Rawn *et al. J Environ Expo Assess* 2024;3:16
DOI: 10.20517/jeea.2024.04

Journal of Environmental Exposure Assessment

Research Article

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Legacy halogenated flame retardants in Canadian human milk from the maternal-infant research on environmental chemicals study

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How to cite this article: Rawn DFK, Sadler AR, Casey VA, Breton F, Sun WF, Feng SY, Arbuckle TE. Legacy halogenated flame retardants in Canadian human milk from the maternal-infant research on environmental chemicals study. *J Environ Expo Assess* 2024;3:16. https://dx.doi.org/10.20517/jeea.2024.04

Received: 25 Jan 2024 **First Decision:** 17 Apr 2024 **Revised:** 27 May 2024 **Accepted:** 31 May 2024 **Published:** 1 Jul 2024

Academic Editors: Stuart Harrad, Jose L Domingo **Copy Editor:** Pei-Yun Wang **Production Editor:** Pei-Yun Wang

Abstract

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) were measured in 298 human milk samples collected from across Canada between 2008 and 2011 as part of the Maternal-Infant Research on Environmental Chemicals study. PBDEs were detected in 100% of the samples analyzed and concentrations ranged from 0.071 to 267 ng·g^{-1} lipid (median 15.6 ng·g^{-1} lipid). The dominant contributors to ΣPBDEs (Σ15, 17, 28, 37, 47, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 160, 183, 190, 209) were PBDE 47 > PBDE 153 > PBDE 99 > PBDE 100 > PBDE 28 > PBDE 209. Previously, PBDE 209 was considered to be a minor contributor to ΣPBDE concentrations in Canadian human milk and, therefore, not reported by our lab. This study showed that when present, PBDE 209 can be an important contributor to ΣPBDEs (range: below detection - 85.3 ng·g^{-1} lipid; median - 0.083 ng·g^{-1} lipid). ΣPBDE concentrations declined slightly in Canadian human milk between the early and late 2000s. HBCD (Σ of α-, β-, and γ-) was observed in 94.0% of the samples measured and concentrations were dominated by α-HBCD (93.3%), with β- (9.7%) and γ- (28.5%) less frequently detected. The maximum ΣHBCD concentration observed

was 7.66 ng·g^{-1} lipid (median value 0.303 ng·g^{-1} lipid). These data suggest that HBCD concentrations similarly decreased in Canadian human milk between the early 2000s and sampling for the present study. Maternal age did not impact the concentrations of these flame retardants in milk. Additionally, other maternal characteristics [e.g., the number of children a woman has had, pre-pregnancy body mass index (BMI), and education level] did not impact concentrations of these brominated flame retardant concentrations.

Keywords: Polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), human milk, pan-Canadian study, human biomonitoring

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are both additive brominated flame retardants (BFRs) that are mixed with polymers present in consumer products (e.g., textiles, electronics, *etc.*) to reduce flammability[1]. By being added and mixed with the polymers, rather than chemically bonded, they are more readily released to the environment throughout their lifecycle. PBDEs were produced as three mixtures named according to the dominant contributors to the total PBDE concentration: the penta-mix, dominated by the penta- and tetra-brominated congeners; the octa-mix, which encompasses significant levels of the octa-, nona- and decabrominated congeners, and the deca-mix, reported to comprise 92%-97% decabromodiphenyl ether (PBDE 209)[2]. The penta-mix and octa-mix were used in different applications (e.g., penta-mix - polyvinylchloride, unsaturated polyesters, rubber, paints, lacquers, and textiles; octa-mix - acrylonitrile butadiene styrene, polyamide, poly butylene terephthalate, polystyrene/high impact polystyrene). While the deca-mix was used for most purposes identified for the other mixtures, it was also used in polyethylene cross-linked polyethylene, polyethylene terephthalate, and polypropylene[3]. The inclusion of PBDEs in these chemicals resulted in their presence in a wide variety of consumer products such as televisions, computers, appliances, smoke detectors, conveyor belts, carpets, textiles, furniture, and the seating upholstery in vehicles[3]. HBCD was used as a replacement for PBDEs[4] primarily in building insulation (expanded and extruded polystyrene), although it was also used to a minor extent in textiles[5].

Although persistence is a desirable quality for flame retardants in consumer products, this characteristic, coupled with their application as additive BFRs, has contributed to their release into the environment during product lifecycles and following disposal[6-8]. Given that many of the products containing PBDEs and HBCD are present within homes and workplaces, they are detected at elevated concentrations in indoor dust and air relative to outdoor environments in global regions free of outdoor sources[9,10]. In addition, BFR levels in the air and dust in offices have been related to the number of electronic devices present[6]. Subsequently, the release of BFRs during recycling of electronic/electrical waste and repair of electronic equipment has been documented as leading to their elevated levels in air and dust where this activity is performed[11,12].

While both of these classes of BFRs are known to be persistent, they also accumulate in the tissues of living organisms, particularly in the lipid tissues[13]. As a result of the bioaccumulation potential, PBDEs and HBCD have been monitored in many different organisms globally, and studies have shown that both of these BFRs biomagnify through food webs[14-22]. In addition, PBDEs and HBCD have been reported in human tissues/fluids throughout the world (e.g., serum, milk) for more than a decade[23-33]. Human milk collected in Sweden over a period of 25 years (1972 to 1997) was analyzed to measure PBDEs and highlighted a dramatic increase in ΣPBDE (Σ of PBDE 28, 47, 66, 85, 99, 100, 153, 154) concentration, from < 0.1 ng·g^{-1} lipid in 1972 to ~4 ng·g^{-1} lipid in 1997[34,35]. Following this study, the same group analyzed a limited number of human milk samples (n = 15) collected in 2000-2001 and found ΣPBDE concentrations

ranged from 0.56 to 7.72 ng·g^{-1} lipid, which shows that concentrations continued to increase into the early 2000s, prior to regulatory action being taken on these chemicals[36]. North American studies measuring PBDEs (consistently including Σ28, 47, 99, 100, 153) in human milk during the early to mid-2000s found concentrations to be higher than observed in Europe during a similar period (range 0.1-2,010 ng·g^{-1} lipid)[37]. Since HBCD has not been as widely studied as PBDEs, temporal trends were not readily established in human milk globally[38,39]. In addition to differences in temporal patterns, concentrations of these legacy BFRs vary among countries and regions.

