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Novel halogenated flame retardants in Canadian human milk from the MIREC study (2008–2011)

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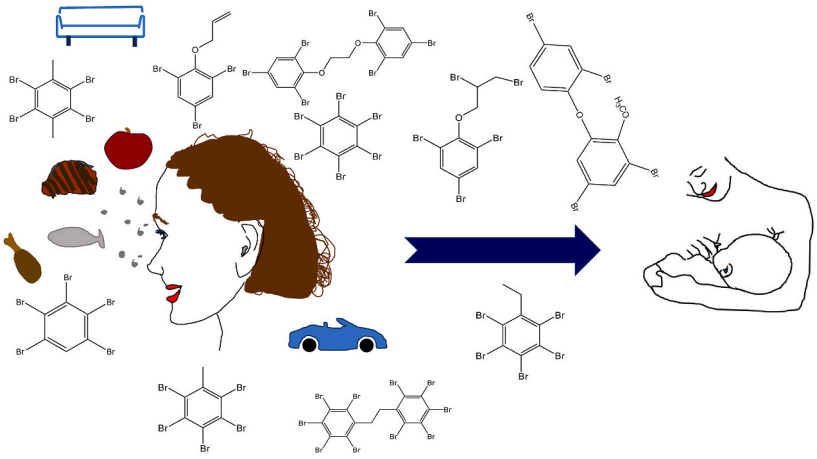
HIGHLIGHTS

- First pan-Canadian study of NHFRs and methoxy-PBDE metabolites in human milk.
- Nine of the 15 measured NHFRs were detected with a frequency more than 9%.
- Two of eight methoxy/MeO-PBDEs measured were observed in the human milk.
- Maximum ΣNHFR/ΣMeO-PBDE concentrations were 6930 pg g^{-1} lipid/ 1600 pg g^{-1} lipid.
- NHFR concentrations correlated with one another in the milk.

GRAPHICAL ABSTRACT



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ABSTRACT

Novel halogenated flame retardants (NHFRs) have been developed to replace those brominated flame retardants that have been restricted due to their persistence, bioaccumulation potential and toxicity, therefore, it is important to determine whether these replacement products are present at detectable concentrations in Canadians. NHFRs were measured in human milk samples (n = 541) collected from across Canada between 2008 and 2011, which is the first pan-Canadian dataset for these chemicals in human milk. Among the 15 measured NHFRs and eight methoxy-polybrominated diphenyl ethers (MeO-PBDEs), nine NHFRs and two MeO-PBDEs (6-MeO-

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Human milk
Pan-Canadian study

PBDE 47 and 2-MeO-PBDE 68) were detected at a frequency of more than 9%. Despite benzene, 1,1′-(1,2-ethanediyl)bis [2,3,4,5,6-pentabromo-]/decabromodiphenylethane [DBDPE] being detected less frequently than the other observed NHFRs, its relative contribution to the sum of nine NHFRs was important when it was present. The maximum ΣNHFR concentration in Canadian human milk was 6930 pg g^{-1} lipid while the maximum ΣMeO-PBDEs was 1600 pg g^{-1} lipid. While most NHFR concentrations were significantly correlated with each other, no relationships between maternal age, parity or pre-pregnancy BMI were identified with ΣNHFR concentrations in the milk. In contrast, maternal age was significantly correlated with ΣMeO-PBDE concentrations ($r = 0.237$, $p < 0.001$). ΣNHFR concentrations were similarly not related to maternal education, although ΣMeO-PBDE concentrations were found to be higher in milk from women who had graduated from trade schools relative to the other education levels considered. NHFR detection frequency and concentrations observed in the Canadian human milk seem to align well with Europe.

1. Introduction

Owing to their physicochemical characteristics, the established flame retardants, polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), have been subject to regulatory action restricting their manufacture and use in Canada and globally (Covaci et al., 2011; Government of Canada, 2022; Government of Canada, 2023). Both of these products have been listed on Annex A of the Stockholm Convention (United Nations Environment Programme, 2019). There is, however, an ongoing need for flame retardant chemicals to ensure that consumer products meet fire safety standards. This has resulted in the development of additional chemicals to replace PBDEs and HBCD. While some halogenated flame retardants beyond PBDEs and HBCD were produced in the 1970s and 1980s (e.g., benzene, 1,2,3,4,5 pentabromo-6-ethyl/pentabromoethylbenzene [PBEB]) (Covaci et al., 2011), benzene, 1,1′-(1,2-ethanediyl)bis [2,3,4,5,6-pentabromo-]/decabromodiphenylethane [DBDPE] production commenced in the early 1990s (Shi et al., 2009). BFR use has drastically increased since that time (Bergman et al., 2012) corresponding to the reduction in use of the legacy BFRs. Many of the replacement halogenated compounds are brominated and are frequently referred to as novel or emerging brominated flame retardants (NBFRs) (e.g., benzene, 1,1′-[1,2-ethanediylbis (oxy)] bis[2,4,6-tribromo-/1,2-bis(2,4,6-tribromophenoxy)ethane [BTBPE], benzene, 1,2,3,4,5,6-hexabromo/hexabromobenzene [HBB], 2,4,6-tribromophenyl allyl ether [TBP-AE/ATE], 1H-Indene, 4,5,6, 7-tetrabromo-2,3-dihydro-1,1,3-trimethyl-3-(2,3,4,5-tetrabromophenyl)- [OBTMPI/OBIND]) (Bergman et al., 2012; Covaci et al., 2011; Demirtepe et al., 2019; Dong et al., 2021; Shi et al., 2016; Tao et al., 2019; Zhou et al., 2014). A more general grouping of novel halogenated flame retardants (NHFRs) includes the chlorine substituted (e.g., dechlorane plus) and those containing both bromine and chlorine (e.g., 1,4-methanobenzocyclooctene,7,8-dibromo-1,2,3,4,11,11-hexachloro-1,4,4a,5,6,7,8,9,10,10a-decahydro/hexachlorocyclopentadienyl-dibromocyclooctane [DBHCTD/HCDBCO]). A paucity of data exist for numerous NHFRs (Falandysz et al., 2022) which may be explained in part by the fact that the NHFRs are structurally diverse and do not belong to a single class of structurally related compounds.

