

Per- and Poly-fluoroalkyl Substances in Circulation in a Canadian Population: Their Association with Serum-Liver Enzyme Biomarkers and Piloting a Novel Method to Reduce Serum-PFAS

Jennifer J. Schlezinger, Anila Bello, Kelsey M. Mangano, Kushal Biswas, Paridhiben P. Patel, Emily Pennoyer, Thomas MS Wolever, Wendy J. Heiger-Bernays, and Dhimiter Bello

Table of Contents

Page 2: Materials and methods for PFAS analyses

Page 3: Table S1. List of PFAS internal standards associated with PFASs

Page 4: Table S2. Multiple Reactions Monitoring (MRM) of PFASs

Page 5: Table S3. Limits of detection for PFASs.

Page 6: Table S4. Association between serum-liver function biomarkers and PFAS exposure (Unadjusted).

Page 7: Table S5. Associations between measures of serum-cholesterol and PFAS exposure (Unadjusted).

Materials and Methods for PFAS Analyses

Materials

High purity liquid chromatography grade solvents, methanol (Cat# BDH85800.400) and acetonitrile (Cat# BDH83639.400) were purchased from VWR International (Radnor, PA). High purity deionized water, >18M Ω , was obtained from a Milli-Q Direct 8 unit (Millipore Sigma). LC-MS grade ammonium acetate was purchased from Sigma Aldrich (Chicago, IL). HPLC analytical column (Luna[®] Omega PS C18, 3 μ m, 100 Å, 100 × 4.6 mm, Part no. 00D-4758-E0) and guard column cartridge (SecurityGuard[™] PS C18, 4 × 3.0 mm, Part No. AJ0-7606) were obtained from Phenomenex (Torrance, CA). A PFAS delay column (Ascentis[®] Express 160 Å FAS Delay, 2.7 μ m, 50 × 4.6mm, Cat#53573-U) was purchased from Supelco[®], Millipore Sigma (Atlanta, GA). Solid phase extraction cartridge (Strata[™] X-AW 33 μ m polymeric weak anion; 60mg/3mL, Cat#8B-S038-UBL) were obtained from Phenomenex Inc (Torrance CA). Polypropylene LC-MS vials (SureSTART Polypropylene Microvials, Cat#6ESV9-1PP) were purchased from Fisher Scientific Company (Hanover Park, IL). A PFAS standard mixture (PFAC-MXF, -MXG, -MXH, -MXI, -MXJ), which contained sixteen PFAS standards quantified in this study [PFBuA, PFHxA, PFHxS, PFHpA, PFOA, PFOS, PFOSA, PFNA, PFDA, PFUdA, 6:2 FTS, Me-FOSAA, Et-FOSAA, L-PFPeS, PFDoA, and PFECHS] were purchased from Wellington Labs LLC (Wilmington, DE); undecafluoro-2-methyl-3-oxahexanoic Acid (GenX) was obtained from Synquest Laboratories (Alachua, FL). Isotopic-labelled PFAS stock solutions (MPFAC-HIF-ES and MPFAC-HIF-IS) were also purchased from Wellington Labs LLC (Wilmington, DE) and used as indicated in Table S2.

Table S1: List of PFAS Internal Standards associated with PFASs

#	PFAS Internal Standards	Abbreviation	Molecular Weight	Molecular Ion (Q1)	Product Ion (Q3)	Associated PFAS
1	Perfluoro-n-13C3-butanoic Acid	M3PFBA	217.01	216	172	PFBA, PFBS*, LPFPeS*
2	Perfluoro-n-(1,2,13C2)-hexanoic acid	M5PFHxA	319.02	318	273	PFHxA, PFHxS*, GenX*
3	Perfluoro-n-(1,2,3,4-13C4)-heptanoic acid	M4PFHpA	368.03	367	322	PFHpA, PFECHS*
4	Perfluoro-n-13C8-octanoic acid	M8PFOA	422	421	376	PFOA
5	Perfluoro-n-13C9-nonanoic acid	M9PFNA	520	519	474	PFNA
6	Perfluoro-1-(13C8)-octanesulfonamide	M8FOSA	507	506	78	PFOSA
7	Perfluoro-1-(1,2,3,4-13C4)-octanesulfonate	MPFOS	526.08	503	80	PFOS
8	Perfluoro-n-(1,2-13C2)-decanoic acid	MPFDA	516.07	515	470	PFDA
9	Perfluoro-n-13C7-undecanoic acid	M7PFUdA	571	570	525	PFUdA
10	Perfluoro-n-13C2-dodecanoic acid	MPFDoA	616	615	570	PFDoA
11	N-methyl-d3-perfluoro-octanesulfonamidoacetic acid	d3-MeFOSAA	574.22	573	419	Me-FOSAA
12	N-ethyl-d5-perfluoro-octanesulfonamidoacetic acid	d5-EtPFOSAA	590.27	589	419	Et-PFOSAA
13	Sodium 1H,1H,2H,2H-(13C2)-Perfluorooctanesulfonate	M2-6:2 FTS	552	529	81	6:2 FTS

