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# Perfluorinated alkyl substances in Canadian human milk as part of the Maternal-Infant Research on Environmental Chemicals (MIREC) study

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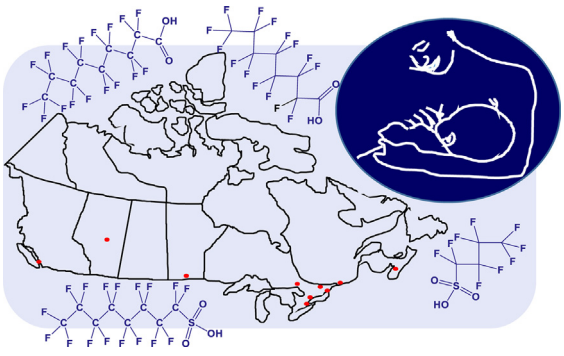
## HIGHLIGHTS

- PFAS were detected in Canadian human milk.
- PFOA and L-PFOS had the greatest contribution to total PFAS measured.
- Primiparous women had higher PFAS concentrations in milk compared to multiparous women.
- No regional differences in PFAS patterns were observed.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Perfluorinated alkyl substances (PFAS) were determined in human milk samples ($n$ = 664) from participants in the Maternal-Infant Research on Environmental Chemicals (MIREC) study. ΣPFAS concentrations (sum of seven PFAS) ranged from 3.1 ng $L^{-1}$ to 603 ng $L^{-1}$, with a median concentration of 106 ng $L^{-1}$ in the Canadian mothers' milk analyzed. These data comprise the first pan-Canadian dataset of PFAS in human milk. Perfluorooctanoic acid (PFOA) and linear perfluorooctanesulfonate (L-PFOS) were the dominant contributors to ΣPFAS in human milk samples. An inverse relationship between ΣPFAS concentrations and age was observed (Spearman correlation - 0.184). Primiparous women had elevated PFAS concentrations in milk relative to women who had children previously ($p < 0.001$). In contrast, the region of maternal birth did not influence ΣPFAS concentrations ($p$ = 0.156). Although China and Norway have observed consistently detectable levels of perfluoroundecanoic acid (PFUdA) in human milk, PFAS with long carbon chains ($n \geq 11$) were not present above method detection limits in Canadian human milk samples analyzed as part of the MIREC study. In conclusion, despite the presence of low levels of environmental contaminants in human milk, Health Canada supports breastfeeding due to the benefits to both infants and mothers.

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## 1. Introduction

A very important consideration of infant mortality and morbidity is whether they have been breastfed, particularly in developing countries (Green et al., 2021; Sánchez et al., 2021). The benefits to the child include the provision of essential nutrients for example, minerals, proteins, lipids and some vitamins (Azad et al., 2021). Human milk also contains prebiotics and chemicals beneficial in microorganism growth and activity promotion. Breastfeeding transfers these compounds to the infant with greater efficiency than milk pumped from the mother (Layuk et al., 2021). Ultimately, breastfed infants are reported to have lower incidence of appendicitis, retinopathy, and certain cancers, while cardiovascular health benefits, lower risk of obesity and childhood infections also have been reported in the literature (Davis et al., 1988; Lapillonne et al., 2021; Pisacane et al., 1995; Sánchez et al., 2021; Vafa et al., 2016; Zhou et al., 2015). Improved cognitive abilities, school attendance and higher income as adults also have been aligned with having been breastfed as infants (Lapillonne et al., 2021; WHO, 2021). In addition to providing essential nutrients for infant development, breastfeeding is thought to provide health benefits to the mother and improve relationships between infants and their mothers (Azad et al., 2021; Lapillonne et al., 2021; Layuk et al., 2021). Due to the benefits observed in breastfed infants, the WHO continues to recommend exclusively breastfeeding infants for the first six months of life (WHO, 2021).

Despite the benefits of breastfeeding, potential exposure to undesirable chemicals is possible through transfer via human milk. Partitioning of lipophilic compounds into the fatty tissues in humans has resulted in much research related to legacy persistent organic pollutants (POPs). The organohalogen compounds (e.g., polychlorinated biphenyls, polychlorinated dioxins/furans, organochlorine insecticides) have been reported in human milk globally for decades and have been determined in Canada since the late 1960s (Fång et al., 2015; Parera et al., 2013; Rawn et al., 2017; Ryan and Rawn, 2014). Infant exposure to PFAS has been strongly associated with immunosuppression, ultimately resulting in a negative impact on infant response to administered vaccines (e.g., measles) (von Holst et al., 2021). In addition, PFAS concentrations have been inversely associated with lipid content in maternal milk which can impact infant growth (Lamichhane et al., 2021).

Perfluoroalkyl substances (PFAS) persist in the environment, bioaccumulate in biological tissues and are subject to long-range transport (Garcia-Barrios et al., 2021; Lin et al., 2022). Toxicity concerns identified with exposure to these chemicals has resulted in a number of individual PFAS being identified as POPs under the Stockholm Convention (UN Environment Programme, 2019). Unlike most POPs, PFAS have both hydrophilic and hydrophobic ends and do not accumulate in the lipids, but rather bind to proteins and are found in protein rich tissues (e.g., blood, liver and kidney) (Ali et al., 2021; Barbarossa et al., 2013; Bjermo et al., 2013; Domingo, 2012). Over the last decade, a number of human milk studies to determine PFAS have been undertaken in European countries including Belgium, France, Italy and Spain (Barbarossa et al., 2013; Cariou et al., 2015; Guerranti et al., 2013; Llorca et al., 2010; Motas Guzmàn et al., 2016; Roosens et al., 2010). In addition, PFAS levels have been investigated in human milk collected from women in multiple Chinese provinces (Liu et al., 2010; So et al., 2006). More recently, PFAS have been reported in human milk from South African women (Macheka et al., 2021). A study also reported PFAS, including perfluorophosphate esters, in human milk collected from women living in a single city in Ontario, Canada (Kubwabo et al., 2013). Numerous investigations into human milk concentrations have focused on the most well characterized PFAS: perfluorooctanesulfonate (L-PFOS) and perfluorooctanoic acid (PFOA) (Barbarossa et al., 2013; Guerranti et al., 2013; Roosens et al., 2010) while other studies have included additional perfluorocarboxylic acids (e.g., perfluorononanoic acid [PFNA]) and sulfonates (e.g., perfluorohexanesulfonate [PFHxS]) (Cariou et al., 2015; Liu et al., 2010; Motas Guzmàn et al., 2016; Zheng et al., 2021).