The widespread usage of NHFRs in consumer products has resulted in their global presence in both abiotic compartments (Demirtepe et al., 2019; He et al., 2021) and biota (Chen et al., 2022; Covaci et al., 2011; Marler et al., 2022). Elevated levels of NHFRs are observed in regions with informal electronic waste handling (Ma et al., 2021) and levels have increased following regulations restricting PBDE manufacture and use (Lee et al., 2022). Despite the use of NHFRs earlier than the 2000s, measurement of these compounds in environmental compartments and biota became of greater interest on a global scale beyond the mid-2000s. Prior to this, much of the focus remained on the determination of PBDEs and HBCD, corresponding with governments considering action to reduce exposure to these BFRs (Kemmlein et al., 2003). Some of the early environmental NHFR reporting was based on work in regions of high industrial activity including electronic manufacturing, where BTBPE and DBDPE were observed in sediment, soil, air and sewage sludge (Shi et al., 2009). PBEB was observed in seals and right whales, while HBB and benzene, 1,2,3,4,5-pentabromo-6-methyl/pentabromotoluene (PBT) were only detected in right whales from eastern US and Canada (Montie et al., 2010). In addition to wildlife (Covaci et al., 2011; Zhang et al., 2022), NHFRs have been measured in food (Sun et al., 2019; Zacs et al., 2021; Zuiderveen et al., 2020) with relatively few data reported in humans (He et al., 2021; Shi et al., 2016; Yamaguchi et al., 1988).

Individual NHFR toxicity studies, despite being somewhat limited in number, have been performed in various organisms with numerous studies being focused on the same product (e.g., DBDPE) owing to its widespread usage, while a lack of toxicity information remains for other NHFRs. In general, NHFRs are not considered to be acutely toxic to organisms (Dong et al., 2021), although there is concern related to their impacts on endocrine function (Liu et al., 2022), similar to PBDEs. DBDPE exposure has been reported to result in oxidative stress in freshwater fish (Feng et al., 2013) and while the impacts observed for DBDPE exposure were lower than those noted for fish treated with PBDE 209, when fish were exposed to both DBDPE and PBDE 209, an additive effect was observed. Exposure to DBDPE has also been reported to reduce hatching rates in zebrafish (Nakari and Huhtala, 2010), however, this observation was not consistent among researchers (Jin et al., 2018). Mitochondrial dysfunction was observed in mice exposed to DBDPE (Shi et al., 2021), while HBB exposure to zebrafish embryos resulted in oxidative stress (Usenko et al., 2016). Curran and coworkers (Curran et al., 2017) observed sex specific changes resulting from 1, 2-dibromo-4-(1,2-dibromoethyl)cyclohexane/ tetrabromoethylcyclohexane (DBE-DBCH/ TBECH) exposure, with thyroid hormones T3 and T4 both increasing in the serum of female rats, but not in males.

Maternal transfer of lipophilic chemicals to their young via human milk is well established in the literature (Čechová et al., 2017; Pratt et al., 2013; Rawn et al., 2017; Ryan and Rawn, 2014; Schreder et al., 2023). Human milk is often the sole food for infants and very young children. Recognizing that NHFR exposure has been increasing globally, women of childbearing age have been and will continue to be exposed to these chemicals via multiple pathways throughout their lives. The Maternal-Infant Research on Environmental Chemicals (MIREC) study was designed to allow for the simultaneous investigation of multiple classes of environmental chemicals in pregnant women across Canada between 2008 and 2011. In addition to the legacy contaminants (e.g., polychlorinated dioxins/furans [PCDD/Fs], polychlorinated biphenyls [PCBs]), perfluoroalkyl substances (PFAS), mirex and dechlorane plus have been reported in Canadian human milk (Rawn et al., 2017; Rawn et al., 2022; Rawn et al., 2023). Sample collection from across Canada allowed researchers to examine whether relative contributions of individual PFAS were consistent across the country (Rawn et al., 2022). Given the paucity of biomonitoring data, NHFRS were analyzed in human milk samples collected as part of this study using a method developed for cow milk (Rawn et al., 2016).

2. Materials and methods

2.1. Study population and sampling

Obstetric clinics with existing research frameworks were the focus

for participant recruitment centre development. Prior to being established as a research centre, each clinic required research ethics board (REB) approval for the study from the clinic's ethics board as well as those of Health Canada and the coordination centre for the MIREC study (Centre hospitalier Universitaire [CHU] Sainte-Justine, Québec). Participants for the study were recruited while women were participating in prenatal clinics (Arbuckle et al., 2013). Using this approach facilitated the connection with participants and follow-up throughout the pregnancy during each trimester visit to the clinic. Prior to participating in the MIREC study, all participants provided written consent. Due to the voluntary nature of the study, participants do not represent a random sample of the population (Table 1).

The MIREC study spanned 10 cities from six provinces (British Columbia, Alberta, Manitoba, Ontario, Québec and Nova Scotia). Half of the urban centres were in Ontario, with each city representing different population densities/cultural backgrounds (Rawn et al., 2022). To be eligible for participation in the study, women had to be 18 years of age or older, less than 14 gestational weeks and have the capacity to communicate in either English or French (Arbuckle et al., 2013). Approximately 59% of the women approached were eligible, with 39% of the eligible women agreeing to participate in the study. The recruitment was performed between 2008 and 2011, resulting in 2001 women being enrolled in the study. Questionnaires about the women's lifestyle, demographic status and health (e.g., pre-pregnancy body mass index [BMI], age and parity) were administered to each participant during their clinic visits. Among the MIREC study participants, 1017 provided human milk samples. Milk collection was performed by participants between two and 10 weeks postpartum (Arbuckle et al., 2013).

2.2. Sample distribution

Given that the study was designed to examine a broad range of environmental chemicals, all analyte classes could not be examined in every sample due to limited sample volumes and laboratory capacities. This resulted in the need for a sample distribution framework to ensure the samples were distributed among laboratories for each of the different chemical classes so that samples from all regions were analyzed for all analytes in a representative manner. The human milk distribution framework was developed using the Canadian Community Health Survey (CCHS) (Statistics Canada, 2013). Multiple factors were considered when the distribution plan was developed; including estimates in terms of the number of participants that would breastfeed their children beyond two weeks of the baby's birth, maternal parity, age and geographical region of the country. The sampling framework also considered the number of samples to be analyzed for each analyte/class of analyte. In addition to the NHFRs and MeO-PBDEs (Fig. 1), the MIREC study resulted in the analysis of a variety of chemical contaminants in the human milk samples collected, including: PCDD/Fs, PCBs, PFAS, organochlorine insecticides, PBDEs, HBCD, phthalates, trace elements, ochratoxin A, monochloropropanediol fatty acid esters and perchlorate (Rawn et al., 2017; Rawn et al., 2022; Rawn et al., 2023; Becalski et al., 2018; Wang et al., 2019; Cao et al., 2021).

