

## Polybrominated diphenyl ethers (PBDEs) in maternal and cord blood plasma of several Northern Canadian Populations

John Jake Ryan<sup>1</sup>, Jay van Oostdam<sup>2</sup>

<sup>1</sup>Bureau Chemical Safety, Health Canada, Ottawa

<sup>2</sup>Management Toxic Substances Division, Health Canada, Ottawa

### Introduction

The pioneering work of Norden and Meironyte<sup>1,2</sup> in Sweden showed that the brominated flame retardants, polybrominated diphenyl ethers (PBDEs), were increasing in human milk in the 1990's in contrast to the more studied persistent organic pollutants (POPs) such as PCBs and dioxins which were decreasing. As a result a great deal of scientific activity<sup>3-6</sup> has focussed on PBDEs in the last few years. New studies have identified their ubiquity in both the environment and man and confirmed their bioaccumulation, persistence, and toxicity. One of the four main characteristics of a POP is transportation from the place of use or origin to more remote regions usually by aerial mechanisms. This transportation of PBDEs along with biomagnification has been shown in environmental biota<sup>7, 8</sup> from the Arctic but to date little or no information is available on the presence of PBDEs and other emerging POPs in northern populations. We showed recently<sup>9</sup> that PBDE congeners were present at ppb (parts per billion; 10<sup>-9</sup>) levels in human milk lipid from samples collected from Inuits residing in Nunavik (northern Quebec). The levels of PBDEs in Nunavik were 2 to 3 times lower than those in southern Quebec and, in 20 samples for each time period, increased from a median value of about 1.7 ppb in 1989-91 to 6.8 ppb in 1996-2000. Other POPs such as PCBs and dioxins decreased in both groups. However no human data on PBDEs, an emerging POP, are available from other parts of Arctic Canada.

The Northern Contaminants Program (NCP) funded by Indian and Northern Affairs Canada has carried out a number of baseline studies in Nunavut and the North West Territories of northern Canada (figure 1) to assess the exposure of indigenous peoples to a variety of chemical classes including POPs and metals. These studies, summarized by Walker et al<sup>10</sup>, have used both maternal and cord human blood plasma as the media from sampling which took place in four phases over the years 1994-1999. Small amounts of individual blood plasma have remained from these investigations. We combined these individual samples into 23 composite samples of maternal and cord blood based mainly on the region and ethnicity of the donors. These composites have been used to study the exposure of northern peoples to PBDEs and to estimate, where possible, the influence of ethnicity, region of collection, and time on such exposure. Comparison is also made between the levels in plasma from northern populations and in human milk from those inhabiting the more numerous south.

## Methods and Materials

**Samples and composites.** Maternal and umbilical cord blood samples were collected from 1994 to 1999 from individuals from various communities in the Northwest (NWT) and Nunavut Territories for measurement of a range of persistent organic pollutants and toxic metals.<sup>10</sup> Blood samples were centrifuged to obtain plasma fractions, which were stored at -20°C in glass containers. After completion of this work, the remnant plasma samples (560 in total) were pooled to form 10 maternal and 13 cord composite samples based on the geographical region in which donors resided (Inuvik and Mackenzie for NWT and Baffin, Kivalliq, and Kitikmeot for Nunavut) and on donor ethnicities (Inuit, Dene/Métis, and Caucasian) (table 1). The number of individual samples in each plasma composite varied between 13 and 61 and the weight of sample for analysis was between 13 and 28 g. A relatively large sample weight is required for these analyses since the compounds studied accumulate in the lipids and the lipid content of blood is relatively low (less than 1 %).



Figure 1- Canadian map showing the northern regions of Nunavut and North West Territories from which maternal and cord human plasma samples were collected between 1994-1999.