The present dataset, collected under the MIREC study, represents the first pan-Canadian investigation into PFAS concentrations in human milk. An additional aim was to identify maternal characteristics associated with elevated levels of PFAS (Table 1).

## 2. Materials and methods

### 2.1. Study population and sampling

During the planning phase of the study, sampling from across Canada was a priority so that a pan-Canadian picture could be developed in terms of maternal/infant exposure to environmental chemicals. Recruiting women during early pregnancy and maintaining clinical contact with them throughout pregnancy was critical for success of the study and, therefore, potential research centres were identified only if clinical obstetrical research frameworks already existed. Prior to qualifying to be a research centre, each site was required to obtain research ethics board approval from Health Canada, the MIREC coordination centre in Centre hospitalier Universitaire Ste. Justine, Québec and the board of the individual research centre. Written consent from each participant was obtained prior to their participation in the study. Once established, the recruitment of women for the MIREC study occurred during prenatal clinics at each research centre selected (Arbuckle et al., 2013). MIREC research centres were established in six provinces and were generally in southern Canada, with Edmonton, Alberta (53.5° latitude) being the most northern community included in the study. Half of the MIREC research centres were located in the most populous province, Ontario (Hamilton, Kingston, Ottawa, Sudbury and Toronto) and represented a variety of population densities and cultural backgrounds. Other centres selected were in Vancouver, British Columbia; Winnipeg, Manitoba; Montréal, Québec and Halifax, Nova Scotia. Participants from each city were volunteers and were not a representative random sample of the population.

To be eligible to participate in the study, women were required to be 18 years or older, <14 weeks gestation and able to communicate in either English or French (Arbuckle et al., 2013). Approximately 59% of approached individuals were eligible to participate while 39% of those eligible agreed to be participants, resulting in a total of 2001 women who enrolled in this study. The participants completed lifestyle and demographic questionnaires during each trimester.

Between 2008 and 2011, pregnant Canadian women were recruited to participate in the pan-Canadian Maternal-Infant Research on Environmental Chemicals (MIREC) study. During the course of this study, maternal exposure to a large suite of chemicals (e.g., legacy POPs, bisphenol A, phthalates, trace elements) was determined through analysis of blood and urine samples collected during each trimester (Arbuckle et al., 2013; Arbuckle et al., 2014; Arbuckle et al., 2016; Fisher et al., 2016). In addition, human milk was collected from the women between two and 10 weeks post-delivery (Arbuckle et al., 2013). Broad spectrum investigations of this type allow researchers the opportunity to develop and examine exposure relationships and consider toxicological outcomes of such exposure in real world situations. This expands the impact of the work for exposure studies beyond investigations focused on a single compound or class of compounds.

Of the 2001 women recruited to participate in the MIREC study, approximately half ($n$ = 1017) provided milk samples. During the sample collection phase, data from the Canadian Community Health Survey (CCHS) (Statistics Canada, 2013) was used to develop a sampling frame for milk among analytical laboratories focused on different chemicals. This was done to ensure that sample distribution among laboratories included sampling from all collection centres in as representative a manner as possible.

Estimates of the number of participants expected to continue breastfeeding beyond two weeks from the date of babies being born were included in the distribution framework. Additional factors used for determining how the samples were distributed included parity, maternal age and the number of samples expected to be measured for each chemical/class of chemical (Table 1). Recruitment and sample collection occurred over four years between 2008 and 2011, which resulted in sample preparation and analyses being done at periodic intervals throughout, and

**Table 1**
Descriptive statistics for participants contributing human milk analyzed for PFAS ($n$ = 664).

| Characteristic | Summary statistics | | | |
|---|---|---|---|---|
| Age | Range: 19–48 years (mean = 32.5) | | | |
| Parity | Range: 1–5 | | | |
| Age group | <30 years | | ≥30 years | |
| Parity | Primiparous | Multiparous | Primiparous | Multiparous |
| Maternal region of birth | | | | |
| Canada | 104 | 59 | 154 | 243 |
| Other | 13 | 10 | 25 | 56 |
| Total | 117 | 69 | 179 | 299 |

extending beyond the collection period. Six hundred and sixty-four (664) samples were identified for PFAS analysis with samples from each of the 10 study centres represented.

Study participants were requested to hand express both fore- and hindmilk; however, if they had trouble in expressing the milk, breast pumps were provided. If a woman required a pump, a new pump was shipped to her, to ensure no cross contamination occurred. Milk samples were collected between two and 10 weeks postpartum. They were collected in pre-cleaned 500 mL wide mouth polypropylene containers. Mothers could express the milk over a period of time and retain the sample in the refrigerator (4 °C) if collected over a number of days. If sample collection took longer, the milk was retained in participants' freezers (−20 °C). Following collection, samples were shipped frozen to the study coordination centre and subsequently transferred to the designated laboratory where an aliquot of each sample for PFAS analysis was transferred to a polypropylene container prior to initiation of sample preparation.