Participants were asked to hand express their milk, however, if they experienced difficulty a manual pump was supplied. Multiple samples from both breasts were collected over several days and samples for NHFR analysis were collected in glass containers.

Table 1
Participant summary information corresponding to NHFR and MeO-PBDE analysis in MIREC study human milk samples.

Characteristic	Summary Statistics			
n = 541				
Age Range:	20–44 years; mean 32.6			
Parity	1–5			
Pre-pregnancy BMI Range:	16.6–48.6 kg m^{-2}; mean 24.6			
Age (years)	<30 years		≥30 years	
Parity	Primiparous	Multiparous	Primiparous	Multiparous
Participant Country of Birth				
Canada	88	44	129	198
Other	11	5	25	41
Total	99 (18%)	49 (9%)	154 (29%)	239 (44%)

2.3. Analytes of interest

Fifteen NHFRs were selected for measurement in human milk to align with information shared in the published literature and accessibility to analytical standards. NHFRs with corresponding acronyms used in the manuscript are listed in Table 2.

In addition, 2',3,4',5-tetrabromo-2-methoxydiphenyl ether or 2-MeO-PBDE 68, originally purchased as 2,3',4,5'-tetrabromo-2-methoxydiphenyl ether (TBMBPE) from LGC Standards (Teddington, Middlesex, UK) was included in the suite of analytes. All samples analyzed for NHFRs and MeO-PBDEs were prepared for analysis within the same

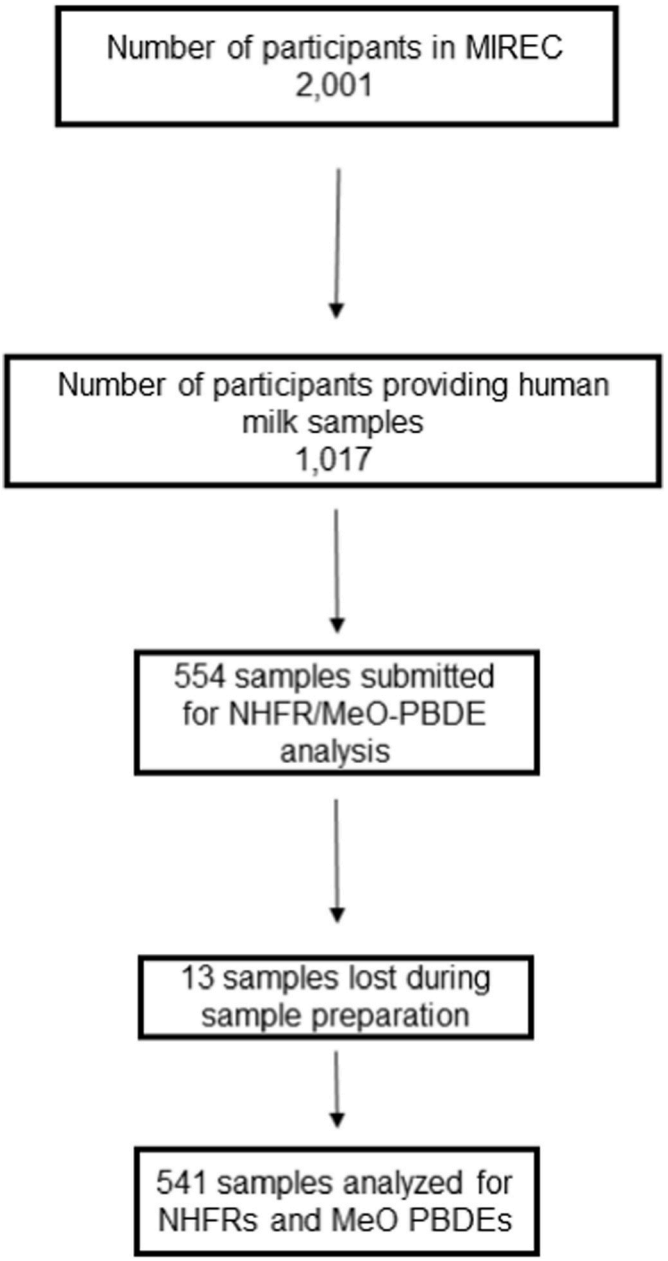

Fig. 1. Study population with samples used for NHFR analysis.

Table 2
List of analytes with corresponding acronyms.

Chemical	Acronym	CAS Number
2,4,6-tribromophenyl allyl ether	TBP-AE/ATE	3278-89-5
2,3,5,6-tetrabromo-*p*-xylene	TBX	23488-38-2
1,2,3,4,5-pentabromobenzene	PBBZ	608-90-2
benzene, 1,2,3,4,5-pentabromo-6-methyl/pentabromotoluene	PBT	87-83-2
benzene, 1,2,3,4,5 pentabromo-6-ethyl/pentabromoethylbenzene	PBEB	85-22-3
benzene, 1,3,5-tribromo-2-(2,3-dibromopropoxy)/2,3-dibromopropyl 2,4,6-tribromophenyl ether	TBP-DBPE/DPTE	35109-60-5
benzene, 1,2,3,4,5,6-hexabromo/hexabromobenzene	HBB	87-82-1
benzene, 1,1′-[1,2-ethanediylbis(oxy)] bis[2,4,6-tribromo-/1,2-bis(2,4,6-tribromophenoxy)ethane	BTBPE	37853-59-1
benzene, 1,1′-(1,2-ethanediyl)bis [2,3,4,5,6-pentabromo-]/decabromodiphenylethane	DBDPE	84852-53-9
1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane/tetrabromoethylcyclohexane	DBE-DBCH/TBECH [α-,β-,γ-, δ-isomers]	3322-93-8
2-bromoallyl 2,4,6-tribromophenyl ether	BATE	99717-56-3
β-1,2,5,6-tetrabromocyclooctane	β-TBCO	3194-57-8
benzene, 1,2,3,4,5-pentabromo-6-(bromomethyl)-/1-bromomethyl-2,3,4,5,6-pentabromobenzene/pentabromobenzylbromide	PBBB	38521-51-6
1H-Indene, 4,5,6,7-tetrabromo-2,3-dihydro-1,1,3-trimethyl-3-(2,3,4,5-tetrabromophenyl)-	OBTMPI/OBIND	1084889-51-9
1,4-methanobenzocyclooctene,7,8-dibromo-1,2,3,4,11,11-hexachloro-1,4,4a,5,6,7,8,9,10,10a-decahydro/ hexachlorocyclopentadienyl- dibromocyclooctane	DBHCTD/HCDBCO	51936-55-1

Health Canada laboratory (Ottawa, ON).

