PERSISTENT ORGANIC POLLUTANT CONCENTRATIONS IN HUMAN MILK FROM CANADA, COMPARISON OF CURRENT WITH PREVIOUS RESULTS

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Introduction

Human exposure to environmental contaminants has been estimated using models that incorporate uptake and elimination of these compounds via multiple pathways (e.g., dietary intake, inhalation, metabolism, excretion, etc.). Biomonitoring, a more direct approach employed in the determination of chemical exposure, provides a measure of internal concentration at the time of sampling[1]. In addition to providing maternal exposure information, analysis of human milk provides insight into the dietary exposure of infants and young children to environmental contaminants[2]. Owing to the ethical considerations and limitations associated with direct sampling of very young humans, the application of biomonitoring results from the mother is beneficial in developing exposure information for both of these subpopulations.

Persistent organic pollutants (POPs) are lipophilic compounds that bioconcentrate and magnify through the food chain. With the knowledge that human milk is a lipid rich food for young children[3], sampling of human milk was undertaken in Canada for a number of lipophilic analytes since the 1960s. The well-established POPs, including polychlorinated dibenzo-*p*-dioxins (PCDDs)/polychlorinated dibenzofurans (PCDFs) were first measured in Canadian human milk in 1981, although PCBs have been studied in human milk since the late 1970s[4]. Temporally, both PCDD/Fs and PCBs have decreased in concentrations in human milk over the periods of study[4].

The Maternal-Infant Research on Environmental Chemicals (MIREC) study was a multi-year project established to determine the exposure of Canadian women to environmental chemicals on a national scale with participant recruitment initiated in 2008[5]. Participants were from ten cities across southern Canada. The MIREC program is the first Canadian study to collect samples for a broad spectrum of analyses (e.g., contaminants, nutrients, biomarkers) on a national scale from women through their pregnancies as well as their newborn children[5]. Biological specimens (e.g., blood, urine) were collected during each trimester of a participant's pregnancy and at the birth, cord blood and meconium were sampled. In addition, collection of personal information (e.g., age, parity, dietary consumption patterns, etc.) was performed via completion of questionnaires at each sampling time. Between two and ten weeks post-partum, participants also provided samples of human milk for chemical analysis: biomarkers, nutritional components, and environmental contaminants, including POPs. This program of study was approved by the research ethics board at Health Canada and the ethics boards in each of the participating centres (e.g., coordination centre, site locations).

Materials and Methods

Human milk samples were expressed by the participants over multiple days between two and 10 weeks post-delivery. Participants were requested to provide a total volume of 200 mL, to allow for analysis of multiple parameters for each participant. Samples were retained in the participants' refrigerators for up to three days, but samples collected over longer periods were stored in their freezers at home until sample collection was complete. Samples were shipped frozen to the coordination centre and sent to the laboratory to prepare aliquots for analysis of the different analyte classes. Samples were thawed prior to development of individual aliquots and each aliquot was re-frozen and stored until ready for extraction and analysis. Of the 1017 milk samples collected, 298 were directed for POP analysis. Analysis of individual samples was performed to ensure that the range in concentration could be determined, in addition to the measurements of central tendency.

The sample aliquots established for POP analysis (~25 g) were thawed and weighed into 250 mL Erlenmeyer flasks to which ^{13}C analogues of PCBs and PCDD/Fs were added prior to homogenisation with acetone: hexane (2:1) using a Polytron, following the method described previously[6]. Extracts were cleaned up initially using

digestion with sulphuric acid followed by column chromatography with Florisil, silica gel and carbopack C: celite. Discrete fractions were collected for analysis containing i) most PCBs and ii) PCDD/Fs plus non-*ortho* substituted PCBs. Analyses were performed using a Micromass Autospec Ultima high resolution mass spectrometer (Waters Corporation, Milford, MA) coupled to an Agilent 6890 gas chromatograph (Missisauga, ON). Separation was achieved using a 30 m J&W DB5 MS column (0.25 mm i.d. x 0.25 µm film thickness) with cool on-column injection[6]. Lipid determination was performed gravimetrically using an aliquot of the raw extract. Concentrations have been corrected for surrogate recoveries.