### 2.2. Extraction and clean up

Individual samples of milk were weighed (2 g) into polypropylene tubes and surrogate standards: $^{18}O_2$ perfluoro-1-hexanesulfonate (L-PFHxS), $^{13}C_8$ PFOA, $^{13}C_4$ L-PFOS, $^{13}C_5$ PFNA (Wellington Laboratories, Guelph, ON, Canada) were added to each tube, tubes were vortexed for 30 s and allowed to sit for 30 min at 4 °C. Aqueous formic acid (1% v/v) (2 mL) (Fluka grade, Fisher Scientific, Ottawa, ON, Canada) was added to each sample, vortexed for 30 s and placed in an ultrasonic water bath for 15 min. Following sonication, acetonitrile (5 mL) (Optima LC/MS grade, Fisher Scientific) was added to each sample and sample tubes were vortexed for a further 30 s and then centrifuged at 10 °C for 12 min at approximately 4000 rpm. The supernatant was transferred to a clean polypropylene tube. A secondary extraction was performed by adding 1 mL acetonitrile: Milli-Q water (minimum resistivity 16 MΩ) (60:40 v/v) to the original sample tube, the tube was vortexed, then sonicated for 5 min followed by centrifugation for 5 min at approximately 4000 rpm (10 °C). The supernatant from the second extraction was added to the first and stored overnight at 4 °C. Samples were removed from the refrigerator, shaken manually 2–3 times and centrifuged 10–15 min at 10 °C at approximately 4000 rpm. The supernatant was transferred to clean polypropylene tubes with 2 mL Milli-Q water.

Clean up was performed using 6 cc, 150 mg Oasis solid phase extraction – weak anion exchange (SPE-WAX) cartridges (Waters Corporation, Mississauga, ON, Canada). Cartridges were installed on a manifold and rinsed with 10 mL 0.1% ammonium hydroxide (ACS grade) (Sigma Aldrich, Oakville, ON, Canada) in methanol (Optima LC/MS grade, Fisher Scientific) followed by 12 mL Milli-Q water and the eluent was discarded. If the samples were cloudy, they were filtered using pre-cleaned 2 μm glass fibre filters before they were added to the top of the cartridges. Following addition to the column, extracts were eluted by gravity, then cartridges were rinsed first with 3 mL 20 mM ammonium acetate (ACS grade) (Sigma Aldrich) in methanol: Milli-Q water (20:80, v/v) at pH 4.0, followed by 6 mL methanol: water (50:50, v/v) and finally 2 mL methanol. Each cartridge was then dried under vacuum for 1 min and the eluent was discarded. Analytes were eluted using 6 mL 2% ammonium hydroxide in methanol by gravity and collected into culture tubes (13 × 100 mm) with 120 μL methanol: ethylene glycol (1:3, v/v) where ethylene glycol was present as a keeper. Samples were then evaporated to near dryness using a gentle stream of nitrogen in a dry block held at 45 °C. Sample extracts were reconstituted in methanol to a final volume of 180 μL and sonicated for 5 min. An aliquot of each extract was placed in a microcentrifuge tube to which the performance standard containing $^{13}C_4$ PFOA, $^{13}C_2$ perfluorodecanoic acid (PFDA) and $^{13}C_8$ L-PFOS (Wellington Laboratories) was added and Milli-Q water was used to dilute the final extract prior to analysis. If particles were observed, samples were centrifuged immediately before they were added to polypropylene inserts in polypropylene chromatography vials.

### 2.3. Analysis

Analyses were performed using an ultra performance liquid chromatograph (UPLC) coupled to a triple quadrupole mass spectrometer (MS/MS). Both the Acquity UPLC coupled to a Quattro Premier XE (Waters) and a Vantage LC-MS/MS (ThermoScientific, St-Laurent, Québec) were utilized for these analyses with an online Oasis 2.5 μm, 2.1 × 20 mm (Waters) and an Acquity BEH C18, 1.7 μm, 2.1 × 100 mm analytical column (Waters) (Table 2). Samples were injected in the partial loop with needle overfill mode with either the 20 or 25 μL loops using between 2 and 12 μL of the sample extract. The analyses were performed following the method described by Tittlemier et al. (Tittlemier et al., 2007) with 5 mM ammonium formate in Milli-Q water and 2:1 (v/v) acetonitrile: methanol as mobile phases A and B, respectively. The capillary voltage was set to −3 kV and source temperature was 140 °C. Nitrogen was both the nebulizer and drying gas, with flows of 20 and 400 L/h, respectively. Argon was used as the collision gas ($2.0 \times 10^{-3}$ mbar).

### 2.4. Quality assurance/quality control

Prior to commencing the work on the MIREC study samples, the laboratory participated in a proficiency test study (PT-PFC Human milk 2009/2010 MTM Research Center, Örebro University, Örebro, Sweden) established to determine PFAS in human milk and an analytical standard. Of the 17 compounds considered in the study, it was determined that interlaboratory comparison of five compounds (perfluoro-*n*-heptanoic acid [PFHpA], PFOA, PFNA, L-PFHxS and L-PFOS) was possible. The present laboratory had results close to the mean values (i.e., less than one standard deviation from the mean) for all analytes in the standard solution and in the 'S' pooled milk sample, however, the PFNA result determined in the 'G' milk pool was one standard deviation from the mean reported value. Proficiency z-scores ranged from zero (PFNA in standard; L-PFOS in 'S' pool) to 1.07 (PFNA in 'G' pool).