The determination of PBDEs and HBCD in human milk is important because these data allow researchers to determine maternal exposure via their milk concentrations. Additionally, human milk is often the sole source of nutrition for infants and very young children, which means it represents an important dietary exposure to BFRs for this demographic. Owing to the fact that infants and very young children are growing and changing rapidly, BFR exposure during this phase of life may impact their development since these compounds are associated with impacts on endocrine function and frequently related to thyroid function disruption[13,40,41]. Although exposure at critical developmental stages may not result in immediate impacts, PBDE exposure can lead to increased risk of disease at later life stages (e.g., diabetes, cancer, neurobehavioural effects)[41-43], and HBCD has also been associated with neurobehavioural impacts and changes in sex-related hormones[40], resulting in effects on behaviour and memory[13].

Despite the presence of persistent organic contaminants, including BFRs, in human milk, the World Health Organization (WHO) recommends that mothers feed children human milk exclusively for the first six months, because of the presence of important nutritional constituents and positive health effects for the mothers and their babies[44]. In addition, breastfed infants are reported to have a lower incidence of obesity later in life[45].

The present study, known as the Maternal-Infant Research on Environmental Chemicals (MIREC) study, was undertaken across Canada between 2008 and 2011 to measure maternal concentrations of environmental chemicals during pregnancy and in the milk following the birth of their babies[46]. The work described herein is focused on the measurement of PBDEs and HBCD in human milk.

EXPERIMENTAL

Study population and sampling

Participants for the MIREC study were recruited from across Canada to allow for a pan-Canadian perspective on maternal environmental chemical concentrations. In addition to providing biomonitoring data on the participants, this work supported the determination of infant dietary exposure via human milk sampling. Research centres were identified as possible recruitment sites if it was determined that existing frameworks for clinical obstetric research were in place prior to being associated with the MIREC study. In advance of confirming any clinic as a MIREC recruitment site or study centre, each clinic was required to obtain ethics board approval from their own research centre, Health Canada's ethics board, and the ethics board of the MIREC coordination centre in CHU Sainte Justine, Québec. Once a clinic was confirmed to be a MIREC study centre, recruitment was undertaken during prenatal clinics[46]. Study centres were established in 10 cities (from six provinces) across southern Canada, from west to east: Vancouver (British Columbia); Edmonton (Alberta); Winnipeg (Manitoba); Hamilton, Kingston, Ottawa, Sudbury and Toronto (Ontario); Montréal (Québec) and Halifax (Nova Scotia).

Eligibility requirements were limited to women aged 18 or above, in the first 14 weeks of gestation, and capable of communicating in either English or French[46]. Among the women approached regarding the

study, 2,001 agreed to participate. As part of the requirement, participants were given lifestyle and demographic questionnaires during each of the first and third trimesters for their completion and approximately 50% (n = 1,017) of the recruited participants provided samples of their milk for analysis of multiple classes of chemicals (e.g., persistent organic pollutants, metals, bisphenol-A).

Health Canada analytical laboratories involved in the human milk analyses did not have the capacity to analyze all of the samples collected as part of the current study. As a result, a plan to distribute the milk samples among analytical laboratories was established for all analytes to ensure that samples from across all sampling sites were analyzed for each class of analyte. Data from the Canadian Community Health Survey (CCHS)[47] and estimates of the number of women who would continue to breastfeed for a period extending beyond two weeks of the birth of their babies were considered as part of the framework. This information was coupled with the number of participants from each of the research centres to enable the development of an estimate of samples available for distribution between laboratories. Statisticians then prepared a sample distribution framework to randomly select samples from each location to be analyzed by each of the laboratories. Factors considered while developing the sampling plan included the number of samples able to be handled by each lab, to reach a target of 300 total samples for legacy persistent organic pollutants (e.g., PBDEs and HBCD), and a representative distribution of ages and number of previous pregnancies/births by participant [Table 1]. The plan was developed in advance of all samples being received, and given that sample collection took place from 2008 until 2011, the plan had to allow for flexibility and to ensure that the pan-Canadian situation would be captured by each laboratory analyzing a subset of the samples. Among the 1,017 participants providing milk, 298 were identified and analyzed for PBDEs and HBCD in the Health Canada laboratory [Figure 1].

Consistent with previous Canadian studies, milk was collected by the study participants, who were asked to hand express their milk (fore- and hindmilk). If participants experienced difficulty with hand expression, a Medela® (Medela International, Zug, Switzerland) manual breast pump was provided, along with instructions for either manually expressing or using the breast pump to collect the milk. Milk samples were collected between two and 10 weeks following delivery of the babies, recognizing that milk was to be analyzed for a variety of different analytes/classes of compounds, 250 mL were requested of participants (125 mL in glass containers, 125 mL in plastic containers). In general, 250 mL were received from participants, although occasionally, the volumes received were lower. Aliquots of the samples for PBDE/HBCD analysis were collected into 500 mL wide-mouth amber I-CHEM® glass jars with fluoropolymer resin-liner polypropylene closure (Thermo Fisher Scientific, Rockwood, TN, USA). Participant instructions for collection allowed for them to express the milk over a period of time, and keep it in their refrigerator (4 °C) if collected over a few days, but if it took a longer period of time, the milk was to be stored at -20 °C in their freezers. Once collected and frozen, the samples were shipped to the coordination centre in Ste. Justine, Québec and then shipped to the laboratory where aliquots of the samples were prepared and then transferred to the laboratories for chemical analysis.

