2), -122.0 (m_c, 2F, CF_2), -123.0 (m_c, 2F, CF_2), -123.5 (m_c, 2F, CF_2), -126.5 (m_c, 2F, CF_2); ^{31}P NMR (CD_3OD, 162 MHz): δ = -5.57.

Extraction procedures of sera samples. Briefly, 1 mL of 0.5M TBAS solution, either adjusted to pH 10 with 30% aqueous NH_3 or without pH adjustment (pH ~3), was

added to 2-3 mL of sera, followed by extraction with two 4 mL aliquots of MTBE. The MTBE aliquots were combined, evaporated to dryness under nitrogen, and reconstituted in 0.14–0.15 mL of methanol. For the analysis of the PFPiAs, the sera samples were extracted using the TBAS solution adjusted to pH 10. For the analysis of all other analytes, the sera samples were extracted using the TBAS solution without pH adjustment. Each of the fifty human sera sample was extracted in duplicate with one procedural blank (HPLC grade water) extracted in company to each sample (n = 50).

Instrumental Analysis. *Liquid Chromatography Details.* Chromatographic separation was performed using a Kinetex C18 column (50 x 4.6 mm, 3 μm; Phenomenex®, Torrance, CA). Analyte quantitation was performed using an API4000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) in the negative electrospray ionization mode, coupled to an Agilent 1100 LC system. Four high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) methods were used for the analysis of the target analytes.

For the analysis of the diPAPs, SAmPAP, 6:2 FTMAP, 4:2 FTS, 6:2 FTS, and 8:2 FTS, the samples were injected as 35 μL injections and analyzed by the following gradient method at 500 μL/min using HPLC grade methanol and water, each prepared into 10 mM ammonium acetate mobile phases: the initial solvent composition at t = 0 min. was 60:40 water:methanol, which changed to 5:95 over a period of 2.5 min. at t = 2.50 min. and held for 3.5 min. to t = 6.00 min., before returning to the initial composition of 60:40 water:methanol at t = 6.50 min. The column was allowed to reequilibrate for 3.5 min. for a total run time of 10 min.

For the analysis of the PFPAs and PFPiAs, the samples were injected as 35 μL injections and analyzed by the following gradient method at 500 μL/min: the initial

solvent composition at t = 0 min. was 70:30 water: methanol, which changed to 5:95 over a period of 5 min. at t = 5.00 min. and held for 2 min. to t = 7.00 min., before returning to the initial composition of 70:30 water:methanol at t = 7.50 min. The column was allowed to reequilibrate for 2.50 min. for a total run time of 10 min.

For the analysis of PFBA, PFPeA, PFHxA, PFHpA, and PFBS, the samples were injected as 25 µL injections and analyzed by the following gradient method at 500 µL/min: the initial solvent composition at t = 0 min. was 80:20 water:methanol, which changed to 5:95 over a period of 3 min. at t = 3.00 min. and held for 2 min. to t = 5.00 min., before returning to the initial composition of 80:20 water:methanol at t = 5.50 min. The column was allowed to reequilibrate for 2.50 min. for a total run time of 8 min.

For the analysis of PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFTeA, PFHxS, PFOS, PFDS, FOSAA, N-MeFOSAA, and N-EtFOSAA, the samples were injected as 25 µL injections and analyzed by the following gradient method at 500 µL/min: the initial solvent composition at t = 0 min. was 35:65 water:methanol, which changed to 5:95 over a period of 3 min. at t = 3.00 min. and held for 2 min. to t = 5.00 min., before returning to the initial composition of 35:65 water:methanol at t = 5.50 min. The column was allowed to reequilibrate for 2.50 min. for a total run time of 8 min.

Mass Spectrometry Details. A list of the analyte-specific multiple reaction monitoring (MRM) transitions and mass spectrometry parameters for all target analytes and their corresponding internal standards is provided in Table S1a-c and S2. For the analysis of diPAPs ($y = x$ only), SAmPAP, and FTSs, two MRM transitions were monitored for quantitation and identity confirmation for each analyte. Three MRM transitions were monitored for 6:2 FTMAP. The most sensitive transition of 6:2 FTMAP (921.0>79.0; [PO_3^-]) was frequently encumbered with interference peaks, especially at

low concentrations; therefore, two additional transitions (921.0>318.7; $[CF_3(CF_2)_4CF_2^-]$ and 921.0>575.0; loss of one 6:2 fluorotelomer tail) were simultaneously monitored.. The peak ratios between the different MRM transitions were consistent within <15% relative standard deviation (RSD) for all the analytes, except for 10:2 diPAP (25% RSD). The PFPAs fragment exclusively to PO_3^- (79 *m/z*) *(4)*, while the PFPiAs fragment to $[F(CF_2)_xPO_2F]^-$ *(5)*. Each of these transitions was monitored for quantitation of the PFPAs and PFPiAs and chemical identification was internally confirmed by standard addition.

Comparison of using single donor versus pooled samples in human sera analysis. Pooled sera samples have been used to obtain representative population-based estimates of concentrations of polyfluorinated and perfluorinated chemicals in humans (*7-10)*. The advantages of pooled samples are reduced analytical costs and lower biosafety costs, since the samples are typically pre-screened for hepatitis and HIV by the commercial supplier. However, human sera analysis using pooled samples does not provide information on the contamination present in individual donors. In this study, a higher number of detects was typically observed in the pooled samples than in the single donor samples, especially for the analytes present in the sub-ppb (µg/L) concentration ranges, such as the diPAPs, FOSAA, N-EtFOSAA, FTSs, PFPiAs, the short chain PFCAs (C4–C6), and PFBS. For the majority of the analytes, no significant differences were observed in the concentrations between the pooled and single donor samples (Mann Whitney *U* test, $p>0.05$, Table S7), except for 6:2 diPAP, N-EtFOSAA, 6:2 FTS, C6/C6 PFPiA, C6/C8 PFPiA, and PFUnA. The choice between using pooled and single donor samples may be dependent on analyte, as well as, the type of data desired (i.e. population-based estimate of the contamination vs. individual contamination).