With each set of MIREC samples prepared for analysis, two method blank samples (2 mL Milli-Q water) were included for quality control purposes. PFAS levels in method blank samples were below method detection limits with each set analyzed or the set was repeated. Additionally, in-house quality control samples were included with sets of samples and results were tracked for ΣPFOS (L-PFOS + PFOS – branched isomers [br-PFOS], PFOA, PFNA and PFHxS). With the exception of one result for each of PFOA, PFNA and PFHxS, all concentrations were within two standard deviations of the mean concentrations. Additionally, method blank samples (20 ng $L^{-1}$, 40 ng $L^{-1}$ or 60 ng $L^{-1}$), cow milk (200 ng $L^{-1}$) and human milk (200 ng $L^{-1}$) were fortified with perfluorobutanesulfonate (PFBS), PFHxS, PFOA, L-PFOS, PFNA and perfluorohexanoic acid (PFHxA) and included in sample sets. Recoveries in spiked blanks ranged from 95.9% to 107%, L-PFOS and PFNA, respectively. Recoveries from cow milk were between 97.5% (PFHxS) and 105% (PFHxA). Slightly lower recoveries of the six PFAS originally used in fortification testing were obtained from fortified human milk samples (85.1%–102%, PFBS and PFHxA), although PFDoA had the highest average recovery from fortified human milk (108%) (Table 2). The average surrogate recovery was 99%, ranging from 91% for $^{13}C_8$ PFOA to 106% for $^{13}C_4$ L-PFOS.

**Table 2**
LC-MS/MS instrumental parameters for analytes with recovery information determined using fortified samples.

| Analyte | Approximate Retention Time (min) | Transitions monitored | | LOD (ng $L^{-1}$) | Analyte Recovery (%) from fortified samples | |
|---|---|---|---|---|---|---|
| | | | | | Cow milk | Human milk |
| PFBS | 3.6 | 299 > 79.8 | 299 > 98.8 | 5 | 98.2 ± 20.8 | 85.1 ± 16.8 |
| PFHxA | 4.6 | 313 > 218.9 | 313 > 118.9 | 10 | 105 ± 24.1 | 102 ± 16.6 |
| PFHxS | 6.1 | 398.8 > 79.8 | 398.8 > 98.8 | 5 | 97.5 ± 14.0 | 92.9 ± 8.81 |
| PFHpA | 6.1 | 362.8 > 318.8 | 362.8 > 168.8 | 5 | – | 96.3 ± 7.70 |
| PFHpS | 7.7 | 448.8 > 79.8 | 448.8 > 98.8 | 5 | – | 87.8 ± 13.0 |
| PFOA | 7.7 | 412.8 > 368.8 | 412.8 > 168.8 | 5 | 103 ± 12.6 | 95.1 ± 11.7 |
| L-PFOS | 9.6 | 498.8 > 79.8 | 498.8 > 98.8 | 10 (5 br-PFOS) | 99.8 ± 20.0 | 95.5 ± 15.5 |
| PFNA | 9.5 | 462.8 > 418.8 | 462.8 > 218.8 | 5 | 99.8 ± 13.5 | 91.9 ± 11.5 |
| PFDA | 11.3 | 512.8 > 468.8 | 512.8 > 268.8 | 5 | – | 98.4 ± 6.10 |
| PFUdA | 13.5 | 562.8 > 518.8 | 562.8 > 268.8 | 7 | – | 93.4 ± 8.77 |
| PFDS | 13.6 | 598.8 > 79.8 | 598.8 > 98.8 | 5 | – | 83.0 ± 10.3 |
| PFDoA | 14.9 | 612.8 > 568.8 | 612.8 > 168.8 | 5 | – | 108 ± 14.4 |
| PFTeDA | 16.7 | 712.8 > 668.8 | 712.8 > 168.8 | 5 | – | 97.4 ± 25.8 |

Although no human milk was available with certified levels of PFAS, serum samples with certified PFAS concentrations were purchased for analysis. The serum was extracted in triplicate a few times throughout the study. Results obtained for PFHxS, PFOA, PFNA were within the certified range each time the serum was tested. ΣPFOS (L-PFOS + br-PFOS isomers) concentrations were over-estimated (111%) during one period of analysis and within the certified range the rest of the time.

Limits of detection (LOD) were established based on 3:1 signal to background noise and were determined with each set of samples. Variability in LOD was observed, depending on the cleanliness of the system. The overall limits of detection were determined to range from 5 ng $L^{-1}$ (PFBS, PFHxS, PFHpA, perfluoroheptanesulfonate [PFHpS], PFOA, br-PFOS, PFNA, PFDA, perfluorodecanesulfonate [PFDS], perfluorododecanoic acid [PFDoA], perfluorotetradecanoic acid [PFTeDA]) to 10 ng $L^{-1}$ (PFHxA, L-PFOS), while the LOD for perfluoroundecanoic acid [PFUdA] was determined to be 7 ng $L^{-1}$.

Originally, the suite of PFAS included 13 compounds: PFBS, PFHxS, PFHpA, PFHpS, PFOA, L-PFOS, br-PFOS, PFNA, PFDA, PFDS, PFUdA, PFDoA and PFTeDA. PFHxA was added to the list of analytes after approximately 90 samples had been analyzed. Following the summary of results for the samples analyzed in the first year ($n$ = 114), it was noted that PFBS, PFHpA, PFHpS, PFDA, PFDS, PFUdA, PFDoA and PFTeDA were either not detected or found at concentrations within the range of LOD reported for individual sets. Based on these results and to help alleviate lab pressures, the analyte list was initially reduced to PFHxS, PFOA, L-PFOS, br-PFOS, PFNA and PFHxA. A subset of the samples, however, was analyzed for all analytes the following year, to establish whether any of the PFAS removed from the regular suite were present at detectable concentrations. PFBS was detected in a sample and, as a result, it was re-established in the routine list of analytes, resulting in analysis of $n$ = 553 samples (83%) for this compound.