During the early work on this study, an additional unexpected peak was observed in some of the extracts and investigated. The presence of 2,2',4,4'-tetrabromo-6-methoxydiphenyl ether (6-MeO-PBDE 47) was identified in human milk extracts based on high resolution mass spectrometric confirmatory analyses. As a result, additional methoxy-PBDEs (2,2',4,4'-tetrabromo-5-methoxydiphenyl ether [5-MeO-PBDE 47], 2,2',4',5-tetrabromo-4-methoxydiphenyl ether [4-MeO-PBDE 49], 2,2',4,4',5-pentabromo-5'-methoxydiphenyl ether [5-MeO-PBDE 99], 2,2',4,4',6'-pentabromo-5-methoxydiphenyl ether [5-MeO-PBDE 100], 2,2',4,5,5'-pentabromo-4'-methoxydiphenyl ether [4-MeO-PBDE 101] and 2,2',4',5,6'-pentabromo-4-methoxydiphenyl ether [4-MeO-PBDE 103]) were added to the list of analytes for the remainder of the study (n = 484 samples).

2.4. Sample extraction and clean up

The method developed for analysis of NHFRs in cow milk, described previously (Rawn et al., 2016) was expanded to the analysis of NHFRs in human milk for the present study. In brief, 10 g of human milk was added to 50 mL polypropylene centrifuge tubes and surrogate standards (^{13}C analogues of PBBZ, HBB, BTBPE and DBDPE) purchased from Wellington Laboratories (Guelph, ON), were added to each sample. After 30 min, 1 mL hydrochloric acid (HCl) (ACS grade, J.T. Baker, Fisher Scientific, Ottawa, ON) was added and each sample was sonicated for 15 min, after which the samples remained on the bench for a further 15 min. Prior to homogenization with an Omni Tissue Homogenizer (1 min), 10 mL purified deionized water (minimum resistivity 18.2 MΩ) and 20 mL acetone: hexane (2:1, v/v) (Omnisolv, EMD Science, Mississauga, ON), were added. Samples were capped and further mixed on a Roto-torque variable speed rotator (Cole-Parmer, Montréal, QC) for 10 min. After mixing, the samples were centrifuged (10 min) at 12,857×*g* in an Eppendorf centrifuge 5810R (Eppendorf, Mississauga, ON), set at 10 °C. The supernatant was then transferred to a 15 mL graduated glass centrifuge tube. The original sample was re-extracted twice; using 10 mL acetone: hexane (2:1) and 8 mL acetone: hexane (2:1), respectively. Each of the supernatants was combined with those from the previous extractions. Sample extracts were concentrated to a volume of 2.5 mL in a water bath (~40 °C) using a gentle stream of nitrogen and diluted to 5 mL with dichloromethane (Omnisolv) and mixed on a vortex mixer. Gravimetric lipid determination was performed by transferring 0.5 mL of each extract to pre-weighed aluminum dishes. The dishes were then placed in a fume hood overnight to passively evaporate prior to weighing the samples the next day to determine lipid content.

Gel permeation chromatography (Gilson GX-271, Middleton, WI, USA) using 35 g of S-X3 biobeads (200–400 mesh) (Bio-Rad) having a mobile phase of 1:1 dichloromethane: hexane (v/v) at a 5 mL min^{-1} flow rate was used as the initial clean up step. Sample extracts were then concentrated to between 5 and 10 mL with rotary evaporation. Additional clean up was performed using 3% water deactivated Florisil (EMD 60–100 mesh; Fisher Scientific, Ottawa, ON) (8 g) and a small layer of anhydrous sodium sulfate on the top and bottom of each column. NHFRs were eluted using 1:1 dichloromethane: hexane (50 mL), followed by rotary evaporation to ~1 mL and cleaned up extracts were then transferred to a graduated centrifuge tube (2 mL) containing 50 μL toluene and concentrated to approximately 40 μL with a gentle stream of nitrogen in a heated water bath (40 °C).

Prior to injection, ^{13}C polybrominated biphenyl (PBB) 52, ^{13}C PBB 153 and ^{13}C PBB 194 from Wellington Laboratories (Guelph, ON, Canada) were added to the sample extracts as performance standards. Samples were diluted to 50 μL using toluene and transferred to glass autosampler vials (amber) with a glass insert, capped and stored at −80 °C until ready for analysis. Despite 554 samples being allocated for NHFR analysis, 13 samples were spilled during sample preparation, therefore, results for 541 samples were obtained.

2.5. Analysis

Analysis was performed using an Agilent 7890A gas chromatograph (Agilent Technologies, Mississauga, ON) coupled to a Waters AutoSpec Premier high resolution mass spectrometer (Waters Corporation, Milford, Massachusetts, USA) operating at 10,000 resolution. A 15 m DB-5MS column (0.25 mm i.d. x 0.10 μm film thickness) coupled to a 5 m, 0.53 mm i.d. deactivated fused silica retention gap (Agilent Technologies) was used for separation of analytes. Initially, the oven was operating at 80 °C and held for 2 min at that temperature, followed by an increase to 170 °C at 20 °C min^{-1} and held for 5.5 min, with a final increase to 320 °C at a rate of 25 °C min^{-1} and held for an additional 10 min. A cool on column injector was used for the analyses, helium was the carrier gas set at a constant flow of 1.2 mL min^{-1}. The mass spectrometer was operated in EI positive ion mode at 36 eV, the trap current was set to 600 μA and the source temperature was 250 °C. One μL of each extract was injected.

The quantification and qualifying ions established for each compound in the analytical standards were used for confirmation of NHFR presence in human milk extracts (Supplementary Table 1). Analyte detection was confirmed based on relative retention time (±0.1 min) and response ratio of two characteristic ions matching with analytical standards (±20%).