**Table 1- characteristics of maternal human blood plasma composites**

Number	Year sampling	Ethnicity	Region	No individuals	Amount plasma, g	extractable lipid %
M1	1998-9	Caucasian	Inuvik	19	22.3	0.62
M2	1998-9	Dene/Metis	Inuvik	40	24.9	0.57
M3	1994-5	Inuit	Kitikmeot	13	24.0	0.67
M4	1994-5	Inuit	Kitikmeot	34	14.6	0.68
M5	1994-9	Caucasian	Mackenzie	24	20.5	0.55
M6	1994-9	Caucasian	Mackenzie	29	23.5	0.63
M7	1994-9	Caucasian	Mackenzie	29	22.9	0.71
M8	1994-5	Dene/Metis	Mackenzie	21	16.6	0.67
M9	1994-5	Dene/Metis	Mackenzie	10	22.4	0.62
M10	1994-9	Inuit	Several	23	27.8	0.57

Analysis Prior to sample extraction and purification, solutions of 500 pg each per congener of the eight most common PBDE congeners (di- up to hepta-) and the deca- (209) carbon-13 labelled surrogate standards were added to each sample to be analysed using the isotope dilution internal standard technique.

Extraction and purification Plasmas were denatured with ethanol and aqueous ammonium sulfate followed by two extractions with hexane. The dried and solvent evaporated hexane extracts were used to calculate the lipid content. The lipid residue, reconstituted in hexane and containing the persistent organic pollutants POPs, was defatted with portions of concentrated sulfuric acid, and purified further on columns of acidic silicate and magnesium silicate (Florisil). The second dichloromethane fraction from Florisil contained the PBDEs and dioxins. These two classes were further purified and separated on a carbon column. The PBDEs were not adsorbed and pass through the carbon with hexane. After evaporating the mixture to dryness, the final extract was reconstituted in 10 :L of toluene containing recovery standards prior to measurement.

#### Measurement by gas chromatography (GC)-mass spectrometry (MS)

The GC contained a DB-5 MS bonded phase capillary column of 15 m length, 0.25 mm id, and 0.1 :m thickness with retention gap. Injection of 1 :L was by the on column method at 80 EC with a fast ramp to 300 EC. The GC column was programmed in steps from 80 EC up to 300 EC in a total run time of about 15 min. The MS was a Kratos Concept 1S operating in the positive electron

impact (EI) mode at 40 eV ionization energy, source temperature of 250 E C, interface temperature of 270 EC, and mass resolution (10 % valley) of 10 K. Up to 14 masses were monitored in each group in the selected ion mode (SIM) usually with two masses for each isotopomer plus a lock and dummy mass. Masses monitored were the  $M^+$  (except deca- which was the  $M^+-2Br$  value) and include the di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, and deca- homologues from a little over 320 amu up to about 810 amu. Identification of each analyte was governed by its GC retention time (within 1.2 seconds of the standard), correct amu ion ratio (within 15% of standard), and a signal to noise ratio of at least 3:1. Under these conditions the absolute detectability for the PBDE congener 47 standard was about 0.5 picograms.

A standard curve was established consisting of a six to eight point concentration level of carbon 12 analytes with constant concentration of carbon 13 isotopomers. Concentrations in the sample were calculated from the standard curve using the isotope dilution internal standard method comprising relative response factors (RRFs), concentration responses, and amounts of whole weight and lipid in the unknown sample. Results were usually expressed in :g/kg (parts per billion;  $10^{-9}$ ; ppb) on a lipid basis. The measurement technique detects all PBDE homologues from di- up to deca- for which 23 carbon - 12 standards are now available. Positive responses existed for about 13 of these congeners and typically the major eight (congeners 28, 47, 85, 99, 100, 153, 154, 183) were summed to express the total or  $\Gamma$  PBDE comprising greater than 95% of PBDEs. Results were automatically corrected for analyte losses using the isotope dilution technique. Recoveries of the carbon-13 surrogates added at the beginning were calculated using the recovery standards added just prior to GC-MS.