Results and Discussion

Total PCB concentrations reported are the Σ of 37 congeners and ΣPCDD/Fs represent all seventeen 2,3,7,8-substituted congeners. Lipid adjusted concentrations are reported for the present work, where lipid content ranged from 0.75% - 7.84% (median = 3.22%). Of the 298 samples extracted and analysed, the PCB fraction of two samples was lost during sample preparation. The majority of sample collection in the present study was performed between 2009 and 2011 with only a few samples collected in 2008.

ΣPCB concentrations ranged from 9.8 ng g^{-1} lipid to 510 ng g^{-1} lipid in the human milk samples collected as part of the MIREC study, while ΣPCDD/F concentrations ranged from 23 ng kg^{-1} lipid to 290 ng kg^{-1} lipid (Table 1). The PCDD/F and PCB concentrations corresponded to the World Health Organization Toxic Equivalency established in 2005 for PCDD/Fs and dioxin-like PCBs (WHO $TEQ_{2005\ PCDD/F + DL\text{-}PCB}$) ranging from 2.2 to 27 ng kg^{-1} lipid.

The detection frequency of the dominant contributors to the overall TEQ, 2,3,7,8 tetrachlorodibenzo-*p*-dioxin and 1,2,3,7,8 penta-substituted dioxin, were 98% and 99%, respectively. Octachlorodibenzodioxin was observed in 100% of the samples analysed and, of the PCDD/F congeners analysed, it was present at the highest concentrations in human milk, similar to observations reported in the literature[7-9].

The dominant contributors to ΣPCBs in human milk were 153 > 138 > 180 and 118. Among these, only PCB 118 contributes to the TEQ and it is the largest mono-*ortho* PCB contributor to TEQ concentrations. The non-*ortho* congeners 126 and 169, however, contribute more greatly to the overall TEQ, despite their relatively lower mean concentrations.

Table 1. PCB (ng g^{-1} lipid), PCDD/F (ng kg^{-1} lipid) and WHO TEQ_{2005} (ng kg^{-1} lipid) concentrations in human milk collected from across Canada between 2008 and 2011

Compounds	Range	Median	Arithmetic Mean	95th Percentile
ΣPCDD	17 – 260	48	56	110
ΣPCDF	6.9 – 110	34	37	65
ΣPCDD/F	23 – 290	57	65	130
Σ PCB_{37}[1]	9.8 – 510	36	50	130
Σ Indicator PCBs[2]	5.2 – 300	19	27	74
WHO TEQ_{PCDD}	0.38 – 13	2.9	3.3	6.4
WHO TEQ_{PCDF}	0.61 – 8.3	1.9	2.1	3.9
WHO $TEQ_{PCDD/F}$	1.6 – 19	4.9	5.4	9.6
WHO $TEQ_{mono\text{-}ortho\ PCB}$	0.04 – 1.5	0.16	0.20	0.43
WHO $TEQ_{non\text{-}ortho\ PCB}$	0.36 – 6.7	1.1	1.3	2.7
WHO $TEQ_{DL\text{-}PCB}$	0.40 – 7.8	1.3	1.5	3.1
WHO $TEQ_{PCDD/F + DL\text{-}PCB}$	2.2 – 27	6.3	6.9	13

[1]Σ of PCB 18, 28, 47, 49, 52, 66, 74, 77, 81, 99, 101, 105, 110, 114, 118, 123, 126, 128, 138, 141, 153, 156, 157, 167, 169, 170, 178, 180, 183, 187, 189, 194, 195, 201, 203, 206, 209

[2]Σ of PCB 28, 52, 101, 138, 153, 180

No significant difference in PCB or PCDD/F concentration over the collection time period was observed (PCB p = 0.630; PCDD/F p = 0.737) in this study (Figure 1). These results may be a function of the narrow time period over which samples were collected (four years), although decreasing trends have been observed within a three-year period in previous Canadian work[4]. The variability observed between sampling locations also was not

large, indicating that PCB and PCDD/F concentrations in Canadian women's milk are fairly uniform across southern Canada, consistent with earlier results[4]. This uniformity in human milk PCB and PCDD/F concentrations, however, has not extended to northern Canada, where elevated concentrations in human milk from Nunavik (northern Quebec) were observed (e.g., median WHO $TEQ_{2005\ PCDD/F}$: 9.3 ng kg^{-1} lipid; WHO $TEQ_{2005\ DL\text{-}PCB}$ 4.0 ng kg^{-1} lipid in 2002) relative to southern Canada (WHO $TEQ_{2005\ PCDD/F}$: 5.8 ng kg^{-1} lipid; WHO $TEQ_{2005\ DL\text{-}PCB}$ 2.3 ng kg^{-1} lipid in 2002)[4].