2.6. Quality assurance/quality control

A reagent blank was included with each set of samples and prepared alongside the unknown samples, to allow for correction of NHFR results for any laboratory background levels. During the period over which the samples were prepared and analyzed, reference materials with certified concentrations of the NHFRs were not available. Cow milk known to be free of the analytes, fortified with NHFRs and MeO-PBDEs, was included in every second set of samples. Fortification concentrations varied by analyte with MeO-PBDEs at a concentration of 0.025 ng g^{-1} except 2-MeO-PBDE 68 which was added at 0.25 ng g^{-1}; PBT, TBP-AE, BATE, PBBZ, TBX, PBEB, TBP-DBPE and HBB were fortified at 0.0625 ng g^{-1}; PBBB, BTBPE and DBHCTD at 0.125 ng g^{-1}; and the DBE-DBCH isomers, β-TBCO and OBTMPI were all fortified at 0.625 ng g^{-1}, while DBDPE was added at 0.3125 ng g^{-1}(sample weight). Average recoveries of analytes from the fortified cow milk ranged from 87.1% to 144%, PBT and OBTMPI respectively. For those sets without the fortified cow milk, a human milk sample internal to the laboratory and available for use as a quality control sample was included in the set. Only TBP-AE and BTBPE were consistently found at concentrations above the limit of detection in the human milk sample, with mean concentrations of 25.4 pg g^{-1} lipid and 18.6 pg g^{-1} lipid, respectively. Concentrations of both TBP-AE and BTBPE were generally within two standard deviations of the mean value. The mean recovery of surrogate standards from the human milk samples tested in the MIREC study ranged from 24.3% (^{13}C DBDPE) to 130% (^{13}C BTBPE). NHFR concentrations were corrected for recovery in all samples.

2.7. Limits of detection

Analyte limits of detection (LOD), were established for each sample individually, using a 3:1 signal to background noise ratio, to account for variability in instrument sensitivity and sample sizes. Average MDLs ranged from 0.047 pg g^{-1} sample; 1.28 pg g^{-1} lipid (PBT) to 33.3 pg g^{-1} sample; 759 pg g^{-1} lipid (γ-, δ-DBE-DBCH), respectively. All results are reported as lipid adjusted concentrations.

2.8. Statistical analysis

Statistical analysis was performed for those analytes observed in the samples at a frequency of detection >5%, using SigmaPlot 12.5 (Systat Software Inc.). Analyte concentrations below the LOD were set to ½ LOD (i.e., LOD/2) for data summary and analysis. Data were examined considering the different participant personal characteristics (e.g., age, parity, pre-pregnancy BMI) in an attempt to determine whether there was any relationship between these parameters and the chemical concentration. In addition, the data were examined to establish whether correlations existed between the different NHFRs in Canadian human milk. The data were not normally distributed, therefore, one-way analysis of variance (ANOVA) tests were performed using Kruskal-Wallis ANOVA on ranks. Relationships were considered statistically significant if the p-value was less than 0.05.

3. Results

Lipid content was determined for each of the samples analyzed in the present investigation. Lipid content ranged from 0.78% to 7.9%. The mean and median lipid content were 3.36% and 3.25% in the milk samples measured, respectively.

Of the 15 NHFRs analyzed in this study, DBE-DBCH (α-, β-, γ-, δ-), BATE, β-TBCO, PBBB, DBHCTD and OBTMPI were detected in five or fewer (i.e., <1%) human milk samples tested. The other NHFRs were detected in human milk at concentrations above the detection limit in 9.4% (DBDPE) to 64% (PBBZ) of the samples tested. 2-MeO-PBDE 68 was detected in 80% of the samples tested and among the MeO-PBDEs added to the method during the study, only 6-MeO-PBDE 47 was frequently observed in human milk samples (47%), while the others (5-MeO-PBDE 47, 4-MeO-PBDE 49, 5-MeO-PBDE 99, 5-MeO-PBDE 100, 4-MeO-PBDE 101, 4-MeO-PBDE 103) were present at detectable concentrations in <5 samples (<1%). Data analysis/interpretation was restricted to NHFRs and MeO-PBDE for which detection frequency was 10% or greater, with the exception of DBDPE which had a 9.4% frequency of detection.

All NHFRs were below the level of detection in 53 of the human milk samples analyzed, while all MeO-PBDEs were below limits of detection in 109 samples. While DBDPE was detected at a lower frequency of detection (9.4%) than TBP-AE (30%), TBX (30%), PBBZ (64%), PBT (39%), PBEB (14%), TBP-DBPE (32%), HBB (34%) and BTBPE (56%), the maximum DBDPE concentration observed in Canadian human milk was more than an order of magnitude higher than the maximum concentration observed for any of the other NHFRs (Fig. 2). The elevated DBDPE LOD relative to the other reported NHFRs impacted its detection frequency, however, when it was present DBDPE had an important contribution to ΣNHFR concentrations (Table 3) in human milk requiring consideration throughout the data analysis.

Median NHFR concentrations in human milk decreased from DBDPE (131 pg g^{-1} lipid) > BTBPE (5.57 pg g^{-1} lipid) > TBP-AE (4.83 pg g^{-1} lipid) > PBBZ (4.50 pg g^{-1} lipid) > HBB (3.03 pg g^{-1} lipid) > TBP-DBPE (1.48 pg g^{-1} lipid) > PBT (0.845 pg g^{-1} lipid) > TBX (0.719 pg g^{-1} lipid) > PBEB (0.710 pg g^{-1} lipid) (Table 3). Most NHFR concentrations were significantly and positively correlated with each other ($p < 0.001$), with an exception occurring between PBBZ and BTBPE (0.068, $p = 0.117$) (Table 4). DBDPE, however, was only found to be significantly positively correlated with four of the NHFRs: TBX (0.116, $p = 0.007$), TBP-DBPE (0.151, $p < 0.001$), HBB (0.158, $p < 0.001$) and BTBPE (0.129, $p = 0.003$).

In addition to being more frequently detected than 6-MeO-PBDE 47, 2-MeO-PBDE 68 was present at elevated concentrations in the human milk (Table 5). 6-MeO-PBDE 47 ranged from < LOD to 156 pg g^{-1} lipid while 2-MeO-PBDE 68 was present in samples at concentrations ranging from <LOD to 1540 pg g^{-1} lipid (Fig. 3), close to an order of magnitude higher than 6-MeO-PBDE 47. MeO-PBDE concentrations, however, were significantly correlated with one another (0.616, $p < 0.001$) in the samples analyzed during this work.