Quality Assurance of Data. *Methanol rinses of blood collection items.* All blood collection items, including storage tubes, bottles, collection bags, needles, and tubings were provided by Tennessee Blood Services Corp. (Memphis, TN). The storage tubes (10 mL) and bottles (250 mL) were rinsed with 3 mL and 50 mL aliquots of HPLC grade methanol respectively. The blood collection bags and the tubings and the needle attached to these bags were cut into small pieces with methanol-rinsed scissors and transferred to 50 mL polypropylene tubes (BD Biosciences, Franklin Lakes, NJ), followed by addition of 40 mL of HPLC grade methanol. All rinses were performed in triplicate ($n = 3$). From the methanol rinses of each item, a 1 mL aliquot was filtered through 0.25 μm nylon syringe filters (Chromatographic Specialties, Brockville, ON) into 1.2 mL low-temperature cryo-vials (VWR International Ltd., Mississauga, ON) and analyzed directly by HPLC-MS/MS without further concentration.

Statistical Analysis. For all statistical tests, any concentrations below the LOD were imputed as the LOD divided by the square root of two. All data were tested for evidence of non-normality using the Shapiro-Wilk *W* test (*p*-values in Table S5ab). Data from the single donor samples were largely non-normally distributed (~90% of the analytes), while data from the pooled samples showed more frequent cases of normal distribution (~60% of the analytes). Non-normally distributed data were logarithmically transformed and re-tested with the Shapiro-Wilk *W* test, but normality only improved for ~10% of the transformed data. The assumption of normality in the data was minimized by using nonparametric methods, such as the Mann-Whitney *U* test to compare analyte concentrations (i.e. temporal, gender, analyte vs. analyte) and the Spearman rank correlation test to test for possible correlations among the target analytes. A *p*-value of 0.05 was chosen as the criterion for statistical significance in all analyses. All statistical

tests were performed using StatsDirect (Version 2.7.8, Cheshire, UK). A summary of the descriptive statistics calculated for all detected analytes is provided in Table S6a-e. A significant concentration difference was observed between the single donor and pooled samples for 6:2 diPAP, N-EtFOSAA, 6:2 FTS, C6/C6 PFPiA, C6/C8 PFPiA, and PFUnA (Mann-Whitney *U* test, $p<0.05$, Table S7), and so their concentrations were considered separately. No significant difference was observed for the remaining analytes (Mann-Whitney *U* test, $p>0.05$, Table S7), and so the concentrations in both sample types were combined for Spearman's rank correlation analyses.

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Table S1a. Multiple reaction monitoring (MRM) transitions and mass spectrometry parameters for all target analytes.

Analyte	Acronym	Mass Transition	Dwell (ms)	Declustering Potential, DP (V)	Collision Energy, CE (V)	Collision Cell Exit Potential, CXP (V)
Polyfluoroalkyl phosphate diester						
4:2 polyfluoroalkyl phosphate diester	4:2 diPAP	589.1>96.9	30	-50	-50	-15
		589.1>343.0	20	-50	-25	-15
4:2/6:2 polyfluoroalkyl phosphate diester	4:2/6:2 diPAP	689.0>96.9	30	-60	-60	-15
6:2 polyfluoroalkyl phosphate diester	6:2 diPAP	789.0>96.9	30	-65	-65	-15
		789.0>443.0	20	-65	-27	-15
6:2/8:2 polyfluoroalkyl phosphate diester	6:2/8:2 diPAP	889.0>96.9	30	-70	-70	-15
8:2 polyfluoroalkyl phosphate diester	8:2 diPAP	989.0>96.9	30	-80	-75	-15
		989.0>543.0	20	-70	-33	-15
8:2/10:2 polyfluoroalkyl phosphate diester	8:2/10:2 diPAP	1089.0>96.9	30	-80	-80	-15
10:2 polyfluoroalkyl phosphate diester	10:2 diPAP	1189.0>96.9	30	-80	-85	-15
		1189.0>643.0	40	-80	-40	-15
10:2/12:2 polyfluoroalkyl phosphate diester	10:2/12:2 diPAP	1289.0>96.9	30	-80	-85	-15
Fluorotelomer mercaptoalkyl phosphate diester						
6:2 fluorotelomer mercaptoalkyl phosphate diester	6:2 FTMAP	921.0>79.0	40	-95	-99	-15
		921.0>318.7	40	-95	-70	-15
		921.0>575.0	40	-95	-50	-15
N-ethyl perfluorooctanesulfonamidoethanol-based phosphate diester						
N-ethyl perfluorooctanesulfonamidoethanol-based phosphate diester	SAmPAP	1203.0>526.0	30	-190	-68	-15
		1203.0>650.0	30	-190	-57	-15

Table S1b. Multiple reaction monitoring (MRM) transitions and mass spectrometry parameters for all target analytes.

Analyte	Acronym	Mass Transition	Dwell (ms)	Declustering Potential, DP (V)	Collision Energy, CE (V)	Collision Cell Exit Potential, CXP (V)
Fluorotelomer sulfonate						
4:2 fluorotelomer sulfonate	4:2 FTS	327.0>81.0	20	-95	-53	-15
		327.0>306.8	20	-95	-30	-18
6:2 fluorotelomer sulfonate	6:2 FTS	427.0>81.0	20	-100	-65	-15
		427.0>406.8	20	-100	-32	-10
8:2 fluorotelomer sulfonate	8:2 FTS	527.0>81.0	20	-100	-72	-14
		527.0>506.8	20	-100	-40	-15
Perfluorooctanesulfonamidoacetate, N-methyl & N-ethyl perfluorooctanesulfonamidoacetate						
Perfluorooctanesulfonamidoacetate	FOSAA	559.9>419.0	20	-40	-45	-15
N-methyl perfluorooctanesulfonamidoacetate	N-MeFOSAA	570.0>419.0	20	-40	-36	-15
N-ethyl perfluorooctanesulfonamidoacetate	N-EtFOSAA	584.0>419.0	20	-50	-36	-15
Perfluorophosphonate and perfluorophosphinate						
C6 perfluorophosphonate	C6 PFPA	399.0>79.0	40	-60	-75	-10
C8 perfluorophosphonate	C8 PFPA	499.0>79.0	40	-70	-80	-10
C10 perfluorophosphonate	C10 PFPA	599.0>79.0	40	-80	-90	-10
C6/C6 perfluorophosphinate	C6/C6 PFPiA	701.0>401.0	40	-95	-75	-10
C6/C8 perfluorophosphinate	C6/C8 PFPiA	801.0>501.0	40	-99	-85	-10
C8/C8 perfluorophosphinate	C8/C8 PFPiA	901.0>501.0	40	-97	-90	-10
C6/C10 perfluorophosphinate	C6/C10 PFPiA	901.0>601.0	40	-92	-90	-10
C8/C10 perfluorophosphinate	C8/C10 PFPiA	1001.0>601.0	40	-97	-97	-10
C6/C12 perfluorophosphinate	C6/C12 PFPiA	1001.0>701.0	40	-92	-98	-10

Table S1c. Multiple reaction monitoring (MRM) transitions and mass spectrometry parameters for all target analytes.