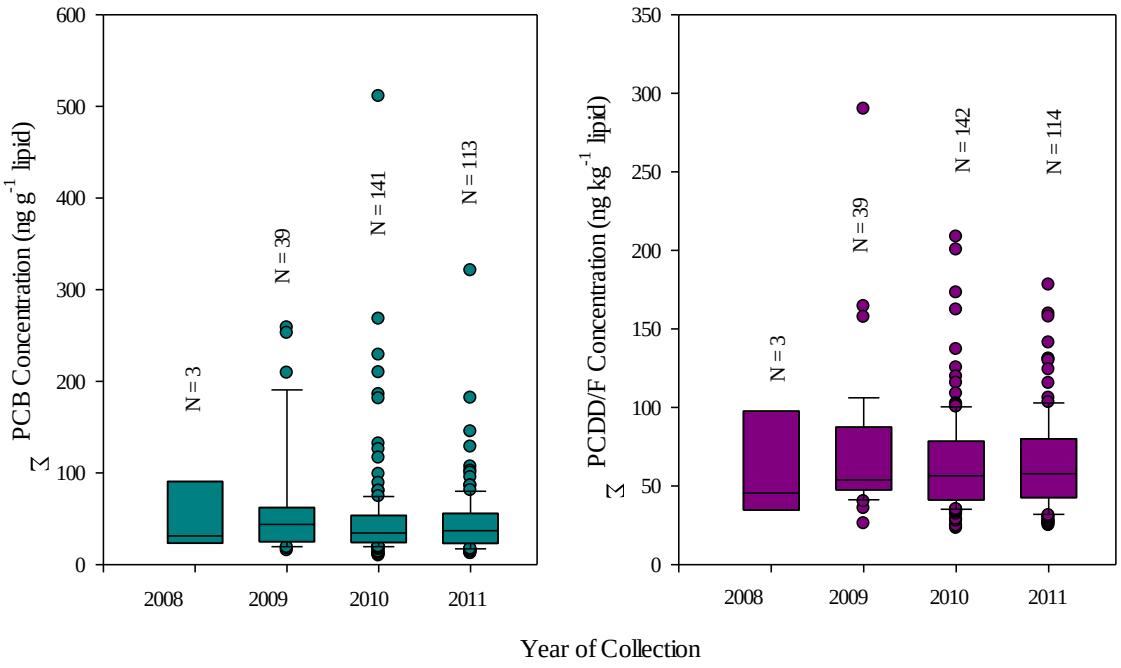

Figure 1: ΣPCB and ΣPCDD/F concentrations in human milk between 2008 and 2011. Boxes indicate 25th, 50th and 75th percentiles. Points indicate data outside of the 10th (⊥) and 90th (┬) percentiles.

Although a decrease in TEQ concentration has been observed in Canadian human milk over time, the results from the present study indicate that the concentrations in Canadian human milk have stabilised (Figure 2). The geometric mean [GM] $WHO_{2005\ TEQPCDD/F\ +\ DL\text{-}PCB}$ concentration measured in human milk during 1992 was 17.6 ng kg^{-1} lipid), with a stepwise decrease in GM concentration to between 5.7 ng kg^{-1} lipid to 6.6 ng kg^{-1} lipid, which were observed as part of the present study, between 2008 and 2011 (Figure 2). The reduction in WHO TEQ concentrations seems to be driven by the decrease in PCDD TEQ concentrations, with less of an impact from the PCDF, non-*ortho* and mono-*ortho* PCB TEQ concentrations over this period of study (~20 years). This decrease in PCB and PCDD/F concentration temporally also has been reported in human milk from Sweden between 1996 and 2006[10].