### Quality Control Measures

Each sample batch contained a laboratory blank to gauge the amount of analyte picked up from the laboratory processing. This amount was subtracted from the total amount in the unknown sample prior to calculation of concentration. As PBDEs are ubiquitous, the contribution of the laboratory blank to the sample was significant and was the limiting factor in the sample limit of detection (LOD). A reference or repeat sample was also analysed in every batch to ensure the analytical process was under control and results compared to previous work and other laboratories. Five of the largest by weight sample composites, four maternal and one cord, were split into two equal portions and analysed by a second independent laboratory. Detection limits for PBDEs in human blood depend on the sample size, its lipid content, and contribution from the laboratory blank. Typically for a 20 mL sample of human blood the limit of detection (LOD) would be about 0.2 ppb for PBDE 47 on a lipid basis. Health Canada has participated successfully in both interlaboratory studies on brominated flame retardants (BFR) sponsored by the Bromine Science Environment Forum BSEF<sup>11</sup>.

### **Results and Discussion**

Generally we were successful in obtaining discrete values for PBDEs in all the maternal and most of the cord samples. PBDEs in the maternal plasma composites averaged on a lipid basis about 23 ppb (table 2) and that in the cord plasma lipid was about 12 ppb (data not tabulated). These values are expressed on a lipid basis with the cord samples containing almost 4 times less extractable lipid

than maternal samples (0.17 % and 0.63 %). However, as the composites were matched according to region and ethnicity and not individually, direct comparison cannot be made between maternal and cord blood. The maternal average level was similar to but lower than the mean value of 60 ppb (median 22 ppb) measured in the lipid of 98 human milks collected 2001-2 from southern Canada. At present there are no Canadian data on PBDEs in human blood but it is reasonable to compare the PBDE content of blood and milk on a lipid basis. Guvenius et al<sup>12</sup> found that the partitioning of PBDEs between individually matched maternal blood and milk when expressed on a lipid basis was close to one. On cursory inspection, there are no obvious correlations for high/low values for any of mother/cord or region/ethnicity factors. In summary the PBDE content of the northern blood samples is positive and somewhat lower than that measured in contemporary southern Canadian samples.

The results of this survey demonstrate that populations residing in the Northwest and Nunavut Territories in Arctic regions of Canada are exposed to PBDEs. The presence of PBDEs in cord blood plasma also indicates that exposure occurs *in utero* in agreement with earlier work<sup>13, 14</sup> on other populations. One distinction between the maternal and cord samples is the ratio of congener 47 to the total PBDEs (table 3). The ratio for maternal blood is about 0.5 and that for cord about 0.8 indicating metabolic change of the PBDE mixture in passing from mother to child. This limited data set also shows that PBDE levels in these northern samples are very similar to PBDE concentrations in southern populations, and that there are no marked differences between the various ethnic groups (Inuit, Dene/Metis, Caucasian) (table 3). The low samples numbers are insufficient to examine whether or not there are differences in PBDEs exposure and body burdens amongst various ethnic groups within regions that consume different diets, but these preliminary data do not support such a conclusion.

There are likely a number of routes of exposure to PBDEs that contribute to the observed plasma concentrations, including environmental contamination and consumption of traditional country foods. However, exposure has also likely occurred via consumption of commercial market foods as it is known that these foods imported mainly from the southern part of Canada and other countries contain PBDEs<sup>14</sup>. The results of this study suggest that a diet consisting of traditional foods does not necessarily result in higher body burdens of PBDEs as has been observed with other legacy POPs such as PCBs.

**Table 2- PBDE congeners in maternal human blood plasma composites collected from northern populations:** values in :g/kg on lipid basis