Compound	Acronym	Mass Transition	Dwell (ms)	Declustering Potential, DP (V)	Collision Energy, CE (V)	Collision Cell Exit Potential, CXP (V)
Perfluorocarboxylate						
Perfluorobutanoate	PFBA (C4)	212.8>168.9	40	-25	-13	-15
Perfluoropentanoate	PFPeA (C5)	262.8>218.97	40	-20	-13	-15
Perfluorohexanoate	PFHxA (C6)	312.8>268.9	20	-20	-13	-15
Perfluoroheptanoate	PFHpA (C7)	362.8>319.0	20	-27	-13	-15
Perfluorooctanoate	PFOA (C8)	413.0>368.9	20	-35	-15	-15
Perfluorononanoate	PFNA (C9)	462.9>419.0	20	-35	-15	-15
Perfluorodecanoate	PFDA (C10)	513.0>470.0	20	-45	-15	-15
Perfluoroundecanoate	PFUnA (C11)	562.8>519.0	20	-45	-15	-15
Perfluorododecanoate	PFDoA (C12)	612.8>569.0	20	-45	-15	-15
Perfluorotridecanoate	PFTrA (C13)	662.8>619.0	20	-45	-15	-15
Perfluorotetradecanoate	PFTeA (C14)	712.8>669.0	20	-45	-15	-15
Perfluorosulfonate						
Perfluorobutanesulfonate	PFBS (C4)	299.0>99.0	20	-55	-65	-15
Perfluorohexanesulfonate	PFHxS (C6)	399.0>99.0	20	-55	-65	-15
Perfluorooctanesulfonate	PFOS (C8)	499.0>99.0	20	-120	-80	-15
Perfluorodecanesulfonate	PFDS (C10)	599.0>99.0	20	-120	-80	-15

Table S2. Multiple reaction monitoring (MRM) transitions and mass spectrometry parameters for all internal standards.

Target Analyte	Internal Standard	Mass Transition	Dwell (ms)	Declustering Potential, DP (V)	Collision Energy, CE (V)	Collision Cell Exit Potential, CXP (V)
Perfluorooctanesulfonamidoacetate, N-methyl & N-ethyl perfluorooctanesulfonamidoacetate						
FOSAA	d_3-N-MeFOSAA	573.0>419.0	20	-40	-36	-15
N-MeFOSAA	d_3-N-MeFOSAA	573.0>419.0	20	-40	-36	-15
N-EtFOSAA	d_5-N-EtFOSAA	589.0>419.0	20	-50	-36	-15
Perfluorinated acids						
PFBA (C4)	$^{13}C_4$-PFBA	217.0>172.0	40	-25	-13	-15
PFPeA (C5)	$^{13}C_2$-PFHxA	314.8>269.8	20	-20	-13	-15
PFHxA (C6)	$^{13}C_2$-PFHxA	314.8>269.8	20	-20	-13	-15
PFHpA (C7)	$^{13}C_4$-PFOA	417.0>372.0	20	-35	-15	-15
PFOA (C8)	$^{13}C_4$-PFOA	417.0>372.0	20	-35	-15	-15
PFNA (C9)	$^{13}C_5$-PFNA	468.0>423.0	20	-35	-15	-15
PFDA (C10)	$^{13}C_2$-PFDA	515.0>470.0	20	-45	-15	-15
PFUnA (C11)	$^{13}C_2$-PFUnA	564.8>520.0	20	-45	-15	-15
PFDoA (C12)	$^{13}C_2$-PFDoA	614.8>570.0	20	-45	-15	-15
PFTrA (C13)	$^{13}C_2$-PFDoA	614.8>570.0	20	-45	-15	-15
PFTeA (C14)	$^{13}C_2$-PFDoA	614.8>570.0	20	-45	-15	-15
PFBS (C4)	$^{18}O_2$-PFHxS	403.0>103.0	20	-55	-65	-15
PFHxS (C6)	$^{18}O_2$-PFHxS	403.0>103.0	20	-55	-65	-15
PFOS (C8)	$^{13}C_4$-PFOS	503.0>99.0	20	-120	-80	-15
PFDS (C10)	$^{13}C_4$-PFOS	503.0>99.0	20	-120	-80	-15

Figure S1. Chromatograms of a standard addition analysis of a human sera sample for the suite of PFPiAs.

