Supplementary Material

Assessment of fetal exposure and maternal elimination to perfluoroalkyl substances

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Reagents and Chemicals. All reagents used for the analysis of poly- and perfluoroalkyl substances (PFASs) were of trace analysis grade. Methyl tert-butyl ether (MTBE) and methanol were purchased from Dima Technology Inc (Richmond Hill, ON, Canada). Sodium carbonate, sodium bicarbonate, ammonium acetate, ammonium hydroxide and tetrabutyl ammonium hydrogen sulfate (TBAHS) used as ion-pair reagent were obtained from Aldrich (St Louis, MO, USA).

PFASs were purchased from following: potassium salt of perfluorobutane sulfonate (PFBS), potassium salt of perfluorohexane sulfonate (PFHxS), potassium salt of perfluorooctane sulfonate (PFOS), and perfluorododecanoic acid (PFDoDA) were purchased from Aldrich (St Louis, MO, USA); perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA) and perfluoroundecanoic acid (PFUnDA) were purchased from Matrix Scientific (Columbia, SC, USA); Perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) were purchased from Fluorochem (Derbyshire, UK); perfluorooctanoic acid (PFOA) was purchased from Strem Chemicals (Bischheim, France). Two internal standards used in this study, including perfluoro-n-[$^{13}C_8$]octanoic acid (M8PFOA), sodium perfluoro-1-[1,2,3,4-$^{13}C_8$]octanesulfonate (MPFOS), were purchased from Wellington Laboratories (Guelph, ON, Canada). Purities of all standards were ≥ 95%.

Oasis WAX extraction cartridges (3cc, 60 mg) were purchased from Waters Company (Milford, MA, USA). The Envi-carbon particle was obtained from Supelco (Bellefonte, PA, USA).

Sample Preparation. Maternal blood (MB), cord blood (CB) and placenta: Prior to extraction, samples of whole blood and placenta were thawed and allowed to return to room temperature; and approximately 10 g fresh weight full thickness placenta samples from multiple sites were freeze-dried with a Telstar Laboratory freeze-dryer Cryodos-80 for 24 h, then dried placenta samples were homogenized, the moisture content of the placenta was recorded to enable reporting of PFAS concentrations on a fresh weight basis. Prior to freeze-drying samples, all placenta samples were washed by using Milli-Q water. Blood and homogenized placenta samples were extracted by ion-pairing method similar to that described by Hansen et al. with some modifications. One milliliter of blood sample (or 0.15 to 0.20 g dry weight homogenized placenta) was added to a 15 mL polypropylene (PP) tube, 5.0 ng of internal standards (MPFOS and M8PFOA, 50 μL, 0.10 ng μL^{-1}), 2.0 mL of 0.25 M sodium carbonate buffer and 1.0 mL of 0.5 M TBAHS (adjusted to pH 10) were added. After through mixing, the extraction was carried out by the addition of 5 mL of MTBE, and the mixture was shaken vigorously for 40 min. The organic layer were separated from the aqueous layer by centrifugation at 3800 (×g) for 5 min and then transferred into a new 15 mL PP-tube. The extraction procedure was repeated with 3 mL of MTBE; the mixture was shaken vigorously for 20 min and combined with the first fraction. The solvent was evaporated to near-dryness under a gentle stream of high-purity nitrogen and then reconstituted with 1.0 mL of methanol. After centrifugation at 3800 (×g) for 2 min, the solution were transferred into an

autosampler vial for UPLC-MS/MS (Waters Acquity ultra performance liquid chromatography equipped with Waters Acquity TQD triple quadrupole mass spectrometer) analysis.

Amniotic fluid (AF): A total of 20 to 50 mL of AF sample was transferred into a 50 mL PP-tube, and then spiked with 3 ng (30 μL, 0.10 ng μL^{-1}) of each internal standard (MPFOS and M8PFOA). The spiked samples were extracted using Oasis WAX extraction cartridges. Prior to loading of the samples, the WAX cartridges were conditioned with 4 mL of 0.1% ammonium hydroxide in methanol, and 4 mL of milli-Q water. The samples were loaded on the conditioned cartridge at a rate of 3 mL min^{-1}. The cartridges were then washed with 4 mL of 25 mM sodium acetate buffer (pH = 4). Then, the target fraction was eluted with 3 mL of 0.1% ammonium hydroxide in methanol with gravity drop. One hundred and fifty milligram of Envi-carbon particle was added into elutes. Then, elutes was performed by shaking the slurry for 10 min, and centrifugation at 3800 (×g) for 8 min. Finally, the extracted solutions were transferred into an autosampler vial for UPLC-MS/MS analysis.

