Polybrominated diphenyl ethers in human milk from Australia

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Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardant compounds that are causing increasing concern as contaminants in biota and the environment. PBDEs are non polar, persistent organic chemicals and thus accumulate in body fat including human milk. Hence, human milk samples have been used to evaluate exposure of mothers and infants to PBDEs in a variety of countries [1,2,3].

Australia is an island continent in which both the levels of industrialization and the population density are relatively low. For the most part, the population base and industry is concentrated along the south eastern coastline. Information regarding the levels of PBDEs in the Australian environment and population is limited and this study represents the first investigation in pooled human milk samples obtained from Australian primiparae women. The aim of this study was to determine PBDEs concentrations in human milk from Australia, and assess geographical trends in the contamination. Furthermore, availability of samples from the early 1990s allowed assessment of historic exposure in one region.

Methods

Sample Collection

In total, 157 human milk samples were collected from 12 regions of Australia during the period March 2002 and September 2003. These were analysed as 17 pooled samples. The regions sampled were as follows with the number of pools shown in parenthesis: *Urban* - Brisbane (1), Sydney (2), Melbourne (4), Adelaide (2), Perth (1), Tasmania (1), Darwin (1); *Rural* - inland Queensland (1); inland NSW (1); Victoria (1); and *Regional* - Newcastle (1); Wollongong (1).

In addition to the samples collected in 2002/03, 24 milk samples that had been previously collected in 1993 by the Key Centre for Applied and Nutritional Measurement were also analysed. These samples were analysed as three pools each containing eight samples and are referred to as Melbourne 1993 A, B and C samples.

Following appropriate ethics application and approval, volunteering mothers were selected using the following criteria: primiparae with a singleton baby aged two - eight weeks; a resident of the area for the past five years; exclusively breastfeeding and willing to provide a minimum of 100 mL of expressed milk. Samples were collected by either direct expression into the glass container or by using a breast pump. They were stored and shipped frozen to the investigators' laboratory using overnight transport. Once collected, samples were pooled according to region with an optimum of 10 samples of equal volume for each pool.

Sample Analysis

Samples were analysed at the National Measurement Institute (NMI), Sydney, Australia for 16 PBDE congeners. At the time of this study, BDE-209 was not routinely analysed at this laboratory, hence it was not included in this study. For inter-laboratory comparison, duplicate pooled samples were analysed by the State Laboratory of NRW, Munster, Germany. The analytical methodology is described elsewhere[4]. Briefly, frozen, pooled human milk samples were thawed, sonicated and shaken to produce a homogeneous sample. A sub sample was spiked with a range of

isotopically labeled surrogate standards. Proteins were denatured with the addition of potassium oxalate then a liquid-liquid extraction was performed with 2:1 acetone:hexane. After cleanup, the extract was concentrated to near dryness. High resolution gas chromatography /high resolution mass spectrometry (HRGC/HRMS) was used to determine the levels of PBDEs in human milk matrices. This method provided data on 16 PBDE congeners determined by the isotope dilution quantification technique. Immediately prior to injection, internal standards were added to each extract. The GCMS resolution, performance and sensitivity were established for each MS run and the recoveries of all isotopically labeled surrogate standards were calculated and reported.

Results and Discussion

Inter-laboratory Comparison

Rural NSW and Melbourne B samples were analysed by both laboratories. Overall there was a good agreement between the results from the two laboratories for the two samples. The largest difference between the two laboratories was observed in both the rural NSW and the Melbourne B pools for congener 99 with a normalized difference of 60 % and 65 %, respectively. For this congener, the levels detected by NMI were higher than those detected by the State Laboratory of NRW in both pools. For congener 47, the levels detected by both laboratories were remarkably similar in both pools. The normalized differences for BDE-47 for rural NSW and Melbourne B pools were 3 and 3.7 %, respectively.

Study Results

PBDE congeners were detected in all samples analysed. Figure 1 represents a summary of results obtained for samples from 2002/03 and 1993. Lipid content was measured in all samples and gave an average concentration of 3.7 ± 0.5% lipid. The mean sum concentration (16 congeners) for the samples collected in 2002/03 was 11 ± 3.2 $ng.g^{-1}$ lipid and the results (mean, minimum, maximum, median and number of detected BDEs in the sample) are shown in Table 1 for the 2002/03 samples. In brief, the levels of sum PBDEs varied by a factor of 3.1 from a minimum of 6.0 $ng.g^{-1}$ lipid detected in the Tasmanian sample to a maximum of 18.7 $ng.g^{-1}$ lipid detected in the rural NSW sample. The data did not show a clear geographical trend, for example related to population density. This finding is consistent with the results of PBDEs in human serum in Australia[5].

