Concentrations of polybrominated diphenyl ethers in plasma samples from postmenopausal women in Quebec, Canada

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Introduction

Organobromine compounds such as polybrominated diphenyl ethers (PBDEs) are now largely distributed in the environment, due to their wide use as flame retardants in electronic equipments, plastics and textiles. Several PBDE congeners, their hydroxylated metabolites and brominatedbisphenol A-analogs induce the estrogen receptor signal transduction pathway *in vitro*[1] and may therefore increase the risk of hormone-related diseases. Studies in Sweden have revealed that PBDEs increased in human breast milk over the last decades[2,3]. However, in recent years a decrease in concentrations has been noted in this country[4]. A similar decline in levels has not been reported in plasma samples from people in North America; to the contrary, limited data for the U.S. population indicate a steep increase in PBDE blood concentrations between 1973 and 2003[5].

In the course of a pilot study investigating possible environmental risk factors of breast cancer, we analysed plasma samples obtained from 110 postmenopausal women living in Quebec (Canada) for several persistent organic pollutants. Here we present the concentrations of four polybrominateddiphenyl ethers, one polybrominated biphenyl (PBB 153) and PCB 153, the latter for comparative purposes.

Material and Methods

We recruited 110 women at a large breast cancer screening clinic located in Quebec City. Women were eligible if 1) they were postmenopausal, 2) they had no history of breast cancer, 3) they had no history of health problems related to steroid hormone metabolism, hepatic, thyroid or adrenal disease, 4) they were not reporting substantial weight loss or gain during the last 6 months (more than 10% of body weight); and 5) they had not taken hormone replacement therapy during the last three months. Women agreeing to participate provided an informed consent including authorization for blood sampling and banking of samples. The research nurse conducted a face-to-face interview and took anthropometric measures (weight, height, skinfold thickness measurements) and collected blood samples (75 ml). Blood specimens were collected in vacutainers with EDTA as the anticoagulant and were kept on ice until transported by the research nurse to the laboratory at the end of each morning and afternoon. Blood was processed within 2-3 hours of collection. Samples were centrifuged and the plasma aliquoted and stored at -80^{o}C in glass vials (pre-washed with hexane) until analysis.

Plasma samples were extracted on an Oasis HLB (540 mg; Waters Corp.) solid phase extraction column according to the method of Sandau et al[6]. Extraction and clean up were completed on a Rapidtrace Automated SPE workstation (Zymark Corp.) and evaporation was performed on a Labconco evaporator (Labconco Corp., Kansas City, MO). Lipids were removed from the extracted sample using a column containing 1 g activated Florisil (60-100 mesh; Fisher, Pittsburgh, PA, USA). The fraction containing the PBDEs was eluted using hexane/dichloromethane (9/1).

Samples were analysed on a Hewlett Packard (HP) 5890 Series II Plus gas chromatograph (GC) equipped with an HP G1512A automatic injector and a HP 5890B mass spectrometer (MS) (Agilent, Wilmington, DE, USA). The mass spectrometer was operated in selected ion monitoring (SIM) mode, using electron capture negative ionisation (ECNI) with methane (99.97%) as the reagent gas. Masses 79 and 81 were monitored for all the brominated

compounds with ^{13}C-PBDE 77 as the internal standard. The target ion was employed for quantification and the confirmation ion from the same isotopic cluster was used to confirm the identity of the compound. The source pressure was 1.8 torr and the source temperature was kept at 150°C. The GC was fitted with a 60 m DB-5 column (5 % phenyl-methylpolysiloxane; 0.25 mm i.d., 0.25 mm film thickness) from J&W Scientific (CA, USA). The carrier gas was helium, and all injections were 2 mL in splitless mode. The injector and transfer line were kept at 275°C and 280°C, respectively.

The lipids were determined enzymatically and the following summation formula was used to calculate the amount of lipids in each plasma sample[7]: TL = 1.677(TC – FC) + FC + TG + PL, where TL= total lipids, TC= total cholesterol, FC= free cholesterol, TG=triglycerides and PL= phospholipids. The quality of the analysis was assured through analysis of certified reference materials and participation in international interlaboratory comparison programmes.

Results and Discussion

Mean age of the participants was 58.3 years (range = 48-76 years). The concentrations of the four PBDE congeners and PBB 153 are shown in Table 1, together with PCB 153 for comparative purposes (lipid based concentrations were not available at the time of writing this extended abstract).