Instrumental Analysis. Concentrations of 11 PFASs, PFBS, PFHxS, PFOS, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA were determined by using Waters Acquity UPLC-MS/MS. Aliquots of five microliters were injected into ACQUITY UPLC BEH C_{18} column (2.1 × 50 mm, Waters, Ireland) at a flow rate of 0.40 mL min^{-1}. The two mobile phases were methanol (A) and 2 mM ammonium acetate in milli-Q water (B), with an initial gradient of 25% A; it was hold

at 25% A for 0.5 min; the gradient increased to 85% A at 5 min; then continuously increased to 100% in 0.1 min; it was hold at 100% A for 1.9 min, and then reverted to 25% A at 9 min; the total running time is 9 min. The MS-MS was operated in electrospray negative ionization mode. The parameters were: Capillary voltage at –2.7 kV, source temperature at 150 °C for all target compounds; desolvation temperature at 350 °C; cone gas flow 20 L hr^{-1}; desolvation gas flow 700 L hr^{-1}. The MS/MS was operated in a multiple reaction monitoring (MRM) mode; the mass transitions monitored were: 299.1 > 80.0 for PFBS, 399.2 > 80.0 for PFHxS, 499.2 > 99.0 for PFOS, 263.2 > 219.1 for PFPeA, 313.2 > 269.1 for PFHxA, 363.2 > 319.1 for PFHpA, 413.2 > 369.1 for PFOA, 463.2 > 419.1 for PFNA, 513.2 > 469.1 for PFDA, 563.2 > 519.1 for PFUnDA, 613.2 > 569.1 for PFDoDA, 421.2 > 376.1 for M8PFOA, 503.2 > 80.0 for MPFOS. When possible, multiple daughter ions were monitored for confirmation, but quantitation was based on a single product ion.

Quality Control and Assurance. Recoveries of the PFASs (10 ng each) spiked into sample matrices ranged from 70 ± 8% to 141 ± 17% for placenta (n = 3) and from 84 ± 12% to 135 ± 1% for whole blood (n = 5). A few exceptions with low recovery were noted: perfluorobutane sulfonate (PFBS) in placenta and whole blood showed abnormal recovery (< 50%). As internal standards, sodium perfluoro-1-[1,2,3,4-$^{13}C_8$]octanesulfonate (MPFOS) and perfluoro-n-[$^{13}C_8$]octanoic acid (M8PFOA) were spiked (5.0 ng each) into all samples prior to extraction. Respective mean recoveries of MPFOS and M8PFOA were 81 ± 7% and 78 ± 6% for placenta

and 102 ± 7% and 98 ± 9% for whole blood (**Table S2**). Method precision was good, with relative standard deviations (RSDs) for each sample matrix were in the range of 1 to 17% for all PFASs (**Table S2**).

The recoveries of PFOS and PFOA were 55 ± 8% and 57 ± 9% (n = 3), respectively (**Table S2**), in AF. Although the recoveries of PFOS and PFOA were low for AF, corresponding internal standards (i.e., MPFOS and M8PFOA) were spiked (level: 0.10 ng mL^{-1} in AF; n = 29) into all samples before extraction. The mean recoveries of MPFOS and M8PFOA were 49 ± 4% and 53 ± 9%, respectively (**Table S2**). The concentrations of PFOS and PFOA in AF samples were corrected by the recoveries of the corresponding internal standard. Method precision was good for all PFASs in AF (RSDs < 10%) (**Table S2**). The limit of quantitation (LOQ) was determined as the lowest concentration of PFC in calibration curve which measured concentration of calibration standards were 70% to 130% of the theoretical concentrations. The LOQs of PFASs were 0.05 ng g^{-1} fresh weight (fw) for placenta, and 0.05 ng mL^{-1} for blood; the LOQs of PFASs in AF ranged from 0.01 to 0.03 ng mL^{-1} (**Table S2**).

Table S1 Characteristics of investigated pregnant women and their fetuses.

	N	range	mean	SD	median
Pregnant Women					
age (year)	27	21-39	30.9	3.44	30.0
predelivery weight (kg)	27	59-105	74.7	9.06	73.3
postdelivery weight (kg)	27	52-92	68.8	8.61	67.8
BMI [a] (kg m^{-2})	27	19.6-47.3	28.6	4.48	26.8
parity [b]	27	0-1	0.18	0.28	0
gestational age at delivery (wks)	27	35-47	40.3	2.3	39.0
Matched Newborn Babies					
BMI (kg m^{-2})	27	9.96-15.2	12.6	1.46	12.6
head circumference (cm)	27	31-35	33.3	1.05	34.0
chest circumference (cm)	27	30-35	32.8	1.04	33.0
sex	27		male: 13, female: 14		

[a] BMI = Body Mass Index, BMI were calculated based on predelivery weight; [b] 82% of pregnant women were primiparous women.

Table S2 The limit of quantitation (LOQ, ng/mL) and mean recoveries (%) of native standards and ^{13}C-labeled internal standards spiked into each category of samples

	Native Standards											Internal Standards	
	PFBS	**PFHxS**	**PFOS**	**PFPeA**	**PFHxA**	**PFHpA**	**PFOA**	**PFNA**	**PFDA**	**PFUnDA**	**PFDoDA**	**MPFOS**	**M8PFOA**
LOQ of PFASs in whole blood and placenta													
	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
LOQ of PFASs in amniotic fluid													
	NC [b]	NC	0.03	NC	NC	NC	0.01	NC	NC	NC	NC	0.03	0.01
Whole blood													
Mean	43	102	84	107	96	93	117	102	135	102	133	102	98
RSDs [a]	2	3	12	13	1	1	7	2	1	2	4	7	9
Placenta													
Mean	35	97	70	118	138	120	111	134	141	115	139	81	78
RSDs	1	17	8	17	17	8	10	11	17	12	16	5	6
Amniotic fluid													
Mean	NC	NC	55	NC	NC	NC	57	NC	NC	NC	NC	50	53
RSDs	NC	NC	8	NC	NC	NC	9	NC	NC	NC	NC	4	9

[a] RSDs = relative standard deviations; [b] NC = not calculated.