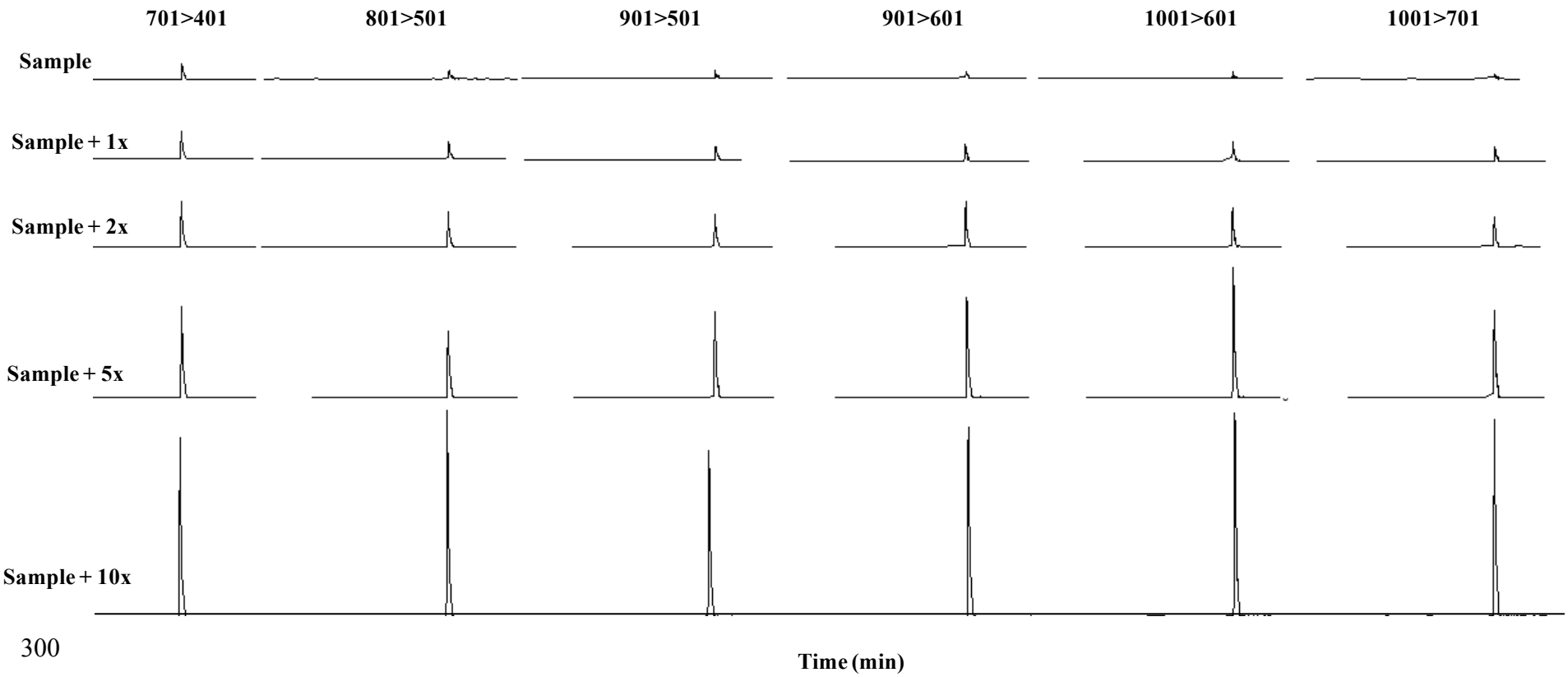

Table S3a. Limits of detection (LODs), limits of quantification (LOQs), and matrix recoveries for the analytes of interest.

Analyte	Instrumental (on column)		Method (20X)		Recovery (%) (n = 3)
	LOD (pg)	LOQ (pg)	LOD (µg/L)	LOQ (µg/L)	
Fluorinated Precursors					
4:2 diPAP	1.75	3.50	0.008	0.015	107 ± 20
6:2 diPAP	1.75	3.50	0.008	0.015	109 ± 22
8:2 diPAP	17.50	26.25	0.075	0.113	87 ± 21
10:2 diPAP	8.75	17.50	0.038	0.075	100 ± 27
6:2 FTMAP	1.75	3.50	0.015	0.038	97 ± 17
SAmPAP	1.75	3.50	0.008	0.02	101 ± 8
Fluorinated Intermediates					
FOSAA	0.88	1.75	0.011	0.023	90 ± 5
N-MeFOSAA	0.18	0.35	0.002	0.005	94 ± 6
N-EtFOSAA	0.35	0.88	0.005	0.011	94 ± 2
4:2 FTS	0.35	0.88	0.005	0.011	90 ± 20
6:2 FTS	0.35	0.88	0.005	0.011	100 ± 21
8:2 FTS	0.35	0.88	0.005	0.011	94 ± 15

Table S3b. Limits of detection (LODs), limits of quantification (LOQs), and matrix recoveries for the analytes of interest.

Analyte	Instrumental (on column)		Method (20X)		Recovery (%) (n = 3)
	LOD (pg)	LOQ (pg)	LOD (µg/L)	LOQ (µg/L)	
Perfluorinated Acids					
C6 PFPA	3.50	8.75	0.009	0.023	86 ± 12
C8 PFPA	1.75	3.50	0.005	0.009	90 ± 11
C10 PFPA	26.25	35.00	0.070	0.093	89 ± 10
C6/C6 PFPiA	0.32	0.65	0.001	0.002	101 ± 32
C6/C8 PFPiA	0.29	0.58	0.001	0.002	105 ± 32
C8/C8 PFPiA	0.47	0.95	0.001	0.003	100 ± 38
C6/C10 PFPiA*	0.88	1.75	0.002	0.005	95 ± 26
C8/C10 PFPiA*	1.75	3.50	0.005	0.009	93 ± 27
C6/C12 PFPiA*	1.75	3.50	0.005	0.009	98 ± 34
PFBA (C4)	0.35	0.88	0.005	0.011	114 ± 17
PFPeA (C5)	0.18	0.35	0.002	0.005	96 ± 9
PFHxA (C6)	0.04	0.18	0.001	0.002	125 ± 11
PFHpA (C7)	0.04	0.18	0.001	0.002	71 ± 3
PFOA (C8)	0.18	0.35	0.002	0.005	91 ± 8
PFNA (C9)	0.18	0.35	0.002	0.005	93 ± 14
PFDA (C10)	0.18	0.35	0.002	0.005	114 ± 15
PFUnA (C11)	0.26	0.35	0.003	0.005	96 ± 13
PFDoA (C12)	0.35	0.88	0.005	0.011	111 ± 23
PFTrA (C13)	0.35	0.88	0.005	0.011	92 ± 18
PFTeA (C14)	0.35	0.88	0.005	0.011	85 ± 24
PFBS (C4)	0.35	0.88	0.005	0.011	80 ± 8
PFHxS (C6)	0.35	0.88	0.005	0.011	106 ± 20
PFOS (C8)	0.18	0.35	0.002	0.005	97 ± 14
PFDS (C10)	0.35	0.88	0.005	0.011	94 ± 15

* Concentrations were not corrected based on corresponding percent distribution in Masurf® 780 standard

Table S4a. Concentrations of all monitored PFCAs and PFSAs observed in NIST SRM 1957 human sera from NIST Certificate of Analysis, an interlaboratory study, and this study.