Extraction and clean-up

Sample preparation followed the protocol for analysis of PBDEs and HBCD described previously for the other persistent organics (e.g., polychlorinated biphenyls)[48]. Briefly, samples (~25 g) were transferred into Erlenmeyer flasks and surrogate standards were added to the samples (^{13}C PBDEs 15, 28, 47, 99, 153, 154, 183 and 209; ^{13}C α-, β- and γ-HBCD) (Cambridge Isotope Laboratories, Andover, MA, USA). An aliquot of each sample (5%) was taken for gravimetric determination of lipid content in each sample. Prior to extraction by homogenizing in 2:1 (v/v) acetone: hexane (Residue analysis grade, EMD; Ottawa, ON, Canada), the samples were set aside on the lab bench for approximately 30 min following the addition of surrogate standards. Lipids present were digested by washing extracts with sequential aliquots of

Table 1. Participant summary information corresponding to the PBDE and HBCD analysis

Characteristic	Minimum	Maximum	Mean	Median
n = 298				
Born in Canada - 84.6%				
Age (years)	21	46	33.6	34
Parity	1	5	-	2
Pre-pregnancy body mass index	16.6	48.6	24.6	23.1
Education level				
Some or all high school	18			
Some college	9			
College or trade school completed	53			
Undergraduate university completed	125			
Graduate degree completed	93			

PBDE: Polybrominated diphenyl ether; HBCD: hexabromocyclododecane.

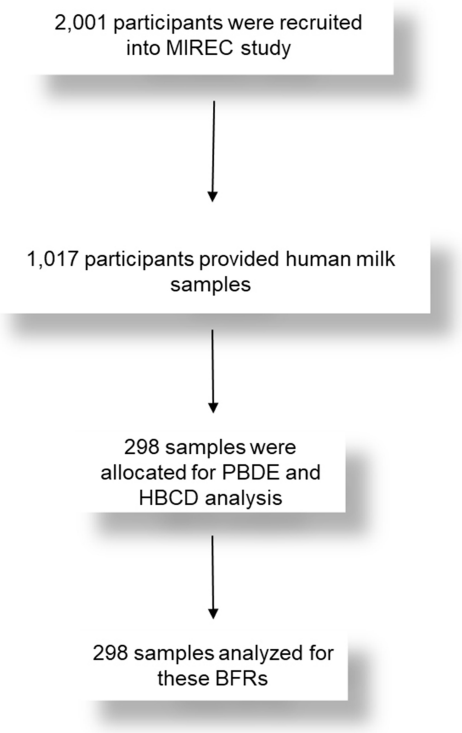

Figure 1. MIREC participant population and samples used for PBDE/HBCD analysis. MIREC: Maternal-Infant Research on Environmental Chemicals; PBDE: polybrominated diphenyl ether; HBCD: hexabromocyclododecane.

concentrated sulphuric acid (ACS grade, EMD; Ottawa, ON, Canada), followed by drying the extracts by passing them through anhydrous sodium sulphate. Column chromatography was performed using activated acidified silica gel, activated Florisil (60-100 mesh) (Fisher Scientific; Ottawa, ON, Canada), and carbon {Carbopack C (60-80 mesh) [Supelco (Bellefonte, PA)]} in series to clean up and fractionate the extracts[21]. Following clean-up, the extracts were evaporated just to dryness, reconstituted in 10 µL of the performance standard and placed into v-vials for gas chromatographic-high resolution mass spectrometric analysis of PBDEs.

Once PBDE analyses were completed, sample extracts were concentrated just to dryness using a gentle stream of nitrogen and diluted with the performance standard, deuterated d_{18} HBCD (α-, β- and γ-), in 100 µL methanol: water (80:20) to allow for LC-MS/MS analysis.

Analysis

PBDE analysis

A Waters Autospec Ultima high resolution mass spectrometer (Waters Corporation, Mississauga, ON) operating at 10,000 resolution, coupled to an Agilent 6890 gas chromatograph (Agilent Technologies, Mississauga, ON), was used for all PBDE analyses (15, 17, 28, 37, 47, 66, 71, 75, 77, 85, 99, 100, 119, 126, 138, 153, 154, 160, 181, 183, 190, 205, 209). Separation of 23 congeners was achieved using a DB-5MS column (15 m × 0.25 mm i.d. × 0.1 µm film thickness; J&W; Agilent Technologies, Mississauga, ON). One µL aliquots were injected using a cool on-column injector, set to track the oven temperature. The starting oven temperature was 80 °C and held for 1 min, after which the temperature was increased at a rate of 32 °C·min^{-1} until it reached 225 °C. Then, the oven was set to increase at 3 °C·min^{-1} to 230 °C where it was held for 1 min and the final increase was set at 40 °C·min^{-1} to a maximum temperature of 295 °C where it remained for 8 min. Helium was the carrier gas set at variable pressure (28-173 kPa), and the mass spectrometer was operating in EI positive ion mode at 50 eV with the trap current set to 650 µA and the source temperature to 250 °C.

HBCD analysis

HBCD analyses were performed using a Waters Acquity ultra-high pressure liquid chromatograph I-Class coupled to a Waters Xevo TQ-XS triple quadrupole mass spectrometer with electrospray ionization operating in the negative ion detection mode. A 2.1 mm × 150 mm Kinetex C18, 2.6 µm column (Phenomenex, USA) was used to separate the three HBCD isomers considered. Mobile phase A (water) and acetonitrile: methanol (2:1) (mobile phase B) were used to separate α-, β- and γ-HBCD using gradient elution, starting with 60% mobile phase B for 1 min, transitioning to 80% mobile phase B by 4 min, holding until 11 min and increasing to 85% mobile phase B by 14 min and remaining there until 16 min, before returning to the starting conditions by 16.1 min, where it was allowed to stabilize until 22 min. The flow rate was 0.175 mL·min^{-1} and the column temperature was set to 25 °C to support complete resolution of the d_{18}, ^{13}C- and native HBCD isomers. The capillary and cone voltage were -2.5 kV and 20 V, respectively. The source temperature was set to 150 °C, while the desolvation temperature was 400 °C. The desolvation gas flow was 1,000 L·h^{-1}, with the cone gas flow set to 170 L·h^{-1}. Argon was used as the collision gas at a flow rate of 0.15 mL·min^{-1}. The transitions monitored for native, ^{13}C, and d_{18} HBCD isomers were: 639 → 79, 641 → 79, 641 → 81, and 643 → 81 (native); 651 → 79, 653 → 79, 653 → 81, and 655 → 81 (^{13}C surrogates); and 656 → 79, 658 → 79, 658 → 81, and 660 → 81 (d_{18}- performance standards), respectively. Dwell times were set to 24 msec.