*These PFAS do not have their own isotope labelled Internal Standard and a surrogate internal standard closest to the analyte retention time and molecular weight/structure was used as indicated in the table.

Table S2. Multiple Reactions Monitoring (MRM) of PFASs

#	List of PFAS	CAS#	Abb.	MW	Molecular Ion (Q1)	Product Ion (Q3)	DP	EP	CE	CXP
1	Perfluoro-n-butanoic Acid	375-22-4	PFBA	214.06	213	169	-32	-10	-13	-10
						101	-32	-10	-29	-16
2	Perfluorohexanoic acid	307-24-4	PFHxA	314.06	313	269	-35	-10	-13	-12
						119	-35	-10	-27	-10
3	Potassium perfluorohexanesulfonate	3871-99-6	PFHxS	438.2	399	98.9	-80	-10	-70	-5
4	Perfluoroheptanoic acid	375-85-9	PFHpA	364.06	363	168.6	-40	-10	-24	-14
5	Perfluorooctanoic acid	335-67-1	PFOA	414	413	168.6	-40	-10	-24	-14
6	Perfluorononanoic acid	375-95-1	PFNA	464.07	463	419	-40	-10	-15	-10
						169	-40	-10	-27	-8
7	Perfluoro-1-octanesulfonamide	754-91-6	PFOSA	499.1	498	80	-75	-10	-99	-10
						98.7	-75	-10	-72	-10
8	Perfluorooctanesulfonate	1763-23-1	PFOS	500	499	98.8	-80	-10	-60	-10
9	Perfluorodecanoic acid	335-76-2	PFDA	514.08	513	469	-45	-10	-15	-10
						269	-45	-10	-25	-10
10	Perfluoroundecanoic acid	2058-94-8	PFuDA	564.08	563	519	-40	-10	-15	-10
						319	-40	-10	-27	-10
11	Perfluorododecanoic acid	307-55-1	PFDoA	614.08	613	569	-45	-10	-17	-13
						293	-45	-10	-14	-10
12	N-methylperfluoro-octanesulfonamidoacetic acid	2355-31-9	Me-PFOSAA	571.2	570	419	-65	-10	-28	-9
13	N-ethylperfluoro-octanesulfonamidoacetic acid	2991-50-6	Et-PFOSAA	585.2	584	419	-70	-10	-29	-21
						526	-70	-10	-26	-11
14	Sodium 1H,1H,2H,2H-Perfluorooctanesulfonate	27619-94-9	6:2 FTS	450	427	407	-50	-10	-33	-15
						81	-50	-10	-67	-13
15	Sodium perfluoro-1-pentanesulfonate	630402-22-1	LPFPeS	372.08	349	80	-75	-10	-66	-7
						99	-75	-10	-48	-7
16	Potassium perfluoro-4ethylcyclohexanesulfonate	335-24-0	PFECHS	502.2	461	99	-60	-10	-54	-7
						381	-60	-10	-37	-15
17	Undecafluoro-2-methyl-3oxahexanoic Acid	13252-13-6	GenX	330.05	329.2	285	-25	-10	-8	-7
						168.8	-25	-10	-18	-8