### 2.5. Statistical analysis

SigmaPlot 12.5 (Systat Software Inc.) was used to perform statistical analyses. Descriptive statistics were performed for all seven PFAS, although histograms were not created for those analytes having detection frequency <1% (PFBS and PFHxA). Concentrations <LOD were set to 1/2 LOD to examine differences between groups (age, parity, maternal region of birth). Relationships were considered statistically significant if the $p$-value was less than 0.05. Spearman correlations were developed to examine the relationship between individual PFAS and ΣPFAS concentrations in human milk versus maternal age. Separate one-way analysis of variance (ANOVA) tests were performed to examine ΣPFAS concentrations observed in human milk as impacted by the region of maternal birth and age. A one-way ANOVA was also performed to examine the relationship between ΣPFAS concentrations and maternal parity. The PFAS data were not normally distributed, therefore, a Kruskal-Wallis ANOVA on ranks was performed followed by Dunn's method. In addition, a two-way ANOVA was performed to allow for a simultaneous comparison of factors (i.e., maternal age and parity).

## 3. Results

Each of the seven compounds that were analyzed throughout the study was detected in at least one sample. Consistent with other reports of PFAS in human milk, PFOA was the most frequently detected analyte (99.5%) with L-PFOS being observed in 95.9% of the samples analyzed. Branched-PFOS isomers were less frequently detected (87.7%), followed by PFHxS (62.5%) and PFNA (61.0%) in the MIREC samples. The detection frequency of PFBS and PFHxA was very low (0.9% and 0.7%, respectively). The relative contribution of each PFAS to ΣPFAS concentrations was relatively stable across Canada (Fig. 1) and PFOA generally contributed the most (~30%) to ΣPFAS concentrations in the human milk measured.

Although PFOA was most frequently detected compound, PFNA was found at the highest concentration (maximum = 396 ng $L^{-1}$) in a single sample (Fig. 2). The maximum concentrations of the other compounds observed in the Canadian human milk samples decreased in the order PFOA (284 ng $L^{-1}$) > PFHxS (167 ng $L^{-1}$) > L-PFOS (142 ng $L^{-1}$) > br-PFOS (132 ng $L^{-1}$) > PFHxA (83.7 ng $L^{-1}$) > PFBS (42.0 ng $L^{-1}$) (Table 3). The measures of central tendency show the predominance of PFOA and L-PFOS in the human milk samples analyzed. The limited number of samples with detectable concentrations of PFBS ($n$ = 5) resulted in the median positive PFBS concentration (35.0 ng $L^{-1}$) exceeding the median concentration of PFOA (34.2 ng $L^{-1}$) in the present study, although when values below the LOD were considered as 1/2 LOD, the median concentration

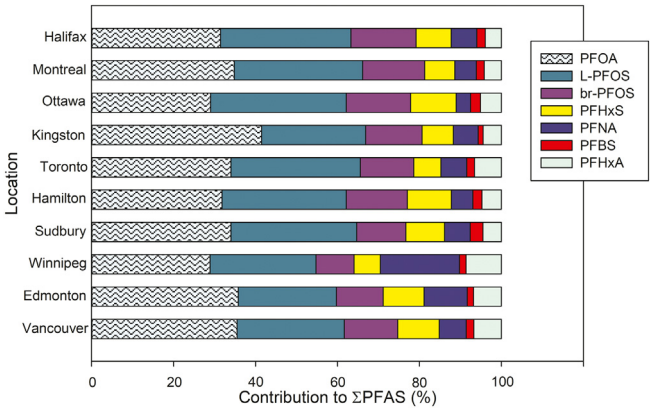


**Fig. 1.** Contribution of individual PFAS to ΣPFAS concentrations (%) in human milk sampled across Canada. ΣPFAS represent Σ of seven individual PFAS reported (PFOA, L-PFOS, br-PFOS, PFHxS, PFNA, PFBS and PFHxA).

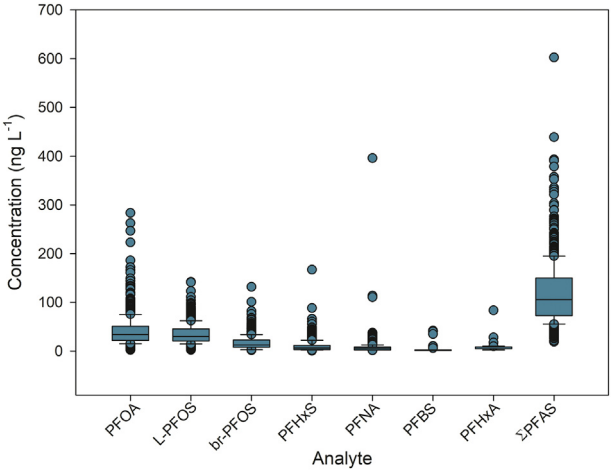


**Fig. 2.** PFAS concentrations (ng $L^{-1}$) in Canadian human milk. (Box indicates 25th, 50th and 75th percentiles. Points indicate data outside of 10th (⊥) or 90th (⊤) percentiles).