Quality assurance/quality control

Two method blanks prepared using reagents alone, following the same protocol as for the unknown samples, were included with each set of samples to allow for the removal of laboratory background concentrations. Additionally, either one or two standard reference materials of human milk containing known concentrations of PBDEs [standard reference material (SRM) 1953 (unfortified), SRM 1954 (fortified)] were included with each set of samples prepared for analysis. For those sets where a single SRM was included, an internal sample of human milk that has been used in the laboratory as an internal quality control (QC) over time was also included. PBDE concentrations and patterns determined in the SRMs were as anticipated, with concentrations generally within two standard deviations of the expected values. The internal QC sample PBDE concentrations were generally within one standard deviation of the mean values.

Although similar certified reference materials with known HBCD concentrations were not available during the work, the HBCD concentrations in the SRMs were determined. Only α-HBCD was frequently detected in both the unfortified and fortified milk, with β- and γ-HBCD generally present below the limits of detection. Concentrations of α-HBCD in the SRMs were within two standard deviations of the mean concentration, similar to the results from testing the laboratory internal QC sample.

Average surrogate recoveries ranged from 35.0% (^{13}C PBDE 15) to 93.3% (^{13}C PBDE 100) in the human milk samples analyzed. HBCD average surrogate recoveries ranged from 68.8% to 83.5% (β-HBCD and γ-HBCD, respectively), while the average α-HBCD recovery was 77.1%. All concentrations in the samples were corrected for recovery.

In addition, representative breast pumps, similar to those sent to participants were examined for background PBDE and HBCD levels. Testing was performed by rinsing the pumps with purified water and the rinse water was prepared for analysis as samples alongside samples of purified water. Any detectable concentrations observed in the pump rinses were considered to be a result of the water used for this testing.

Limits of detection

Instrumental limits of detection (LOD) were determined for each PBDE based on a 3:1 signal to background noise ratio for each sample individually and method detection limits (MDLs) were then calculated to account for variation in instrument sensitivity and sample sizes. Average PBDE MDLs ranged from 0.023 pg·g^{-1} sample; 0.769 pg·g^{-1} lipid (PBDE 15) to 0.712 pg·g^{-1} sample; 23.5 pg·g^{-1} lipid (PBDE 209), respectively. PBDE 183 had the second highest MDL observed in the samples (0.187 pg·g^{-1} sample, 6.32 pg·g^{-1} lipid). Limits of detection for the individual HBCD isomers were determined using low-level standards and adjusted for sample weight and instrument sensitivity, resulting in MDLs of 0.617 pg·g^{-1} sample, 21.6 pg·g^{-1} lipid (α-HBCD); 0.385 pg·g^{-1} sample, 13.4 pg·g^{-1} lipid (β-HBCD); and 0.693 pg·g^{-1} sample, 24.0 pg·g^{-1} lipid (γ-HBCD) in the human milk samples.

Statistical analysis

Statistical analyses were performed using SigmaPlot 12.5 (Systat Software Inc.). For those compounds below the MDL in any sample, the analyte concentrations were adjusted to 1/2 MDL (i.e., MDL/2) established for the samples prior to initiating data summary and statistical analysis. In addition to developing descriptive statistics of these data, they were also studied to consider whether concentrations were related to some of the personal characteristics of the participants (e.g., age, number of children the participant had prior to this pregnancy/parity). The concentration 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.

RESULTS AND DISCUSSION

The lipid content determined in the human milk samples ranged from 0.75% to 7.84%, with mean and median lipid concentrations of 3.26% and 3.22%, respectively. PBDEs and HBCD are lipophilic compounds; therefore, results are reported on a lipid-adjusted basis throughout this manuscript. While PBDEs were detected in all 298 of the milk samples collected from across Canada, HBCD was detected in 94.0% (n = 280) of the samples. PBDE 47 was the dominant contributor to ΣPBDE concentrations, followed by 153 > 99 > 100 > 28 > 209. Consistent with previous HBCD concentrations determined in Canadian serum[30], α-HBCD was the predominant isomer (93.3%) in human milk samples, with β-HBCD and γ-HBCD detected in 9.7% and 28.5% of the samples, respectively.

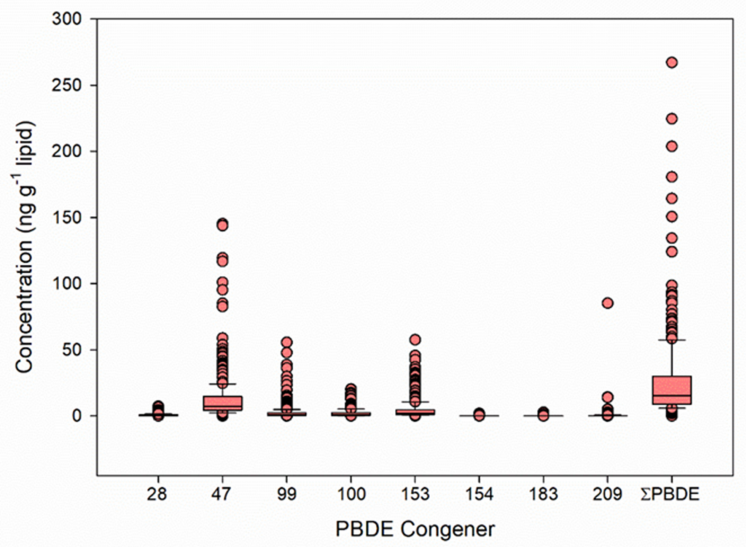

Figure 2. Selected PBDE congeners and ΣPBDE (Σ20 congeners) concentrations (ng·g^{-1} lipid) in human milk from the MIREC study (*n* = 298). Box indicates 25th, 50th (median), and 75th percentiles. Points indicate data outside of the 10th and 90th percentiles. PBDE: Polybrominated diphenyl ether; MIREC: Maternal-Infant Research on Environmental Chemicals.