Table 1: Concentrations of PBDEs, PBB 153 and PCB 153 in plasma samples (pg/ml) from 99 postmenopausal in Quebec, Canada.

Compounds	Geometric Mean	Arithmetic Mean	Min	Max	LOD	% above LOD
PBDEs/PBB						
PBDE 47	45	289	< 4	15516	4	99
PBDE 99	8	93	< 2	6236	2	83
PBDE 100	6	49	< 2	3184	2	82
PBDE 153	9	39	< 2	1727	2	97
PBB 153	< 2	3	< 2	80	2	29
PCBs						
PCB 153	281	326	39	1269	10	100

Note: LOD=limit of detection. A value equal to half the limit of detection was substituted for nondetects to calculate mean values.

When analysing PBDEs there is always a great risk of having elevated blanks caused by background contamination in the laboratory. Pre-packed Florisil columns were found to be a substantial and variable source of PBDEs in blanks and therefore Florisil columns were prepared manually in the laboratory. Water blanks were continuously monitored when analysing these samples and the average level found for PBDE 47 was 16 pg/ml. For the other PBDE congeners these values were lower or the compound was not detected in the blank. The blank values were subtracted from the calculated values before levels were reported.

PBDE 47 was the dominating PBDE determined in these samples and it was detected in 99% of the samples. PBDE 99 was the second most abundant congener but it was detected in only 82% of the samples as opposed to PBDE 153 which was detected in 97% of the samples. PBDE 47 and 99 were highly correlated (Pearson's r = 0.91 on ln-transformed values; see Fig. 1).

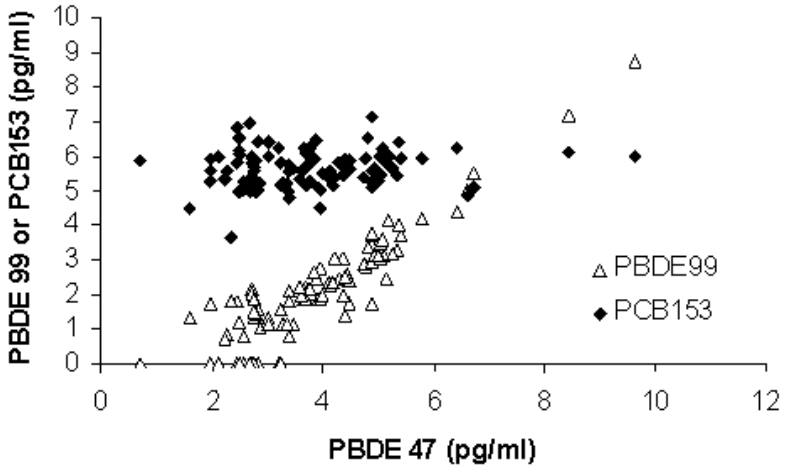

Figure 1. Correlations between plasma concentrations of PBDE 47 and PBDE 99 or PCB 153 (ln-transformed values) in 99 postmenopausal women from Quebec, Canada.

The arithmetic mean values were considerably higher than the geometric mean values for the PBDEs due to a few samples with extremely high levels. The maximum value observed for PBDE 47 was 15.6 ng/ml (Table 1). This concentration is more than 10 times higher than the highest value observed for PCB 153 (1.27 ng/ml plasma). In the samples with the extreme levels of PBDEs, several other brominated compounds were observed. These were, however, not identified due to lack of standards.

PBDE concentrations were not correlated to PCB 153 (Figure 1), indicating that the sources of exposure are different for PCBs and PBDEs. While exposure pathways for PBDEs have not been elucidated, diet may not be the most important contributor. Direct exposure through inhalation and dermal contact with these chemicals that are still being used may have a substantial contribution[8].

PBB153 was detected in only 29 % of the samples and was neither correlated to PBDEs nor to PCB 153. Levels were also considerably lower than those of the PBDEs supporting previously reported low levels of PBBs[8].

The fact that the geometric mean concentration of PBDE 47 is only 6 times lower than that of PCB 153 clearly indicates the need to monitor PBDEs in human samples on a regular basis. There is also a need to identify the most important sources of human exposure to the PBDEs in the general population.

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