Maternal age (years) was considered in terms of whether it had an impact on Σ9 NHFR and Σ2 MeO-PBDE concentrations in human milk. NHFR concentrations in milk were not significantly correlated with maternal age (Spearman correlation 0.016, $p = 0.709$). In contrast to the

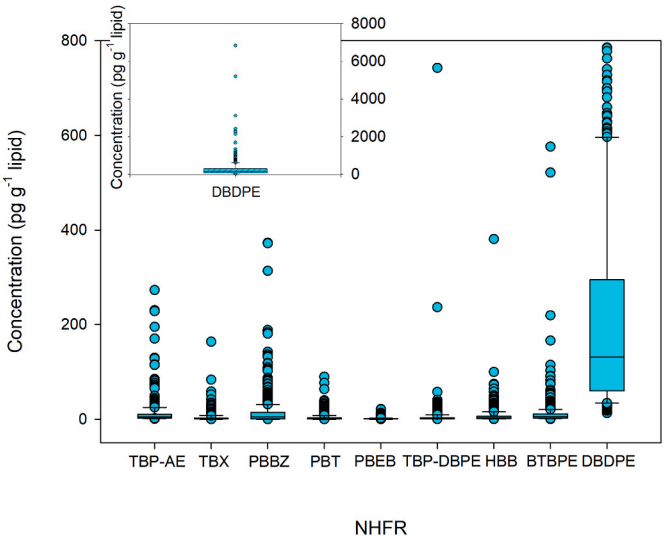

Fig. 2. Concentrations of NHFRs in Canadian human milk from the MIREC study. Box indicates 25th, 50th and 75th percentiles. Points indicate data outside of 10th and 90th percentiles.

Table 3
NHFR concentrations (pg g^{-1} lipid) in Canadian human milk samples from the MIREC study (n = 541).

NHFR	Detection Frequency (%)	Minimum[a]	Maximum	Mean	Standard Deviation	Median	Geometric Mean
TBP-AE	30	<LOD (0.839)	273	12.0	25.8	4.83	5.84
TBX	30	<LOD (0.064)	164	3.24	9.72	0.719	1.04
PBBZ	64	<LOD (0.105)	373	14.6	35.2	4.50	3.45
PBT	39	<LOD (0.081)	89.7	3.40	7.83	0.845	1.16
PBEB	14	<LOD (0.109)	21.5	1.28	2.01	0.710	0.790
TBP-DBPE	32	<LOD (0.201)	743	5.48	33.9	1.48	1.87
HBB	34	<LOD (0.194)	381	7.14	19.0	3.03	3.49
BTBPE	56	<LOD (0.238)	576	12.4	37.4	5.57	5.50
DBDPE	9.4	<LOD (12.9)	6830	266	482	131	139
ΣNHFRs	90[b]	–	6930	325	495	203	201

[a] (LOD/2).
[b] 90% of the samples had at least one NHFR detected.

Table 4
Spearman correlation coefficients between individual NHFRs with p-values of <0.001.

	TBX	PBBZ	PBT	PBEB	TBP-DBPE	HBB	BTBPE
TBP-AE	0.385	0.194	0.413	0.419	0.355	0.288	0.191
TBX		0.425	0.475	0.468	0.474	0.406	0.148
PBBZ			0.444	0.244	0.369	0.399	0.068[a]
PBT				0.481	0.603	0.496	0.145
PBEB					0.481	0.479	0.223
TBP-DBPE						0.571	0.230
HBB							0.186

[a] Not significant (p = 0.117).

NHFRs, age was significantly and positively correlated with ΣMeO-PBDE concentrations (Spearman correlation 0.237, p < 0.001).

One-way ANOVA were performed to examine ΣNHFR and ΣMeO-PBDE concentrations in terms of other maternal characteristics (e.g., parity, pre-pregnancy BMI). Median ΣNHFR concentrations did not vary significantly based on the number of children a women had borne (parity = 0, 1, 2, 3+) (p = 0.777) and similarly ΣMeO-PBDE concentrations in human milk did not vary significantly corresponding to a woman's parity (p = 0.910) for this dataset. Similarly, no significant difference between median ΣNHFR concentrations was observed in relation to pre-pregnancy body mass index (<20, 20–25, >25–30, >30–35, >35, no BMI information provided) (p = 0.140), consistent with the ANOVA results considering the ΣMeO-PBDEs and pre-pregnancy BMI (p = 0.311).

An additional factor, maternal education, was considered to explore the differences in ΣNHFR and ΣMeO-PBDE concentrations in human milk. Maternal education was broken down into eight categories (obtaining Grade 8 or less, having some high school, completing high school having earned the diploma, having taken some college classes, receipt of an earned college diploma, completing trade school and obtaining the diploma, earning an undergraduate university degree and having earned a graduate degree (Master's, Ph.D.)). All participants, among the contributors of human milk for NHFR analysis had completed Grade 8 with relatively few who had not completed high school (n = 5). Similarly, few participants had obtained trade school diplomas (n = 9). While median ΣNHFR concentrations in the maternal milk were not found to be statistically different based on the level of the mothers' education (ANOVA p = 0.168), median ΣMeO-PBDEs were found to be statistically, although weakly, significantly different (ANOVA p = 0.03) for this factor. The highest median ΣNHFR concentration was observed in milk from women who completed trade school (344 pg g^{-1} lipid) followed by those university educated (211 pg g^{-1} lipid, both undergraduate and graduate degrees), those who completed college (198 pg g^{-1} lipid), those who graduated from high school (143 pg g^{-1} lipid) and those with incomplete high school (139 pg g^{-1} lipid) or college (137 pg g^{-1} lipid). The highest median ΣMeO-PBDE concentrations were similarly observed in milk from the women having earned a trade school diploma (48.0 pg g^{-1} lipid) > those having university degrees (20.8 pg g^{-1} lipid [graduate degree] ≈ 20.0 pg g^{-1} lipid [undergraduate]) > college educated (15.8 pg g^{-1} lipid [some college] > 14.5 pg g^{-1} lipid [obtained college diploma]) > some high school (8.59 pg g^{-1} lipid) > earned high school diploma (8.30 pg g^{-1} lipid) (Fig. 4).