Concentrations

Although PBDEs were present at detectable concentrations in all 298 of the Canadian human milk samples analyzed, three congeners were observed in relatively few samples. PBDEs 126 and 205 were observed in only two samples (< 1%), while PBDE 181 was present at detectable concentrations in 22 samples (7.4%). As a result of the very low detection rate of these three congeners, they have been removed from the data analysis and calculation of total PBDE concentrations.

ΣPBDE (Σ of 20 congeners: 15, 17, 28, 37, 47, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 160, 183, 190 and 209) concentrations ranged from 0.071 to 267 ng·g^{-1} lipid in the human milk from the MIREC study [Figure 2, Table 2]. ΣHBCD (Σα-, β-, γ-) concentrations ranged from below detection to 7.66 ng·g^{-1} lipid in the samples analyzed, with a median concentration of 0.303 ng·g^{-1} lipid [Figure 3, Table 2].The dominant PBDE congeners contributing to ΣPBDE concentrations observed in human milk (47, 153, 99, 100, 209) as part of the present study are similar to those identified for Canadian human serum collected in 2007 where 47 > 153 > 99 > 100 > 209 were reported (geometric mean: 22, 9.4, 4.6, 4.1 and 1.1 ng·g^{-1} lipid, respectively)[30]. Consistent with the widespread use of the penta-mix in North America, PBDE 47 (geometric mean: 7.97 ng·g^{-1} lipid) contributed approximately half (47%) of the ΣPBDE (geometric mean 16.8 ng·g^{-1} lipid) concentrations in the Canadian human milk measured [Table 2] and 45% of ΣPBDE (geometric mean: 47 ng·g^{-1} lipid) in the pooled serum samples from individuals aged 20 to 39 years as part of Cycle 1 of the Canadian Health Measures Survey (CHMS)[30].

PBDE 209 was observed in the serum of Canadian women of childbearing age as part of the CHMS work, although it had not been reported by our laboratory in human samples prior to that time. Although PBDE 209 was detected in 70.5% of the samples measured, the maximum PBDE 209 concentration observed in this work (85.3 ng·g^{-1} lipid) exceeded the maximum concentration of other congeners that generally contributed more to ΣPBDE concentrations (e.g., 99, 100, Table 2). PBDE 209 was not detected in all of the milk samples as part of the present study; when present, it contributed ≈ 3.3% to ΣPBDE concentrations.

Table 2. Concentrations (ng·g^{-1} lipid) of selected PBDE congeners, total PBDEs (Σ20 congeners) and α-, β- and γ-HBCD and Σ (α-, β-, γ-) HBCD in human milk (2008-2011)

Congener	Detection frequency (%)	Minimum	Maximum	Median	Geometric mean	Arithmetic mean	Standard deviation
PBDE 28	100	0.0001	7.45	0.550	0.566	0.835	0.901
PBDE 47	100	0.005	145	7.44	7.97	13.6	19.6
PBDE 99	100	0.006	55.6	1.09	1.28	2.83	6.14
PBDE 100	100	0.001	20.3	1.17	1.27	2.29	3.17
PBDE 153	99.3	< MDL (0.004)	57.5	2.01	2.41	5.03	8.22
PBDE 154	99.3	< MDL (0.003)	1.93	0.077	0.084	0.157	0.264
PBDE 183	98.0	< MDL (0.001)	2.78	0.043	0.043	0.082	0.201
PBDE 209	70.5	< MDL (0.001)	85.3	0.083	0.071	0.635	5.08
ΣPBDE[1]	100	0.071	267	15.6	16.8	26.5	33.2
α-HBCD	93.3	< MDL (0.004)	7.26	0.271	0.247	0.425	0.655
β-HBCD	9.7	< MDL (0.001)	0.801	0.006	0.008	0.024	0.084
γ-HBCD	28.5	< MDL (0.001)	6.39	0.010	0.012	0.097	0.454
ΣHBCD[2]	94.0	< MDL (0.010)	7.66	0.303	0.316	0.547	0.871

[1]Sum of 20 congeners: 15, 17, 28, 37, 47, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 160, 183, 190, and 209 (PBDE 126, 181, and 205 have been removed due to low detectability). [2]Sum of α-HBCD, β-HBCD, and γ-HBCD. PBDE: Polybrominated diphenyl ether; HBCD: hexabromocyclododecane; MDL: method detection limit.

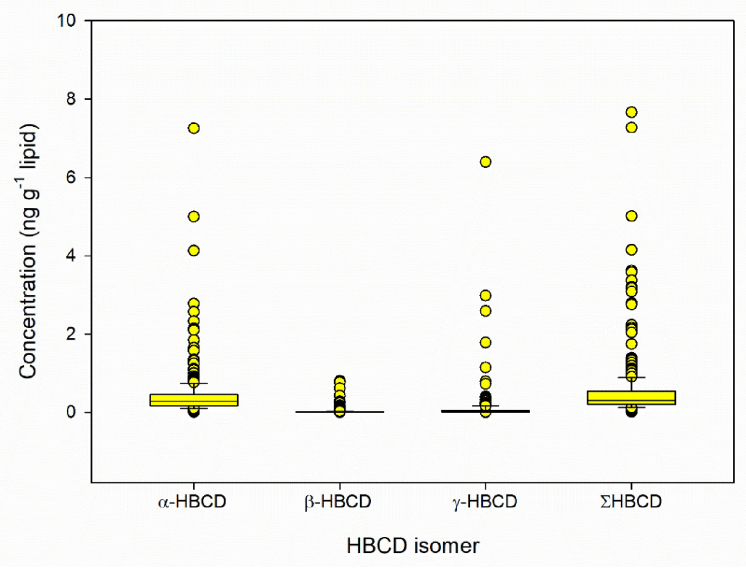

Figure 3. α-, β- γ-HBCD and Σ(α-, β-, γ-) HBCD concentrations (ng·g^{-1} lipid) in human milk samples. Box indicates 25th, 50th (median), and 75th percentiles. Points indicate data outside of the 10th and 90th percentiles. HBCD: Hexabromocyclododecane.