Analyte	Reported Concentrations (µg/L) NIST Certificate of Analysis[1]	Interlaboratory Study[2]	Measured Concentrations[3] (µg/L)
PFBA	*	<LOD or <LOQ	nd
PFPeA	*	<LOD or <LOQ	0.23±0.09
PFHxA	*	<LOD or <LOQ	0.08±0.02
PFHpA	0.305±0.036	0.28–0.33	0.27±0.10
PFOA	5.00±0.40	4.08–5.86	5.06±0.86
PFNA	0.880±0.068	0.76–0.97	0.88±0.10
PFDA	0.39±0.10	0.29–0.53	0.33±0.06
PFUnA	0.174±0.031	0.11–0.22	0.15±0.02
PFDoA	*	0.16–0.20	0.02±0.01
PFTrA	*	<LOD or <LOQ	0.03±0.00
PFTeA	*	<LOD or <LOQ	nd
PFBS	*	<LOD or <LOQ	nd
PFHxS	4.00±0.75	3.01–6.49	3.49±0.94
PFOS	21.1±1.2	19.5–38.0	13.66±1.13
PFDS	*	0.15–0.49	0.22±0.05

[1] Data obtained from certificate of analysis available on the NIST website: www.nist.gov/srm.

[2] Data obtained from ref. *(6)*.

[3] Data obtained from replicate analysis (n = 4) of SRM1957 in the present study.

* Concentrations of PFBA, PFPeA, PFHxA, PFDoA, PFTrA, PFTeA, PFBS, and PFDS are not reported on the NIST certificate of analysis.

nd = nondetects (i.e. analytes were either not detected or concentrations were below their corresponding LODs)

Table S4b. Concentrations of all other target analytes monitored in NIST SRM 1957 human sera from this study (n = 4).

Analyte	Measured Concentrations (µg/L)
4:2 diPAP	0.05±0.01
4:2/6:2 diPAP	0.15±0.04
6:2 diPAP	0.31±0.09
6:2/8:2 diPAP	0.13±0.05
8:2 diPAP	0.14±0.05
8:2/10:2 diPAP	nd
10:2 diPAP	nd
6:2 FTMAP	nd
N-EtFOSE phosphate	nd
FOSAA	0.16±0.02
N-MeFOSAA	0.74±0.06
N-EtFOSAA	0.15±0.01
4:2 FTS	0.03±0.01
6:2 FTS	0.02±0.01
8:2 FTS	0.09±0.03
C6 PFPA	nd
C8 PFPA	nd
C10 PFPA	nd
C6/C6 PFPiA	0.003±0.001
C6/C8 PFPiA	0.006±0.001
C8/C8 PFPiA	nd
C6/C10 PFPiA*	0.011±0.001
C8/C10 PFPiA*	nd
C6/C12 PFPiA*	nd

nd = nondetects (i.e. analytes were either not detected or concentrations were below their corresponding LODs)

* Concentrations were not corrected based on corresponding percent distribution in Masurf® 780 standard

Table S5a. *P*-values from Shapiro-Wilk *W* test to analyze data for evidence of non-normality. A *p*-value of 0.05 is the chosen criterion of statistical significance such that if the test statistic is below 0.05 ($p<0.05$), the null hypothesis may be rejected, and the data are unlikely to be normally distributed. If the test statistic is above 0.05 ($p>0.05$), the Shapiro-Wilk *W* test can only conclude there is no evidence of non-normality.

Analyte	Type of Sample	Type of Data	
		Data without log transformation	Log-transformed data
4:2 diPAP	Single donor	<0.0001	<0.0001
	Pooled	*	*
4:2/6:2 diPAP	Single donor	<0.0001	<0.0001
	Pooled	<0.0001	<0.0001
6:2 diPAP	Single donor	<0.0001	0.0547[a]
	Pooled	0.0122	0.2566[b]
6:2/8:2 diPAP	Single donor	<0.0001	0.0001
	Pooled	0.0027	0.0494
8:2 diPAP	Single donor	<0.0001	<0.0001
	Pooled	0.0486	0.0476
FOSAA	Single donor	<0.0001	0.001
	Pooled	0.0242	0.6837[b]
N-MeFOSAA	Single donor	<0.0001	<0.0001
	Pooled	0.0009	0.5203[b]
N-EtFOSAA	Single donor	0.0001	<0.0001
	Pooled	0.1183[b]	0.7616[b]
4:2 FTS	Single donor	<0.0001	<0.0001
	Pooled	<0.0001	<0.0001
6:2 FTS	Single donor	<0.0001	<0.0001
	Pooled	0.4333[b]	0.061[a]
8:2 FTS	Single donor	<0.0001	0.2636[b]
	Pooled	0.0324	0.2043[b]
C6/C6 PFPiA	Single donor	<0.0001	<0.0001
	Pooled	<0.0001	0.0109
C6/C8 PFPiA	Single donor	<0.0001	0.0065
	Pooled	<0.0001	0.0023
C8/C8 PFPiA	Single donor	<0.0001	<0.0001
	Pooled	<0.0001	<0.0001
C6/C10 PFPiA	Single donor	<0.0001	<0.0001
	Pooled	<0.0001	0.1284[b]
C8/C10 PFPiA	Single donor	<0.0001	<0.0001
	Pooled	<0.0001	<0.0001
C6/C12 PFPiA	Single donor	<0.0001	<0.0001
	Pooled	<0.0001	<0.0001

* Test cannot be performed due to 100% non-detection in the samples.

[a] Test was not quite significant; cannot assume there is no evidence of non-normality.

[b] No evidence of non-normality.

Table S5b. *P*-values from Shapiro-Wilk *W* test to analyze data for evidence of non-normality. A *p*-value of 0.05 is the chosen criterion of statistical significance such that if the test statistic is below 0.05 ($p<0.05$), the null hypothesis may be rejected, and the data are unlikely to be normally distributed. If the test statistic is above 0.05 ($p>0.05$), the Shapiro-Wilk *W* test can only conclude there is no evidence of non-normality.