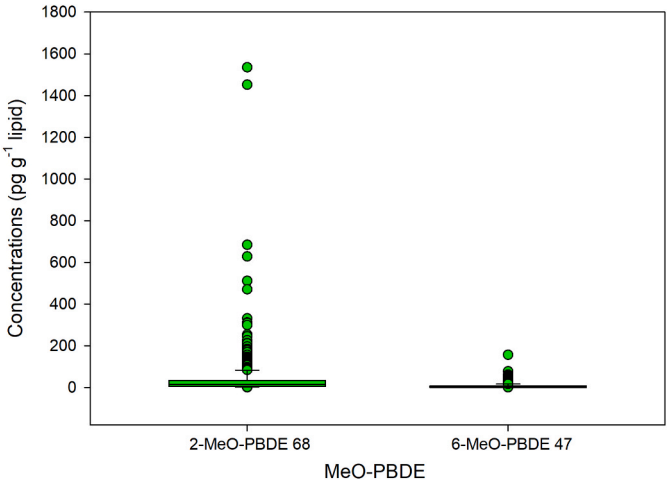

Fig. 3. MeO-PBDE concentrations in human milk samples. Box indicates 25th, 50th and 75th percentiles. Points indicate data outside of 10th and 90th percentiles.

Table 5
MeO-PBDE concentrations (pg g^{-1} lipid) in human milk.

MeO-PBDE	Detection Frequency (%)	Minimum	Maximum	Mean	Standard Deviation	Median	Geometric Mean
2-MeO-PBDE 68[b]	80	<LOD[a] (0.253)	1540	39.4	111	15.1	12.3
6-MeO-PBDE 47[c]	47	<LOD (0.190)	156	6.17	11.4	2.14	2.55
ΣMeO-PBDEs	80[d]	–	1600	45.7	117	19.0	16.8

[a] (LOD/2).
[b] n = 541.
[c] n = 484.
[d] 80% of the samples had at least one MeO-PBDE detected.

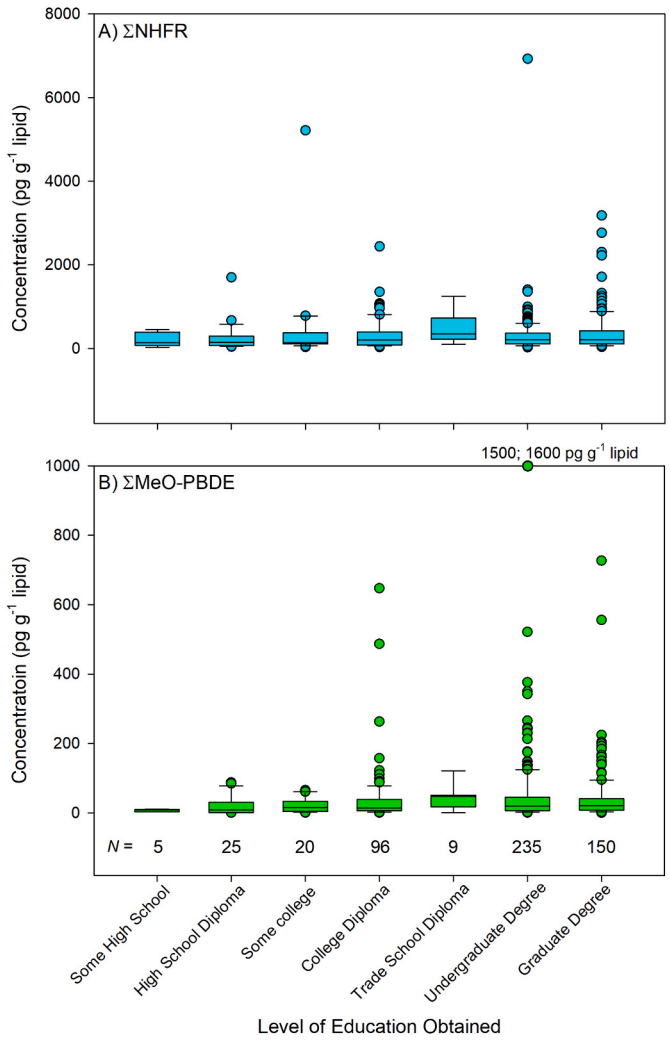

Fig. 4. A) ΣNHFR and B) ΣMeO-PBDE concentrations in milk broken down by education level. Box indicates 25th, 50th and 75th percentiles. Points indicate data outside of 10th and 90th percentiles. One participant did not provide education information.

4. Discussion

With restrictions on the use of the historical halogenated flame retardants, development of replacement FRs has occurred and usage of these newer products has expanded over time (Lee et al., 2022; Ma et al., 2023). NHFR presence has been observed in air and other environmental compartments globally (Covaci et al., 2011; Hou et al., 2021; Lee et al., 2022; Shunthirasingham et al., 2018; Xie et al., 2023). Given their use patterns, NHFRs have also been studied and observed at detectable levels in indoor environments where dust has been established as a critical route of exposure (Besis et al., 2023; Pasecnaja et al., 2021; Qi et al., 2014; Sahlström et al., 2015; Shoeib et al., 2012; Strid et al., 2014). While NHFR exposure can result from both inhalation and ingestion of house dust, diet has been identified as the second most important pathway of exposure, behind dust inhalation (Li et al., 2015).

While limited data reporting NHFR concentrations in humans are available in the literature, DBDPE has been reported in human milk from a number of countries, although its detection frequency (4–10%) was somewhat lower than other NHFRs (e.g., α-, β-DBE-DBCH [20–100%], BTBPE [28–40%]) in human milk from the UK (Tao et al., 2017). The low DBDPE detection frequency (9.4%) relative to eight of the other analytes (14–64%) in the present study is consistent with UK observations, although in contrast all DBE-DBCH isomers were observed in <1% of the MIREC samples. Human milk collected from Sherbrooke, Québec, Canada also resulted in a similarly low detection frequency (8.6%) of DBDPE, but BTBPE was not detected in any of these samples (Zhou et al., 2014), whereas in the present study BTBPE was detected in >50% of the samples analyzed. It is interesting that the reported detection frequency of DBDPE is low relative to other NHFRs in Canada and the UK, but when it is present, concentrations observed are elevated over the other NHFRs considered which contrasts with observations from China where DBDPE was observed in 100% of the samples collected (Shi et al., 2016).

While the detection frequency was similar for DBDPE in human milk between the UK and Canada, maximum reported concentrations (DBDPE: 250 ng g^{-1} lipid, BTBPE: 56 ng g^{-1} lipid) in human milk from the UK collected in 2010 (Tao et al., 2017), were elevated over maximum concentrations observed across Canada in the MIREC study (6830 pg g^{-1} lipid [6.83 ng g^{-1} lipid]; 576 pg g^{-1} lipid [0.576 ng g^{-1} lipid], DBDPE and BTBPE, respectively). DBDPE concentrations in human milk from New Zealand (Mannetje et al., 2013) and Ireland (Pratt et al., 2013), in contrast, were below detection in all samples analyzed.