Relationships between compounds

Strong Spearman correlations were found between the concentrations of each of the dominant, lower brominated PBDE congeners (tri- through penta-: 28, 47, 99 and 100) observed in Canadian human milk. While the concentrations of these lower brominated congeners were strongly correlated with each other ($P > 0.790$), the relationship was much weaker between these congeners and the hexabrominated PBDE 153 [Table 3]. In contrast, PBDE 209 concentrations were not well correlated with any of the other congeners having important contributions to ΣPBDEs (i.e., 28, 47, 99, 100, 153). This difference aligns with the broader usage of the decabromodiphenyl ether in consumer products, which extended beyond the applications of

Table 3. Spearman correlations between individual predominant contributing PBDE congeners in Canadian human milk (*P* values < 0.001 unless indicated)

Congener	47	99	100	153	209
PBDE 28	0.922	0.791	0.832	0.353	0.074; *P* = 0.200
PBDE 47		0.920	0.907	0.389	0.061; *P* = 0.292
PBDE 99			0.858	0.401	0.075; *P* = 0.197
PBDE 100				0.592	0.085; *P* = 0.142
PBDE 153					0.039; *P* = 0.503

PBDE: Polybrominated diphenyl ether.

the other, lower brominated congeners.

ΣHBCD concentrations in the human milk analyzed were not correlated with ΣPBDE concentrations (r = 0.051, *P* = 0.377). This observation may be a result of HBCD being used primarily in insulation for buildings (e.g., polystyrene)[5], rather than in electronics and other consumer products.

Impact of maternal characteristics

Neither ΣPBDE (r = 0.019, *P* = 0.739) nor ΣHBCD (r = 0.084, *P* = 0.148) concentrations were correlated with maternal age in the human milk samples analyzed. This differs from legacy polychlorinated biphenyls (PCBs), where relationships between maternal age and chemical concentration in human milk (r = 0.385, *P* < 0.001) were observed[48]. It is, however, consistent with observations in human milk from the MIREC study for novel halogenated flame retardants (Σ9 NHFRs) which were also not correlated with age (r = 0.016, *P* = 0.709)[49]. The Spearman correlation was determined between maternal age and concentrations of the sum of nine novel halogenated flame retardants {Σ of 2,4,6-tribromophenyl allyl ether (TBP-AE/ATE); 2,3,5,6-tetrabromo-*p*-xylene (TBX); 1,2,3,4,5-pentabromobenzene (PBBZ); benzene, 1,2,3,4,5-pentabromo-6-methyl/pentabromotoluene (PBT); benzene, 1,2,3,4,5 pentabromo-6-ethyl/pentabromoethylbenzene (PBEB); benzene, 1,3,5-tribromo-2-(2,3-dibromopropoxy)/2,3-dibromopropyl 2,4,6-tribromophenyl ether (TBP-DBPE/DPTE); benzene, 1,2,3,4,5,6-hexabromo/hexabromobenzene (HBB); benzene, 1,1'-[1,2-ethanediylbis(oxy)] bis[2,4,6-tribromo-/1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), and benzene, 1,1'-(1,2-ethanediyl)bis [2,3,4,5,6-pentabromo-/decabromodiphenylethane (DBDPE)}[49].

An ANOVA was performed to examine the relationship between ΣPBDE and ΣHBCD concentrations with characteristics of the participants who provided milk for these analyses [e.g., parity, pre-pregnancy body mass index (BMI), and education level]. A woman's parity (1, 2, 3, 4+) impacted neither median ΣPBDE concentrations (*P* = 0.483) nor ΣHBCD concentrations (*P* = 0.366). This lack of relationship between parity and median concentrations is similarly consistent with the results of the NHFR concentrations in samples from this cohort (*P* = 0.777)[49]. Pre-pregnancy BMI (< 20, 20-25, > 25-30, > 30-35, > 35, no information provided) was not a factor that impacted ΣPBDE (*P* = 0.372) or ΣHBCD (*P* = 0.073) concentrations. The education level of the participants was broken down into a variety of categories [having some/completing high school, having earned the diploma, having taken some college classes, having earned college or trade school diploma, having earned an undergraduate university degree, and having earned a graduate degree (Master, Ph.D.)]. The ΣPBDE and ΣHBCD concentrations were not significantly impacted by this factor (*P* = 0.878 and *P* = 0.745, respectively). This result is again consistent with the lack of relationship between ΣNHFR concentrations and the groups of individuals with different levels of education (*P* = 0.168)[49].

Temporal trends

Both of the BFRs examined in the present study have been previously measured in Canadian human milk. The prior work from our laboratory did not include PBDE 209 because it was not consistently present and, at the time, it was thought to be a minor contributor to overall PBDE concentrations[28]. Total PBDE concentrations previously were reported as a total of seven congeners (Σ28, 47, 99, 100, 153, 154, 183) with concentrations ranging from 0.552 to 596 ng·g^{-1} lipid (median 2.99 ng·g^{-1} lipid; geometric mean 3.54 ng·g^{-1} lipid) for samples collected from across southern Canada in 1992 and from 0.841 to 956 ng·g^{-1} lipid (median 22.1 ng·g^{-1} lipid; geometric mean 25.2 ng·g^{-1} lipid) for the 2002 collection[28]. Median/geometric mean ΣPBDE concentrations (19.9 and 21.1 ng·g^{-1} lipid, respectively) in human milk collected from Hamilton, Ontario, in 2005 indicated that PBDE concentrations had stabilized[28]. The sum of concentrations for these same seven congeners in the present study, 2008-2011 collection, as part of the MIREC study, ranged from 0.473 to 257 ng·g^{-1} lipid (14.6 and 15.5 ng·g^{-1} lipid, median and geometric mean, respectively), suggesting that PBDE concentrations in human milk began to decline relative to concentrations observed in 2002.