The WHO $TEQ_{2005\ PCDD/F\ +\ DL\text{-}PCB}$ concentrations observed in the present study (2.2 to 27 ng kg^{-1} lipid) are within the range measured in individual human milk samples collected in France during 2007 (WHO $TEQ_{2005\ PCDD/F\ +\ DL\text{-}PCB}$ 2.6 - 52 ng kg^{-1} lipid)[11]. Although the maximum PCDD/F concentrations observed in human milk from Canada collected between 2008 and 2011 (Figure 1) are higher than reported in other recent European studies[7,8], the Canadian measurements of central tendency (e.g., GM, mean, median) are similar to or lower than reported concentrations, which is consistent with our measurement of these compounds in samples from individual women rather than analysis of pooled samples. The Canadian WHO TEQ_{2005} concentrations also exceeded concentrations determined in individual human milk from New Zealand that was collected between 2007 and 2010 (WHO $TEQ_{2005\ PCDD/F}$ 1.4 – 11 ng kg^{-1} lipid)[12]. In each year of the present study, with the exception of 2008, when there were only a very limited number of samples collected (n = 3), the large number of samples collected may have contributed to the wide range in concentrations observed in the present study.

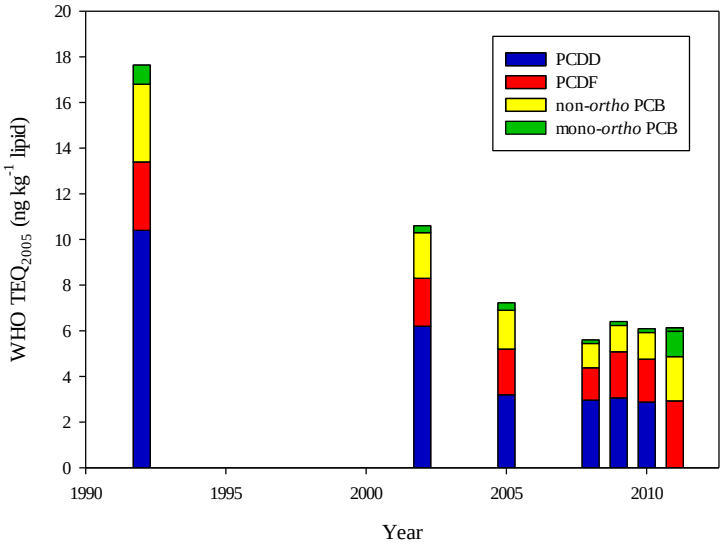

Figure 2: Geometric mean WHO TEQ_{2005} concentrations (ng kg^{-1} lipid) in Canadian human milk between 1992 and 2011.

Acknowledgements

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References:

1) Rawn DFK, Ryan JJ, Sadler AR, Sun W-F, Haines D, Macey K, van Oostdam J. (2012); *Environment Int*. 47: 48-55.

2) Jakobsson K, Fång J, Athanasiadou M, Rignell-Hydbom A, Bergman Å. (2012); *Environment Int*. 47: 121-130.

3) Croes K, Colles A, Koppen G, Govarts E, Bruckers L, Van de Mieroop E, et al. (2012); *Chemosphere*. 89: 988-994.

4) Ryan JJ, Rawn DFK. (2014); *Chemosphere*. 102: 76-86.

5) Arbuckle TE, Fraser WD, Fisher M, Davis K, Liang CL, Lupien N, Bastien S, Velez M.P., et al. (2013); *Paediatric and Perinatal Epidemiology*. 27: 415-425.

6) Rawn DFK, Sadler AR, Quade SC, Sun W-F, Kosarac I, Hayward S, Ryan JJ. (2012); *Chemosphere*. 89: 929-935.

7) Pratt IS, Anderson WA, Crowley D, Daly SF, Evans RI, Fernandes AR, Fitzgerald M, Geary M., et al. (2012); *Chemosphere*. 88: 865-872.

8) Vigh É, Colombo A, Benfenati E, Håkansson H, Berlund M, Bódis J, Garai J. *Sci. Tot. Environ*. (2013); 449: 336-344.

9) Bianco G, Zianni R, Anzillotta G, Palma A, Vitacco V, Scrano L, Cataldi TRI. (2013); *Anal. Bioanal. Chem*. 405: 2405-2410.

10) Lignell S, Aune M, Darnerud PO, Cnattingius S, Glynn A. (2009); *Environmental Research*. 109: 760-767.

11) Focant J-F, Fréry N, Bidondo M-L, Eppe G, Scholl G, Saoudi A, Oleko A, Vandentorren S. (2013); *Sci. Tot. Environ*. 452-453: 155-162.

12) Mannetje A, Coakley J, Bridgen P, Brooks C, Harrad S, Smith AH, Pearce N, Douwes J. (2013); *Sci. Tot. Environ*. 458-460: 399-407.