The additional NHFRs analyzed in Irish human milk (HBB, BTBPE) were below the level of detection in 100% of the samples analyzed (Pratt et al., 2013). Human milk collected from primiparous women living in Tanzania in 2012 were similarly free of detectable levels of all NHFRs analyzed in that work (BTBPE, HBB, TBP-DBPE, PBEB, PBT) (Müller et al., 2016). HBB and PBEB, however, were observed in 100% and ≈50% of the samples, respectively from a New Zealand study (Mannetje et al., 2013). Despite HBB and PBEB having a higher detection frequency in human milk from New Zealand relative to those established in Canadian samples, maximum concentrations in Canada (381 pg g^{-1} lipid and 21.5 pg g^{-1} lipid, HBB and PBEB, respectively) were approximately five to seven times higher than those observed in New Zealand (73.7 pg g^{-1} lipid, 3.10 pg g^{-1} lipid, HBB and PBEB, respectively) (Mannetje et al., 2013). Human milk collected between 2003 and 2006 in three countries from Europe; Norway, the Netherlands and Slovakia was reported to have detectable levels of HBB, PBT and PBBZ in 44%, 87% and 97% of the samples collected, respectively while PBEB was observed infrequently (2%) (Čechová et al., 2017). Although the NHFRs were present at detectable concentrations in >40% of the samples, the 95th percentile concentrations (<200 pg g^{-1} lipid) (Čechová et al., 2017) remained within the range of the Canadian concentrations.

Human milk from China had consistently elevated concentrations of the NHFRs relative to the concentrations observed in the present Canadian study. DBDPE detection frequency in human milk from China was 100%, and present at concentrations ranging from 2450 to 21,800 pg g^{-1} lipid (2.45–21.8 ng g^{-1} lipid) (Shi et al., 2016), exceeding the results from the Canadian MIREC study by more than a factor of three. PBT was similarly observed in 100% of the samples tested in the Chinese study, while HBB (93%), BTBPE (90%) and TBP-DBPE (86%) detection frequencies were elevated over the present study (39%, 34%, 56% and 32%, respectively). While a difference was noted in the detection frequency, Canadian maximum concentrations of these NHFRs were generally in the same order of magnitude (maximum concentration PBT, HBB, BTBPE, TBP-DBPE; 89.7, 381, 576 and 743 pg g^{-1} lipid, respectively) as reported in Chinese milk (PBT, HBB, BTBPE, TBP-DBPE; 517, 500, 922 and 179 pg g^{-1} lipid, respectively) (Shi et al., 2016).

Among the eight MeO-PBDEs analyzed in the present study, only two (2-MeO-PBDE 68 and 6-MeO-PBDE 47) were detected in Canadian human milk (Table 4). These results are consistent with observations in four human milk samples obtained from a milk bank in Texas, USA, where both 2-MeO-PBDE 68 (3 of 4 samples) and 6-MeO-PBDE 47 (1 of 4 samples) were observed (Butryn et al., 2015), with concentrations ranging from 0.048 ng g^{-1} lipid to 0.284 ng g^{-1} lipid 2-MeO-PBDE 68 and 0.148 ng g^{-1} lipid 6-MeO-PBDE 47.

These MeO-PBDEs have been similarly observed in human milk collected in Tunisia and while 2-MeO-PBDE 68 was the most frequently observed MeO-PBDE in this Canadian study, the opposite was true in the

Tunisian samples with 6-MeO-PBDE 47 being reported in 81% of the samples analyzed (Ben Hassine et al., 2015). The maximum concentrations of the two MeO-PBDEs in milk from the Tunisian women (3.02 ng g^{-1} lipid 6-MeO-PBDE 47 and 1.94 ng g^{-1} lipid 2-MeO-PBDE 68) were higher than the maximum concentrations observed in the MIREC study samples. Among the MeO-PBDEs studied in human milk from Spain, only 6-MeO-PBDE 47 was in common with the current work, and it was detected in 21% of the samples analyzed, although the maximum concentration reported (15,000 pg g^{-1} lipid) (Lacorte and Ikonomou, 2009) is approximately 100 times higher than observed in the Canadian study (156 pg g^{-1} lipid). 6-MeO-PBDE 47 was observed in most (8 of 9) human milk samples collected from Okinawa, Japan where the median concentration was 0.19 ng g^{-1} lipid (190 pg g^{-1} lipid) and the maximum concentration was 530 pg g^{-1} lipid (Fujii et al., 2014). While the detection frequency was lower in Canadian human milk, the concentrations observed in the samples were within the range reported for the Japanese study.

5. Conclusion

The present work is the first pan-Canadian study to examine these compounds in human milk and has confirmed detectability of nine NHFRs (TBP-AE, TBX, PBBZ, PBT, PBEB, TBP-DBPE, HBB, BTBPE and DBDPE). Although DBDPE was present with a relatively low frequency of detection, when present it was an important contributor to ΣNHFR concentrations, which suggests future monitoring of this HFR will be important. Maximum concentrations of the other detected NHFRs were present at concentrations approximately a factor of 10 lower than DBDPE or less, consistent with observations from other studies. Concentrations of all NHFRs were below the LOD in 53 samples analyzed (~10%) and all MeO-PBDEs were below LOD in 109 samples (20%). The presence of 2-MeO-PBDE 68 and 6-MeO-PBDE 47 observed in Canadian human milk samples collected as part of this study is consistent with observations of MeO-PBDEs from other regions of the world. While detected and reported in human milk from different countries, NHFR and MeO-PBDE concentrations have not been well defined by global region.

No relationships between analyte concentrations in human milk from this study and maternal age or parity were established, which may be a function of the relatively new uses of these chemicals relative to legacy environmental organic contaminants, in contrast to the relationship between maternal age and MeO-PBDE concentration. These data provide maternal exposure levels to NHFRs while their use may be expanding globally and will allow risk assessors to consider potential impacts of dietary exposure to infants at a critical time in development. Additional work to determine NHFR and MeO-PBDE concentrations in human milk will be required in the future to establish temporal trends of dietary infant exposure during the period of breastfeeding.

CRediT authorship contribution statement

Dorothea F.K. Rawn: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Catherine Corrigan:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Cathie Ménard:** Formal analysis, Investigation, Methodology. **Wing-Fung Sun:** Formal analysis, Investigation, Methodology. **François Breton:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Tye E. Arbuckle:** Conceptualization, Funding acquisition, Project administration, 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.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.141065.

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