While ΣPBDE concentrations appear to be declining, not all individual congeners follow this trend. A comparison of the previous data from our laboratory (1992, 2002 and 2005) with the data from the present work does show similar patterns for several of the congeners considered. The lowest median concentrations of PBDE 47, 99, 100, and 154 were observed in 1992, followed by increased concentrations in 2002, and appeared to stabilize by 2005, followed by a decline observed in the present work [Table 4]. Median PBDE 28 concentrations increased between 1992 and 2002, reaching the highest concentration observed in 2005, followed by a decline, while a comparison of the median PBDE 183 concentrations over time did not show a clear trend. In contrast to the other congeners investigated, the median PBDE 153 concentration determined in human milk from the present study was higher than the values reported in 1992, 2002 or 2005, although the results from 2002 and 2005 suggested the beginning of a decline [Table 4]. This variability in temporal trends among PBDE congeners is consistent with what has been reported for human milk collected in Sweden[50], although the pattern observed is similar to that observed in the serum of Swedish mothers where a decrease in PBDEs 47, 99, and 100 was observed and an increase in PBDE 153 was determined[51].

An additional study that measured PBDE concentrations (Σ four congeners: 47, 99, 100, and 153) in human milk from two discrete communities in Canada (Kingston, Ontario in 2003/2004 and Sherbrooke, Québec in 2008/2009) has also been reported[52]. PBDE concentrations ranged from 2.7 to 108 ng·g^{-1} lipid and 2.9 to 119 ng·g^{-1} lipid, with median concentrations of 11 and 15 ng·g^{-1} lipid in Kingston and Sherbrooke, respectively[52]. The Σ47, 99, 100, and 153 congeners in the present study had a wider concentration range than those observed in the two cities (0.02-251 ng·g^{-1} lipid), albeit with only slightly elevated median (13.7 ng·g^{-1} lipid) and geometric mean (14.7 ng·g^{-1} lipid) concentrations than those reported for Kingston and Sherbrooke. The Canadian data over time suggest that PBDE concentrations peaked in Canadian human milk during the early 2000s. These observations are consistent with the timing of the reviews of tetra-, penta-, hexa- and heptabrominated diphenyl ethers as part of the Stockholm Convention, which listed them as persistent organic pollutants in 2009[53].

HBCD was determined previously in human milk from one community in northern Canada (Nunavik) at two time points (1989-1991 and 1996-1999) and one location in southern Canada (Hamilton, Ontario) in 2005[28]. The early HBCD analysis of milk from Nunavik measured α-HBCD alone [0.1-0.6 ng·g^{-1} lipid (1989-1991) and 0.1-13.3 ng·g^{-1} lipid (1996-1999)][28]. In contrast, the analysis of samples collected from Hamilton in 2005 involved the determination of α-, β- and γ- isomers. ΣHBCD concentrations in those samples ranged from 0.1-28.2 ng·g^{-1} lipid, with median and geometric mean concentrations of 0.7 and 0.6 ng·g^{-1} lipid, respectively. The results of the pan-Canadian samples collected in the present study ranged from below the

Table 4. Median PBDE concentrations of select individual congeners in Canadian human milk over time, previous data taken from Ryan and Rawn (2014)[28]

Compound	Median concentration (ng·g^{-1} lipid)			
	1992	2002	2005	Present study
PBDE 28	0.108	0.948	1.40	0.550
PBDE 47	1.42	12.9	12.1	7.44
PBDE 99	0.507	3.30	2.55	1.09
PBDE 100	0.228	1.91	1.52	1.17
PBDE 153	0.304	1.32	1.11	2.01
PBDE 154	0.042	0.174	0.147	0.077
PBDE 183	0.173	0.121	0.172	0.043

PBDE: Polybrominated diphenyl ether.

method detection limits to 7.66 ng·g^{-1} lipid [median 0.303 ng·g^{-1} lipid (303 pg·g^{-1} lipid)]. These data suggest that HBCD concentrations declined in Canadian human milk from the early investigations into HBCD concentration to the collection period covered in the present study, similar to the declining temporal trend for PBDEs.

Comparison with international studies

PBDE concentrations remained elevated in Canadian human milk relative to other countries during the sample collection period. This observation is consistent with the conclusions of a systematic review of global data for these compounds in human milk collected between 2000 and 2012[54]. European countries continue to have lower PBDE concentrations in human milk [Table 5] in comparison to Canadian results. South American PBDE concentrations are similarly low relative to the concentrations reported in North America [Table 5]. The selection of representative congeners used for the determination of ΣPBDE concentrations, however, makes the direct comparison between regions challenging. While some areas of Asia have lower PBDE concentrations in human milk relative to Canada, other regions show elevated levels. HBCD concentrations in Canadian human milk seem to be within the range reported internationally [Table 6]. In contrast to the PBDEs, HBCD concentrations in human milk collected from some European countries are elevated over concentrations reported in the present study, where collection occurred between 2008-2011.

CONCLUSIONS

PBDE concentrations declined in Canadian human milk between the early 2000s and the collection period of the present study (2008-2011). Maximum HBCD concentrations determined in the present study were lower than the previous maximum values measured in Canadian human milk. Concentrations of these BFRs in human milk were not correlated with maternal age. Parity (1, 2, 3, 4+) and pre-pregnancy BMI (< 20, 20-25, > 25-30, > 30-35, > 35) did not significantly impact PBDE levels (P = 0.483, P = 0.372, parity and pre-pregnancy BMI, respectively) or HBCD concentrations (P = 0.366, P = 0.073, parity and pre-pregnancy BMI, respectively). Maternal education level similarly did not affect PBDE (P = 0.878) or HBCD (P = 0.745) concentrations in the human milk from the MIREC study. Ongoing monitoring of these BFRs is required to establish whether a continued decrease in concentration over time is occurring in Canadian human milk, following regulatory action on a global scale.