No	28	47	85	99	100	153	154	183	Σ PBDE
M1	0.8	15.1	0.4	4.2	1.6	2.1	0.2	1.4	25.8
M2	1.2	12.6	0.8	4.9	4.1	6.7	0.7	1.1	31.9
M3	0.2	4.8	0.8	7.6	2.1	2.5	0.9	0.8	19.6
M4	0.3	5.5	0.9	9.5	1.7	1.9	0.7	0.5	21.0
M5	0.5	6.7	0.2	3.0	0.9	0.8	ND(0.4)	0.9	13.1
M6	1.4	23.5	1.1	11.5	4.1	3.3	0.9	0.8	46.5
M7	1.1	7.2	0.2	2.8	1.1	1.5	0.3	1.1	15.3
M8	0.9	7.9	ND(.05)	3.7	1.0	0.8	ND(0.4)	0.6	14.9
M9	0.8	10.3	0.2	3.7	1.1	1.0	0.3	0.5	18.0
M10	1.1	15.6	0.4	4.8	2.3	2.0	0.5	0.6	27.2
<b>Mean</b>	<b>0.8</b>	<b>10.9</b>	<b>0.5</b>	<b>5.6</b>	<b>2.0</b>	<b>2.3</b>	<b>0.5</b>	<b>0.8</b>	<b>23.3</b>
Range	0.2-1.4	4.8-23.5	ND-1.1	2.8-11.5	1.0-4.1	0.8-6.7	ND-0.9	0.5-1.4	13.1-46.5

**Acknowledgement** Funding for this study is gratefully acknowledged to the Northern Contaminants Program, Indian and Northern Affairs Canada. Nicole G. Beaudoin (Food Research Division, Health Canada) helped with the tedious task of preparation of the plasma pools from hundreds of residual amounts of human plasma. The environmental analytical company, AXYS, of Sydney, BC is thanked for sample archiving, some cataloguing, and delivery of the individual blood vials.

<b>Table 3- Mean concentrations of PBDEs in maternal and cord plasmas by ethnic groups;</b> values in :g/kg (ppb) in lipid basis				
<b>Ethnic Group</b>	<b>n</b>	<b>Congener 47</b>	<b>Total PBDEs (n=7)</b>	<b>Ratio 47/Total</b>
<b>Maternal Plasma</b>				
Inuit	3	8.6	22.6	0.38
Dene/Metis	3	10.3	21.6	0.48
Caucasian	4	13.1	25.1	0.52
<b>Cord Plasma</b>				
Inuit	5	12.4	17.5	0.71
Dene/Metis	4	6.7	8.6	0.78
Caucasian	4	9.8	11.6	0.84

## References

1. Meironyté D, Norén K, Bergman Å. (1999) J Toxicol Environ Health 58, 329-341.
2. Norén K, Meironyté D. (2000) Chemosphere 40, 1111-1123.
3. Darnerud P O, Eriksen GS, Jóhannesson T, Larsen PB, and Viluksela M. (2001) Environ Health Perspect 109, 49-68.
4. Birnbaum L, Staskai DF. (2004) Environ Health Perspect 112, 9-17.
5. Hites RA. (2004) Environ Sci Technol 38, 945-956.
6. Gill U, Chu I, Ryan JJ, Feeley M. (2004) Rev Environ Contam Toxicol 182, 55-96.
7. Ikonomou MG, Rayne S., and Addison R. F. (2002) Environ Sci Technol 36, 1886-1892.
8. Wolkers H, Van Bavel B, Derocher AE, Wiig Ø, Kovacs KM, Lydersen C, Lindström G. (2004) Environ Sci Technol 38, 1667-1674.
9. Pereg D, Ryan JJ, Ayotte P, Muckle G, Patry B, Dewailly E. (2003) Organohalogen Cpds 61, 127-130.
10. Walker JB, Seddon L, McMullen E, Houseman J, Tofflemire K, Corriveau A, Weber J-P, Mills C, Smith S, Van Oostdam J. (2003) Sci Total Environ 302:27-52.
11. Ryan JJ, Patry B, Mills P, Beaudoin NG. Organohalogen Cpds 58 (2002) 173-176.
12. Guvenius DA, Aronsson A, Ekman-Ordeberg G, Bergman Å, Norén K. (2003) Environ Health Perspect 111, 1235-1241.
13. Mazdai A, Dodder NG, Abernathy MP, Hites RA, Bigsby RM. (2003) Environ Health Perspect 111, 1249 -1252.
14. Ryan JJ, Patry B. (2001) Organohalogen Cpds 51, 226 -229.