Analyte	Type of Sample	Type of Data	
		Data without log transformation	Log-transformed data
PFBA (C4)	Single donor	<0.0001	<0.0001
	Pooled	0.7788[b]	0.8161[b]
PFPeA (C5)	Single donor	<0.0001	0.0002
	Pooled	*	*
PFHxA (C6)	Single donor	<0.0001	<0.0001
	Pooled	0.0048	0.0301
PFHpA (C7)	Single donor	<0.0001	<0.0001
	Pooled	0.5644[b]	0.5239[b]
PFOA (C8)	Single donor	0.1385[b]	0.0338
	Pooled	0.2385[b]	>0.9999[b]
PFNA (C9)	Single donor	0.0784[a]	0.0997[a]
	Pooled	0.0827[a]	0.6286
PFDA (C10)	Single donor	0.0001	<0.0001
	Pooled	0.2721[b]	0.6403[b]
PFUnA (C11)	Single donor	<0.0001	0.0022
	Pooled	0.1059[b]	0.9254[b]
PFBS (C4)	Single donor	<0.0001	<0.0001
	Pooled	<0.0001	<0.0001
PFHxS (C6)	Single donor	<0.0001	0.1724[b]
	Pooled	0.7754[b]	0.3600[b]
PFOS (C8)	Single donor	<0.0001	0.0139
	Pooled	0.9626[b]	0.9799[b]
PFDS (C10)	Single donor	0.0001	<0.0001
	Pooled	0.0031	0.0349

* Test cannot be performed due to 100% non-detection in the samples.
[a] Test was not quite significant; cannot assume there is no evidence of non-normality.
[b] No evidence of non-normality.

Table S6a. Summary of descriptive statistics for all detected analytes. For the purposes of calculating means, values below the LOD were assigned a value of zero and values below the LOQ were used unaltered. For analytes that were detected in <20% of the samples, mean concentrations were not calculated and only the range is reported. Concentrations are reported in ng/L (ppt).

(ng/L)	Analyte				
	4:2 diPAP	**4:2/6:2 diPAP**	**6:2 diPAP**	**6:2/8:2 diPAP**	**8:2 diPAP**
All single donor samples (*n* = 40)					
Mean	*	*	72.07	34.65	110.31
SE	*	*	15.13	8.68	48.05
Range	<LOD–21.51	<LOD–100.54	<LOD–388.55	<LOD–303.05	<LOD–1801.74
% <LOD	88	85	18	48	68
% <LOQ	98	90	50	93	78
Male single donor samples (*n* = 20)					
Mean	*	*	87.14	42.85	91.96
SE	*	*	25.53	16.60	39.45
Range	<LOD–21.51	<LOD–100.54	<LOD–388.55	<LOD–303.05	<LOD–777.24
% <LOD	85	80	25	55	70
% <LOQ	95	90	30	85	75
Female single donor samples (*n* = 20)					
Mean	*	*	57.00	26.46	128.65
SE	*	*	16.24	5.19	88.82
Range	<LOD–9.23	<LOD–55.54	<LOD–328.29	<LOD–70.54	<LOD–1801.74
% <LOD	90	90	10	40	65
% <LOQ	100	90	20	100	80
All pooled samples (*n* = 10)					
Mean	*	*	131.81	49.06	133.59
SE	*	*	37.85	19.20	38.80
Range	-	<LOD–163.94	30.97–346.46	<LOD–157.03	<LOD–323.36
% <LOD	100	90	0	40	40
% <LOQ	100	90	0	70	50

* Mean concentrations and standard error were not reported due to the low frequency of detection in the samples (<20%).

- Range was not reported due to 100% non-detection in the samples

Table S6b. Summary of descriptive statistics for all detected analytes. For the purposes of calculating means, values below the LOD were assigned a value of zero and values below the LOQ were used unaltered. For analytes that were detected in <20% of the samples, mean concentrations were not calculated and only the range is reported. Concentrations are reported in ng/L (ppt).

(ng/L)	Analyte					
	FOSAA	**N-MeFOSAA**	**N-EtFOSAA**	**4:2 FTS**	**6:2 FTS**	**8:2FTS**
All single donor samples (*n* = 40)						
Mean	64.30	356.99	50.28	*	7.64	37.75
SE	15.22	71.24	6.92	*	1.26	6.16
Range	<LOD–432.35	<LOD–1997.72	<LOD–173.54	<LOD–17.94	<LOD–29.54	<LOD–162.49
% <LOD	35	10	18	90	46	5
% <LOQ	43	10	18	95	69	13
Male single donor samples (*n* = 20)						
Mean	44.24	241.29	41.25	*	5.91	45.18
SE	16.25	72.15	7.93	*	1.70	9.56
Range	<LOD–305.55	<LOD–1509.43	<LOD–134.61	<LOD–15.83	<LOD–18.39	<LOD–154.12
% <LOD	50	15	25	95	58	11
% <LOQ	50	15	25	100	68	11
Female single donor samples (*n* = 20)						
Mean	84.36	472.69	59.31	*	9.28	30.68
SE	25.38	119.24	11.20	*	1.82	7.78
Range	<LOD – 432.35	<LOD–1997.72	<LOD–173.54	<LOD–17.94	<LOD–29.54	7.32 – 162.49
% <LOD	20	5	10	85	35	0
% <LOQ	35	5	10	90	70	15
All pooled donor samples (*n* = 10)						
Mean	64.20	443.66	69.19	*	23.74	73.68
SE	13.56	108.22	6.78	*	5.37	21.03
Range	25.93–166.58	146.76–1355.58	43.27–119.86	-	<LOD–47.25	9.12 – 230.70
% <LOD	0	0	0	100	20	0
% <LOQ	0	0	0	100	30	20

* Mean concentrations and standard error were not reported due to the low frequency of detection in the samples (<20%).

\- Range was not reported due to 100% non-detection in the samples

Table S6c. Summary of descriptive statistics for all detected analytes. For the purposes of calculating means, values below the LOD were assigned a value of zero and values below the LOQ were used unaltered. For analytes that were detected in <20% of the samples, mean concentrations were not calculated and only the range is reported. Concentrations are reported in ng/L (ppt).