Table 5. International PBDE concentrations (ng·g^{-1} lipid) reported in human milk

Country	*n*	Range	Median	Congeners included	Year(s) of collection	Ref.
North America						
Canada	298	0.473-257	14.6	28, 47, 99, 100, 153, 154, 183	2008-2011	Current study
Canada	298	0.071-267	15.6	Σ20 PBDE congeners	2008-2011	Current study
USA	31	4.79-18.6		15, 28, 47, 99, 153	2016-2020	Jung *et al.*, 2023[55]
	10		8.78 (non-firefighters)			
	21		9.89 (firefighters)			
USA	50	1.46-1,170	15.0	28, 47, 85, 99, 100, 139, 153, 154, 183	2019	Schreder *et al.*, 2023[56]
South America						
Brazil	200	0.14-6.5 (wet weight, ww)	2.33 (geometric mean, ww)	28, 47, 99, 100, 153, 154, 183	2019-2020	Souza *et al.*, 2022[57]
Colombia	60	0.33-16.4	1.28	28, 47, 99, 100, 153, 154, 183	2014-2015	Torres-Moreno *et al.*, 2023[58]
Europe Scandinavia						
Denmark	438	1.22-111	4.90	28, 47, 99, 100, 153, 154, 183	1997-2002	Antignac *et al.*, 2016[31]
Finland	22	1.47-19.0	5.19	28, 47, 99, 100, 153, 154, 183	1997-2002	Antignac *et al.*, 2016[31]
Sweden	198	0.33-73	1.2; 1.3; 1.4; 1.8 (different locations)	47 99 100 153	2000-2004	Glynn *et al.*, 2011[25]
		< 0.1-17	0.3, 0.2, 0.2, 0.5			
		< 0.08-18	0.3, 0.2, 0.3, 0.4			
		0.2-8.0	0.6, 0.5, 0.6, 0.7			
Other						
Czech Republic	231	0.05-0.99 (PBDE 47) 0.15-1.31 (PBDE 99) 0.15-0.79 (PBDE 153) (5-95 percentile)	0.15, 0.19 (2 locations, PBDE 47) Not calculated (PBDE 99) 0.32 (1 location only calculated, PBDE 153)	47, 99, 153 individually	2019-2021	Parizek *et al.*, 2023[59]
France	96	0.45-15.3	1.47	28, 47, 99, 100, 153, 154, 183	2011-2014	Antignac *et al.*, 2016[31]
Ireland	16[1]	1.7-24	2.5	28, 47, 99, 100, 153, 154, 183, 209	2016-2018	Wemken *et al.*, 2020[32]
Asia						
China (Shanghai)	36	7.9-2,980 (pg·g^{-1} ww)	32 pg·g^{-1} ww	28, 47, 99, 100, 153, 154, 183, 209	2018-2019	Lin *et al.*, 2022[60]
China	105	0.458-157	1.1	28, 47, 99, 100, 153, 154, 183, 209	2018	Zhao and Shi, 2021[33]
Japan	40	< 0.2-69	1.5	28, 47, 99, 100, 153, 154	2005-2006	Fujii *et al.*, 2012[61]
Philippines	30	0.61-11	2.6	15, 28, 47, 99, 100, 153, 154, 183, 196, 197, 207, 209	2008	Malarvannan *et al.*, 2013b[26]
Vietnam	33	0.24-250	0.57, 0.73, 2.3, 3.2, 84 (by site)	15, 28, 47, 99, 100, 153, 154, 183, 196, 197, 206, 207, 209	2007	Tue *et al.*, 2010[23]
Middle East						
Saudi Arabia	75	0.2-3.6	2.8 (quartile 1-3)	47, 99, 153, 209, identified	Not specified	Yakout *et al.*, 2023[62]

[1]Pooled samples. PBDE: Polybrominated diphenyl ether.

Table 6. International HBCD concentrations (ng·g^{-1} lipid) reported in human milk

Country	*n*	Range	Median	HBCD isomers included	Year(s) of Collection	Ref.
North America						
Canada	298	0.010-7.66	0.303	Σα-, β-, γ-	2008-2011	Current study
Europe Scandinavia						
Denmark	435	0.02-28.7	0.31	α-	1997-2002	Antignac *et al.*, 2016[31]
Finland	22	0.03-2.19	0.31	α-	1997-2002	Antignac *et al.*, 2016[31]
Sweden	178	0.09-10	0.3, < 0.4, 0.4, 0.4 (by region)	Not specified (GC-MS analysis)	2000-2004	Glynn *et al.*, 2011[25]
Other						
Czech Republic	231	0.25-17.1 (ng·mL^{-1})	Not calculated	α-	2019-2021	Parizek *et al.*, 2023[59]
France	41	0.22-4.21	0.56	α-	2011-2014	Antignac *et al.*, 2016[31]
Ireland	16[1]	0.83-3.6	1.8	Σα-, β-, γ-	2016-2018	Wemken *et al.*, 2020[32]
Asia						
China	105	2.84-196	7.64	Σα-, β-, γ-	2018	Zhao and Shi, 2021[33]
Philippines	30	< 0.01-0.91	0.19	Σα-, β-, γ-	2008	Malarvannan *et al.*, 2013b[26]
Vietnam, some individuals involved in recycling	33	0.07-7.6	0.33, 0.42, 0.38, 0.36, 2.0 (by site)	Σα-, β-, γ-	2007	Tue *et al.*, 2021[23]

[1]pooled samples. HBCD: Hexabromocyclododecane; GC-MS: gas chromatography-mass spectrometry.

DECLARATIONS

Acknowledgements

The authors thank the participants in the study and their families. The authors also acknowledge the MIREC Study Group, in particular the site investigators, in addition to their field staff and the coordinating centre. The authors thank Sue Quade for carefully reviewing the manuscript and tracking reference formatting.

Authors' contributions

Made substantial contributions to the conception and design of the study and performed data analysis and interpretation: Rawn DFK, Arbuckle TE

Performed data acquisition, as well as providing administrative, technical, and material support: Sadler AR, Casey VA, Breton F, Sun WF, Feng SY

Availability of data and materials

The data that has been used is confidential.

Financial support and sponsorship

MIREC was funded by Health Canada's Chemicals Management Plan, the Canadian Institute of Health Research (grant MOP-81285), and the Ontario Ministry of the Environment.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Participants consented to participate in the study and ethics Board approval was obtained from Health Canada's ethics board, the ethics board of the MIREC coordination centre at CHU Sainte Justine, Québec, and the ethics board at each of the research centres (Vancouver, Edmonton, Winnipeg, Hamilton, Kingston, Ottawa, Sudbury, Toronto, Montréal and Halifax) (REB 2006-027H).

Consent for publication

Not applicable.

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