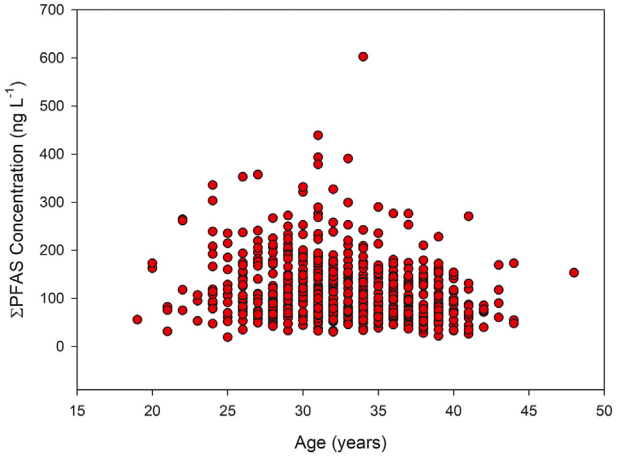


**Fig. 3.** Relationship between ΣPFAS concentrations (ng $L^{-1}$) in human milk and the age (years) of participants.

was reduced to 1/2 the LOD (2.5 ng $L^{-1}$). In the vast majority of samples with compounds present at detectable levels, the impact of how results below the LOD were treated was very minor. In contrast, those PFAS with few samples having PFAS at detectable levels (PFBS and PFHxA), the choice of treatment of data for those below the level of detection, had an exaggerated influence on the measures of central tendency (Table 3).

PFOA and L-PFOS had the greatest impact on ΣPFAS concentration distribution (see Supplementary information Fig. S1). The sample density profile for L-PFOS was distributed more broadly among the range in concentrations observed which is somewhat different from what was observed for PFOA and br-PFOS isomers. Few samples were found to have PFHxS and PFNA at concentrations above 20 ng $L^{-1}$. Ultimately, the histograms were right skewed with few samples exhibiting mid-range concentrations, and only sporadic samples with concentrations in the upper range. The greatest degree of variation in sample concentrations was observed in the PFOS and PFOA histograms.

A weak inverse relationship between maternal age and ΣPFAS concentration was observed in the present work (Spearman correlation $\rho = -0.184$). Despite a statistically significant relationship (ANOVA $p$-value $= 0.018$), the pattern was not visually apparent (Fig. 3). A statistically significant relationship, however, was established between concentrations in human milk and maternal parity ($p < 0.001$) (Fig. 4). Primiparous women

**Table 3**
PFAS concentrations (ng $L^{-1}$) in Canadian human milk samples ($n = 664$).

| Compound | LOD | Detection frequency (%) | Range | Treatment of LOD | Mean | Standard deviation | Geometric mean | Median |
|---|---|---|---|---|---|---|---|---|
| PFOA | 5 | 99.5 | <LOD – 284 | >LOD only | 41.4 | 30.7 | 33.8 | 34.2 |
| | | | | LOD = 0 | 41.3 | 30.8 | – | 34.1 |
| | | | | LOD = 1/2 LOD | 41.3 | 30.7 | 33.5 | 34.1 |
| | | | | LOD = LOD | 41.3 | 30.7 | 33.6 | 34.1 |
| L-PFOS | 10 | 95.9 | <LOD – 142 | >LOD only | 35.7 | 20.4 | 30.6 | 30.2 |
| | | | | LOD = 0 | 35.5 | 20.5 | – | 30.0 |
| | | | | LOD = 1/2 LOD | 35.5 | 20.5 | 30.1 | 30.0 |
| | | | | LOD = LOD | 35.6 | 20.5 | 30.3 | 30.0 |
| br-PFOS isomers | 5 | 87.7 | <LOD – 132 | >LOD only | 18.5 | 13.8 | 14.9 | 14.5 |
| | | | | LOD = 0 | 16.8 | 14.2 | – | 13.0 |
| | | | | LOD = 1/2 LOD | 17.1 | 13.9 | 12.6 | 13.0 |
| | | | | LOD = LOD | 17.0 | 13.7 | 13.5 | 13.0 |
| PFHxS | 5 | 62.5 | <LOD – 167 | >LOD only | 13.0 | 12.9 | 10.0 | 9.30 |
| | | | | LOD = 0 | 9.37 | 12.4 | – | 6.70 |
| | | | | LOD = 1/2 LOD | 10.1 | 11.9 | 6.80 | 6.70 |
| | | | | LOD = LOD | 10.9 | 11.5 | 8.25 | 7.20 |
| PFNA | 5 | 61.0 | <LOD – 396 | >LOD only | 10.4 | 20.3 | 8.20 | 7.60 |
| | | | | LOD = 0 | 6.96 | 17.3 | – | 6.08 |
| | | | | LOD = 1/2 LOD | 7.83 | 17.0 | 5.60 | 6.10 |
| | | | | LOD = LOD | 8.69 | 16.8 | 7.05 | 6.10 |
| PFBS[a] | 5 | 0.9 | <LOD – 42.0 | >LOD only | 26.8 | 17.0 | 20.8 | 35.0 |
| | | | | LOD = 0 | 0.242 | 2.92 | – | 0.00 |
| | | | | LOD = 1/2 LOD | 2.34 | 2.83 | 2.08 | 2.50 |
| | | | | LOD = LOD | 4.43 | 2.92 | 4.13 | 5.00 |
| PFHxA[b] | 10 | 0.7 | <LOD – 83.7 | >LOD only | 34.8 | 33.4 | 25.4 | 22.8 |
| | | | | LOD = 0 | 0.241 | 3.76 | – | 0.00 |
| | | | | LOD = 1/2 LOD | 6.21 | 4.21 | 5.56 | 5.00 |
| | | | | LOD = LOD | 12.2 | 5.86 | 11.1 | 10.0 |
| ΣPFAS[c] | | 100 | 3.1 – 603 | >LOD only | 110 | 66.3 | 93.4 | 95.6 |
| | | | | LOD = 0 | 110 | 66.3 | 93.3 | 95.6 |
| | | | | LOD = 1/2 LOD | 119 | 65.3 | 105 | 106 |
| | | | | LOD = LOD | 128 | 64.6 | 115 | 112 |

[a] n = 553.
[b] n = 578.
[c] ΣPFAS is the sum of the seven individual PFAS reported.