(ng/L)	Analyte					
	C6/C6 PFPiA	**C6/C8 PFPiA**	**C8/C8 PFPiA**	**C6/C10 PFPiA[a]**	**C8/C10 PFPiA[a]**	**C6/C12 PFPiA[a]**
All single donor samples (*n* = 40)						
Mean	3.65	7.67	*	19.88	*	12.19
SE	1.32	1.91	*	4.77	*	6.01
Range	<LOD–50.24	<LOD–60.96	<LOD–22.19	<LOD–133.95	<LOD–48.73	<LOD–225.12
% <LOD	50	28	95	58	95	80
% <LOQ	58	30	98	58	98	80
Male single donor samples (*n* = 20)						
Mean	5.71	9.74	*	26.59	*	20.87
SE	2.51	3.19	*	7.73	*	11.56
Range	<LOD–50.24	<LOD–60.96	<LOD–22.19	<LOD–133.95	<LOD–48.73	<LOD–225.12
% <LOD	40	15	95	45	95	70
% <LOQ	45	15	95	45	95	70
Female single donor samples (*n* = 20)						
Mean	1.60	5.60	*	13.18	*	*
SE	0.65	2.07	*	5.38	*	*
Range	<LOD–12.02	<LOD–36.67	-	<LOD–86.56	<LOD–5.68	<LOD–47.86
% <LOD	60	40	100	70	95	90
% <LOQ	70	45	100	70	100	90
All pooled donor samples (*n* = 10)						
Mean	23.20	37.86	*	140.35	*	*
SE	19.81	27.35	*	115.98	*	*
Range	<LOD–201.41	4.36–283.38	<LOD–50.73	<LOD–1182.50	<LOD–891.02	<LOD–957.44
% <LOD	10	0	90	30	90	90
% <LOQ	20	0	90	30	90	90

* Mean concentrations and standard error were not reported due to the low frequency of detection in the samples (<20%).

\- Range was not reported due to 100% non-detection in the samples

[a] Concentrations were not corrected based on corresponding percent distribution in Masurf® 780 standard

Table S6d. Summary of descriptive statistics for all detected analytes. For the purposes of calculating means, values below the LOD were assigned a value of zero and values below the LOQ were used unaltered. For analytes that were detected in <20% of the samples, mean concentrations were not calculated and only the range is reported. Concentrations are reported in ng/L (ppt).

(ng/L)	Analyte							
	PFBA	**PFPeA**	**PFHxA**	**PFHpA**	**PFOA**	**PFNA**	**PFDA**	**PFUnA**
All single donor samples (*n* = 40)								
Mean	35.06	72.59	49.62	97.16	2001.42	694.89	416.75	218.67
SE	7.81	17.91	18.94	15.27	182.67	59.95	59.63	48.49
Range	<LOD–227.56	<LOD–502.04	<LOD–718.22	<LOD–416.60	190.15–5163.96	108.23–1581.31	<LOD–1561.41	<LOD–1439.77
% <LOD	43	35	40	13	0	0	5	20
% <LOQ	45	35	40	13	0	0	5	20
Male single donor samples (*n* = 20)								
Mean	45.76	68.65	36.58	100.59	2466.50	782.63	464.47	188.66
SE	14.09	24.39	15.26	20.11	285.47	93.99	92.70	52.03
Range	<LOD–227.56	<LOD–402.86	<LOD–288.42	<LOD–299.50	329.87–5163.96	108.23–1581.31	<LOD–1561.41	<LOD–757.02
% <LOD	45	40	50	15	0	0	5	30
% <LOQ	45	40	50	15	0	0	5	30
Female single donor samples (*n* = 20)								
Mean	24.36	76.53	62.67	93.73	1536.34	607.14	369.04	248.68
SE	6.29	26.85	34.95	23.49	180.89	71.48	75.94	82.77
Range	<LOD–89.91	<LOD–502.04	<LOD–718.22	<LOD–416.60	190.15–3650.99	129.22–1456.65	25.31–1172.39	<LOD–1439.77
% <LOD	40	30	30	10	0	0	0	10
% <LOQ	45	30	30	10	0	0	0	10
All pooled donor samples (*n* = 10)								
Mean	37.46	*	38.61	83.18	1760.65	703.72	294.84	261.56
SE	3.88	*	2.13	13.58	307.54	80.05	15.44	42.52
Range	37.65 – 57.30	-	32.52 – 55.98	24.64– 161.75	613.75– 3978.95	444.92– 1303.42	229.10– 405.90	121.53– 577.79
% <LOD	0	100	0	0	0	0	0	0
% <LOQ	0	100	0	0	0	0	0	0

* Mean concentrations and standard error were not reported due to the low frequency of detection in the samples (<20%).

\- Range was not reported due to 100% non-detection in the samples

Table S6e. Summary of descriptive statistics for all detected analytes. For the purposes of calculating means, values below the LOD were assigned a value of zero and values below the LOQ were used unaltered. For analytes that were detected in <20% of the samples, mean concentrations were not calculated and only the range is reported. Concentrations are reported in ng/L (ppt).

(ng/L)	Analyte			
	PFBS	**PFHxS**	**PFOS**	**PFDS**
All single donor samples (*n* = 40)				
Mean	*	1249.05	12263.19	39.89
SE	*	202.69	3794.29	6.36
Range	<LOD–59.60	27.99 – 6795.84	143.96 – 119559.05	<LOD–155.26
% <LOD	85	0	0	35
% <LOQ	85	0	0	38
Male single donor samples (*n* = 20)				
Mean	*	1419.63	13295.01	36.53
SE	*	250.53	5991.11	8.14
Range	<LOD–59.60	185.63–4362.86	143.96 – 119559.05	<LOD–118.74
% <LOD	85	0	0	35
% <LOQ	85	0	0	35
Female single donor samples (*n* = 20)				
Mean	*	1078.46	11231.37	43.25
SE	*	320.68	4805.89	9.93
Range	<LOD–53.68	27.99–6795.84	778.07–75979.13	<LOD–155.26
% <LOD	85	0	0	35
% <LOQ	85	0	0	40
All pooled donor samples (*n* = 10)				
Mean	16.78	1193.81	4442.95	51.34
SE	8.55	177.07	462.25	3.76
Range	<LOD–58.64	353.24–2039.20	2318.33 – 7209.94	40.76–82.39
% <LOD	70	0	0	0
% <LOQ	70	0	0	0

* Mean concentrations and standard error were not reported due to the low frequency of detection in the samples (<20%).