Table S3: Limits of detection (LOD) for PFASs

Analyte	Lowest Detectable std concentration (ng/ml)*	S/N ratio	LOQ (ng/mL)	LOD (ng/ml)	R2
PFBA	0.025	5.5	0.023	0.014	0.9995
PFHxA	0.0125	4.8	0.013	0.008	0.9998
PFHxS	0.0125	5.2	0.012	0.007	0.9998
PFHpA	0.0125	5	0.013	0.008	0.9998
PFOA	0.0125	4.6	0.014	0.008	0.9995
PFNA	0.0125	3.9	0.016	0.010	0.9997
PFOSA	0.025	4.4	0.028	0.017	1
PFOS	0.025	6.5	0.019	0.012	1
PFDA	0.0125	5.0	0.012	0.007	0.9994
PFUdA	0.0125	3.3	0.019	0.011	0.9999
PFDoA	0.0125	4.2	0.015	0.009	0.9996
Me-FOSAA	0.05	3.5	0.071	0.043	0.9999
Et-FOSAA	0.125	5.5	0.114	0.068	1
6:2 FTS	0.01	4.1	0.012	0.007	0.9999
L-PFPeS	0.0125	4.5	0.014	0.008	0.9999
PFECHS	0.025	8.5	0.015	0.009	0.9998
GenX	0.025	5.2	0.024	0.014	0.9999

* 100 µl of serum was analyzed in a final total volume of 200 µL.

Table S4. Association between serum-liver function biomarkers and PFAS exposure (Unadjusted).

PFAS	LB ^a	GGT		AST		ALT	
	β ^b	p-value ^c	β	p-value	β	p-value	
PFBuA		0.25	0.04	-0.05	0.55	0.12	0.33
PFHxA		0.12	0.07	0.05	0.19	0.12	0.09
PFHpA		-0.02	0.57	0.01	0.80	0.02	0.69
PFOA		0.10	0.34	0.04	0.52	0.00	0.97
PFNA		0.07	0.25	-0.02	0.66	-0.05	0.41
PFDA		0.08	0.09	-0.03	0.31	-0.06	0.25
PFUdA		-0.01	0.77	-0.04	0.11	-0.08	0.03
PFHxS		-0.01	0.92	0.04	0.25	0.15	0.01
PFOS		0.01	0.93	-0.04	0.44	-0.09	0.31
GenX		0.17	0.06	0.03	0.55	0.07	0.49
PFOSA		-0.02	0.39	-0.02	0.12	-0.06	0.02
Total PFAS		0.07	0.48	-0.01	0.93	-0.04	0.68
NASEM PFASs ^d		0.05	0.66	-0.02	0.78	-0.04	0.76

a. LB: Liver injury biomarker

b. β = Change in U/L of serum liver biomarker per ln-ng PFAS increase in serum.

c. Multivariable linear regression

d. NASEM PFASs include PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFOS. Me-FOSAA concentrations were below the LOD.

Table S5. Associations between measures of serum-cholesterol and PFAS exposure (Unadjusted). β , mmol/ μ g PFAS in serum.

PFAS \ CB ^a	Total cholesterol		LDL		HDL	
	β^b	p-value ^c	β	p-value	β	p-value
PFBuA	-0.008	0.854	-0.007	0.891	-0.052	0.453
PFHxA	0.030	0.180	0.013	0.625	0.076	0.034
PFHpA	0.004	0.783	-0.004	0.800	0.057	0.007
PFOA	0.016	0.652	0.021	0.639	0.040	0.494
PFNA	0.028	0.162	0.027	0.272	0.048	0.140
PFDA	-0.005	0.790	-0.003	0.879	-0.008	0.772
PFUdA	0.009	0.505	0.020	0.226	-0.011	0.596
PFHxS	0.000	0.999	-0.010	0.706	0.036	0.291
PFOS	0.011	0.701	0.032	0.386	0.004	0.932
GenX	0.032	0.324	0.028	0.487	0.074	0.157
PFOSA	0.001	0.942	0.006	0.591	-0.005	0.721
Total PFAS	0.021	0.541	0.026	0.546	0.058	0.302
NASEM PFASs ^d	0.023	0.546	0.045	0.340	0.031	0.624

a. CB: Cholesterol biomarker

b. β = change in mmol of cholesterol biomarker per ln-ng PFAS increase in serum.

c. Multivariable linear regression

d. NASEM PFASs include PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFOS. Me-FOSAA concentrations were below the LOD.