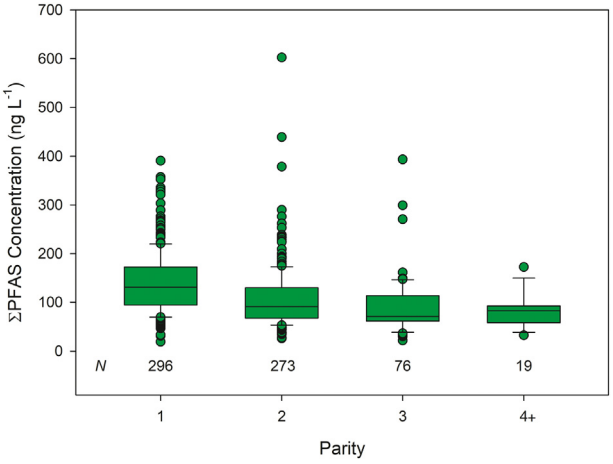


**Fig. 4.** ΣPFAS concentration (ng $L^{-1}$) in human milk according to maternal parity. (Box indicates 25th, 50th and 75th percentiles. Points indicate data outside of 10th (⊥) or 90th (⊤) percentiles).

had higher ΣPFAS milk concentrations than multiparous women (ANOVA $p < 0.001$); similarly, milk from women who had one child before participating in the MIREC study had higher ΣPFAS milk concentrations than women who had two children prior to the MIREC study (Mann-Whitney rank sum test $p = 0.003$). The decrease in PFAS concentrations observed in milk from women who had previously had a child relative to primiparous women may be a function of maternal transfer to the children born before the MIREC cohort, which could occur via breastfeeding or through transfer to the fetus during pregnancy. The interaction between maternal age and parity was not statistically significant (Two Way ANOVA $p = 0.894$). No relationship was found between maternal region of birth and ΣPFAS concentrations (ANOVA $p = 0.156$) (supplementary information Fig. S2).

## 4. Discussion

The broad usage of PFAS in consumer products, coupled with their persistence in the environment and bioaccumulation potential, is consistent with the detection of these compounds in human tissues. Chemical transfer from mother to infant via human milk has been reported in the literature for many years, particularly for the traditional persistent organic pollutants (Ryan and Rawn, 2014).

Consistent with the observations reported in the literature, PFOA contributed the most to ΣPFAS concentrations in Canadian human milk that was collected as part of the MIREC study. Median ΣPFAS concentrations observed in the present study (106 ng $L^{-1}$) are similar to concentrations recently reported in the US (121 ng $L^{-1}$) (Zheng et al., 2021), although the US study was undertaken in 2019 and included a greater number of PFAS. Similar to Canadian human milk, the PFAS with ≥8 carbons contributed more to US ΣPFAS concentrations in human milk than those PFAS having shorter carbon chains. Median L-PFOS and PFOA concentrations in the present study (30.0 ng $L^{-1}$ and 34.1 ng $L^{-1}$, respectively) are similar to those reported in the US (30.4 ng $L^{-1}$ and 13.9 ng $L^{-1}$, respectively) (Zheng et al., 2021). Maximum PFOS and PFOA concentrations reported in human milk from Spain (865 ng $L^{-1}$ and 907 ng $L^{-1}$, respectively) (Llorca et al., 2010) were higher than the maximum concentrations observed in Canadian milk collected as part of the MIREC study between 2008 and 2011 (ΣL-PFOS + br-PFOS isomers 273 ng $L^{-1}$, PFOA 284 ng $L^{-1}$). Maximum PFOS concentrations reported in German (309 ng $L^{-1}$) and Hungarian (639 ng $L^{-1}$) human milk (Völkel et al., 2008) also exceeded maximum concentrations in the Canadian samples. PFOA detection frequency was low (16%) in the German study, however, positive reporting was based on concentrations above the limit of quantification (200 ng $L^{-1}$) (Völkel et al., 2008) and PFOA was present in concentrations above 200 ng $L^{-1}$ in >1% of the samples in the present study. In other work, human milk collected from Germany had similar PFOS concentrations (<30 ng $L^{-1}$–110 ng $L^{-1}$; median 40 ng $L^{-1}$) to those observed in the present study (L-PFOS <10 ng $L^{-1}$–142 ng $L^{-1}$; median 30.2 ng $L^{-1}$) (Fromme et al., 2010). PFOA was detected infrequently (2%) in the German samples which contrasts with the Canadian human milk where PFOA was observed in >99% of the samples although this difference in detectability appears to be a function of the elevated limit of detection in the earlier work (Fromme et al., 2010). Average concentrations (232 ng $L^{-1}$ and 77.7 ng $L^{-1}$, PFOS and PFOA, respectively) reported in human milk from Japan in 1999 (Tao et al., 2008) were elevated over the concentrations reported in Canada collected in 2008–2011 (L-PFOS 35.5 ng $L^{-1}$, PFOA 41.3 ng $L^{-1}$). Mean PFOS concentrations (46.1 ng $L^{-1}$) in human milk collected in India; however, were similar to those observed in the present study (35.7 ng $L^{-1}$ L-PFOS, 18.5 ng $L^{-1}$ br-PFOS), although the frequency of detection was lower in the Indian milk (85%; LOD = 11 ng $L^{-1}$) (Tao et al., 2008) than in the Canadian samples (95.9%; LOD = 10 ng $L^{-1}$).