- Range was not reported due to 100% non-detection in the samples

Table S7. *P*-values from Mann-Whitney *U* test to compare concentrations between single donor and pooled sera samples and for gender differences. A *p*-value of 0.05 is the chosen criterion of statistical significance such that if the test statistic is below 0.05 ($p<0.05$), the null hypothesis may be rejected, and there is a significant difference between the two groups of data. If the test statistic is above 0.05 ($p>0.05$), there is no significant difference between the two groups of data. The Mann-Whitney *U* test was used to compare the concentrations observed in the single donor and pooled sera samples, and the concentrations observed in male and female single donor samples. In the gender comparison analysis, a one-sided *p*-value was calculated to test whether the concentrations observed in female donors were lower as compared to male donors.

Analyte	Type of Comparison		
	Single donor vs. Pooled	Female (F) vs. Male (M)	
	***p*-value (two-sided)**	***p*-value (two-sided)**	***p*-value (one-sided; F<M)**
4:2 diPAP	0.6211	0.6050	0.3025
4:2/6:2 diPAP	0.8581	0.5335	0.2668
6:2 diPAP	0.0246	0.9734	0.4867
6:2/8:2 diPAP	0.6848	0.6105	0.3053
8:2 diPAP	0.0751	0.6423	0.3212
FOSAA	0.1914	0.1382	0.0691
N-MeFOSAA	0.0699	0.2661	0.1331
N-EtFOSAA	0.0264	0.2999	0.1499
4:2 FTS	0.2589	0.5768	0.2884
6:2 FTS	0.0051	0.1995	0.0998
8:2 FTS	0.1285	0.2354	0.1177
C6/C6 PFPiA	0.0377	0.1496	0.0748
C6/C8 PFPiA	0.0065	0.1233	0.0617
C8/C8 PFPiA	0.4612	0.4872	0.2436
C6/C10 PFPiA	0.1707	0.1302	0.0651
C8/C10 PFPiA	0.4612	*	*
C6/C12 PFPiA	0.8322	0.1257	0.0628
PFBA (C4)	0.2010	0.4484	0.2242
PFPeA (C5)	*	0.7476	0.3738
PFHxA (C6)	0.1187	0.3486	0.1743
PFHpA (C7)	0.7469	0.7581	0.3790
PFOA (C8)	0.6242	0.0122	0.0061
PFNA (C9)	0.8392	0.1738	0.0869
PFDA (C10)	0.8734	0.4568	0.2284
PFUnA (C11)	0.0363	0.5871	0.2935
PFBS (C4)	0.2005	0.8984	0.4492
PFHxS (C6)	0.4224	0.1081	0.0540
PFOS (C8)	0.8955	0.4612	0.2306
PFDS (C10)	0.1780	0.7748	0.3874

***** Test was not performed due to 100% non-detection in the samples.

Table S8. *P*-values from Mann-Whitney *U* test to compare concentrations of 6:2 and 8:2 FTS observed in pooled human sera collected in 2002 and 2009. A *p*-value of 0.05 is the chosen criterion of statistical significance such that if the test statistic is below 0.05 ($p<0.05$), the null hypothesis may be rejected, and there is a significant difference between the two groups of data. If the test statistic is above 0.05 ($p>0.05$), there is no significant difference between the two groups of data. The Mann-Whitney *U* test was used to compare the concentrations observed in the single donor and pooled sera samples, and the concentrations observed in male and female single donor samples. In the gender comparison analysis, a one-sided *p*-value was calculated to test whether the concentrations observed in female donors were lower as compared to male donors.

Analyte	Type of Comparison
	2002 pooled sera[a] vs. 2009 pooled sera[b]
	p-value (two-sided)
6:2 FTS	0.3915
8:2 FTS	0.8968

[a] Data obtained from ref. *(11)*.
[b] Data obtained from this study.

Table S9. Spearman's rank correlation coefficient *r*-values and *p*-values from Spearman's rank correlation test to analyze two groups of data for correlation. A *p*-value of 0.05 is the chosen criterion of statistical significance such that if the test statistic is below 0.05 ($p<0.05$), the null hypothesis may be rejected, and there is a significant correlation between the two groups of data. If the test statistic is above 0.05 ($p>0.05$), there is no significant correlation between the two groups of data. The value of *r* always falls between -1 and +1. The closer *r* falls to +1 or -1, the greater the correlation. The closer *r* is to 0, the lesser the correlation. In each cell, the top row represents the test performed on the concentrations between single donor samples and the bottom row represents the test performed on the concentrations between the pooled samples.

Single donor / Pooled	C6/C6 PFPiA	C6/C8 PFPiA	C8/C8 PFPiA	C6/C10 PFPiA	C8/C10 PFPiA	C6/C12 PFPiA
C6/C6 PFPiA	n/a	r=0.76; p<0.0001 r=0.83; p=0.0047	*	r=0.66; p<0.0001 r=0.50; p=0.1548	*	r=0.48; p=0.0019 *
C6/C8 PFPiA	r=0.76; p<0.0001 r=0.83; p=0.0047	n/a	*	r=0.78; p<0.0001 r=0.83; p=0.0047	*	r=0.56; p=0.0002 *
C8/C8 PFPiA	*	*	n/a	*	*	*
C6/C10 PFPiA	r=0.66; p<0.0001 r=0.50; p=0.1548	r=0.78; p<0.0001 r=0.83; p=0.0047	*	n/a	*	r=0.60; p<0.0001[a]
C8/C10 PFPiA	*	*	*	*	n/a	*
C6/C12 PFPiA	r=0.48; p=0.0019 *	r=0.56; p=0.0002 *	*	r=0.60; p<0.0001[a]	*	n/a

n/a Correlation tests were not performed for the concentrations of the same analyte.

* Correlation tests were not performed due to the large number of non-detects observed for these analytes.

[a] The correlation test to compare C6/C10 and C6/C12 PFPiA was performed on the concentrations combined from the single donor and pooled samples as the Mann-Whitney *U* test showed no significant difference in their concentrations from both sample types ($p>0.05$, Table S7).