In addition to concentration variability between countries, detection rates also differed among studies. While PFUdA was either not detected or observed at concentrations similar to the detection limit in the current work, it was frequently observed in human milk from China (Liu et al., 2010; So et al., 2006). PFDA was similarly observed frequently in Chinese human milk (Liu et al., 2010; So et al., 2006); however, it was not detected in any of the Canadian samples analyzed during the first year of the study and was subsequently removed from the analysis. Consistent with the results from China, the PFAS profile in human milk from Norway included the detection of PFUdA (Thomsen et al., 2010). The presence of longer chain PFAS (e.g., PFUdA, PFDoA) in human milk recently collected from South African women (Macheka et al., 2021) has been reported similar to recent results reported for human milk collected in the US (Zheng et al., 2021), which contrasts with the observations in the Canadian work. Differences in PFAS concentrations were observed between regions in China (Liu et al., 2010). The individual compound contribution to ΣPFAS concentrations in the Canadian human milk did not differ by region, where similar profiles were observed regardless of collection site (Fig. 1).

The Government of Canada, in partnership with industry, has been working to reduce the long chain PFAS since 2006 (Government of Canada, 2018). As part of this effort, industry agreed to reduce long chain PFAS content in perfluorochemical products marketed in Canada. The timeframe of this effort is consistent with the lower frequency of detection of the longer chain PFAS in the Canadian human milk from this study between 2008 and 2011.

The inverse relationship established between maternal age and ΣPFAS concentrations in human milk was consistent with the relationships developed for each of the five most frequently detected PFAS in the present study. Individual PFAS concentrations were weakly correlated with age (see supplementary information Table S1). In addition, an examination of maternal age based on separate age groups (i.e., <26 years, 26–30 years, 31–35 years, 36–40 years and >40 years) was possible using the data from this study. A statistically significant difference ($p < 0.001$) was obtained following the completion of an ANOVA to examine ΣPFAS concentrations by age group. The age group with the highest median ΣPFAS concentrations was 26–30 year olds (115 ng $L^{-1}$), followed by decreasing concentrations in each of the following age groups (31–35 years [107 ng $L^{-1}$]; 36–40 years [95.8 ng $L^{-1}$] and >40 years [74.3 ng $L^{-1}$]). This pattern also was observed for L-PFOS and PFOA individually. These results contrast with the observations of the lipophilic polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/Fs) and polychlorinated biphenyls (PCBs) which were found to increase in human milk corresponding to an increase in maternal age (Rawn et al., 2017).

Parity was associated with decreased ΣPFAS concentrations in the human milk measured as part of this study. Prior breastfeeding has been associated with lower PFAS concentrations by some authors, however, in some studies reduced PFAS concentrations were not significantly different in human milk from primiparous, relative to multiparous women (Tao et al., 2008; Kim et al., 2011; Brantsæter et al., 2013; Papadopoulou et al., 2015; Lamichhane et al., 2021). The median ΣPFAS concentration

determined in the milk of primiparous women was elevated and statistically different from the results obtained for multiparous women (Fig. 4). ΣPFAS concentrations, however, were not statistically different among milk from the multiparous women (i.e., women having 2, 3 or 4+ children). A statistically significant interaction between maternal age and parity was not observed.

Despite PFAS exposure occurring over time via multiple pathways, including water and food consumption, dust ingestion, inhalation and dermal exposure, the median PFAS concentrations observed in the milk from women belonging to the 31–35, 36–40 and >40 year age groups were lower than concentrations in the 26–30 year age group. This observation may be related to previous pregnancies in women >30 years of age and breastfeeding of other infants. In addition to breastfeeding, women having had prior pregnancies can result in the lowering of maternal PFAS concentrations (Brantsæter et al., 2013; Papadopoulou et al., 2015). In addition, the length of time a woman breastfeeds an infant results in decreased PFAS concentrations in human milk (Thomsen et al., 2010). Although no statistically significant interaction between age and parity was found in the present study, the relative number of primiparous women (63%) in the 26–30 year age group was greater than each of the older age groups. Relatively fewer women from each of the older age groups were primiparous (37%, 32%, 33%, 31–35, 36–40 and >40 years, respectively), which may have contributed to this observation in the present study.

## 5. Conclusion

Similar to global observations, PFOA and L-PFOS were the predominant contributors to ΣPFAS concentrations in Canadian human milk samples collected as part of the MIREC investigation. Analysis of PFHpA, PFHpS, PFDA, PFDS, PFUdA, PFDoA and PFTeDA was limited to the first year of the study as they were not present at concentrations above detection limits in >100 samples tested. Each of the seven PFAS consistently analyzed throughout the four years of the study (PFOA, L-PFOS, br-PFOS, PFHxS, PFNA, PFBS and PFHxA) were detected in at least one sample. The relative contribution of these seven compounds to ΣPFAS levels was consistent across Canada with no clear regional differences observed among the Canadian sampling sites. ΣPFAS concentrations in milk from primiparous women were significantly higher than in milk from multiparous women ($p < 0.001$). Maternal region of birth did not impact ΣPFAS concentrations, although age was inversely but weakly correlated with the ΣPFAS concentrations in milk (Spearman correlation −0.184) from Canadian women.

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## CRediT authorship contribution statement

Dorothea F.K. Rawn: Funding acquisition; Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Validation; Writing – original draft; Writing – review & editing: Guy Dufresne: Methodology; Investigation; Validation: Geneviève Clément: Supervision; Validation; Writing – Review: William D. Fraser: Conceptualization; Writing – review: Tye E. Arbuckle: Funding acquisition; Conceptualization; Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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