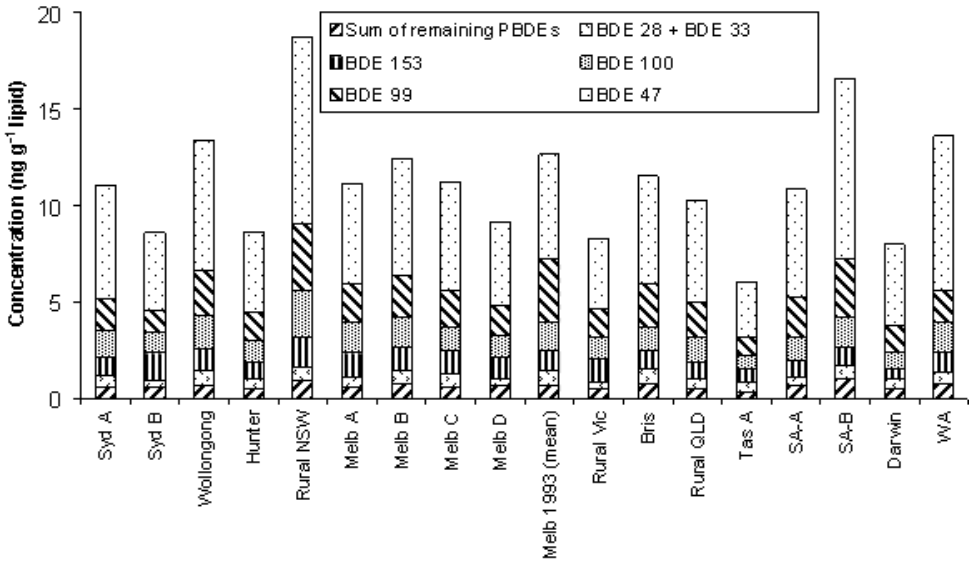

Figure 1. Summary of results obtained for samples from 2002/03 and 1993

Specific PBDE congeners, BDE 47, 99, 100,153 and 154 are known to dominate samples obtained from both environmental and human sources [1,2,3,6] . Similarly in this study, these were the dominant congeners detected in all samples from both 1993 and 2002/03. The concentration of BDE- 47 varied by a factor 3.4 from a minimum

concentration of 2.8 ng g^{-1} detected in the Tasmanian pool to a maximum concentration of 9.6 ng.g^{-1} detected in the rural NSW sample. For samples collected in 2002/03 the dominant congeners, BDE 47, 99, 100, 153 and 154 contributed an average of 50, 17, 12, 10 1 and 1 %, respectively, to the total concentration for each pooled sample. For 1993, these congeners contributed 42, 26, 12, 8 and 2 %, respectively, to the total.

This study represents the first to determine the levels of PBDE congeners in pooled human milk obtained in Australia. For this study the determination of a relationship between age and PBDE levels in Australia was not possible as pooled samples were used. The levels of PBDEs reported in this study are consistent with findings reported internationally. On a worldwide basis, the levels of PBDE compounds detected in Australian human milk are higher than those observed in continental European samples and Japan but are lower than those observed in North America [1,7,8]. There is little information about the exact sources or exposure pathways to PBDEs in Australia or internationally. As BDE 47 was the dominant congener this suggests that a significant proportion of exposure in Australia may be caused by the commercial Penta-BDE product which consists of 50-62 % penta-BDE[9]. Further investigation of these samples including analysis of individual samples may be warranted in order to determine the exact sources and the levels of these compounds in the Australian population.

The concentration of PBDEs in the historic samples ranged from 11 – 15 ng.g^{-1} lipid (13 ± 2.1 ng.g^{-1} lipid, data shown in Table 2). Interestingly we found no systematic differences between the analytical results obtained for the recently collected samples and those that were collected in 1993. In contrast to our results many other studies have found that the levels of PBDEs have increased from the early 1990s until 2002[8]. Unfortunately we have little information about these historic samples except that they were collected in the Melbourne region around 1993. Hence these few results do not provide good evidence on historic levels of PBDEs in human milk in Australia.

Table 1. Minimum, maximum, mean and median for PBDE compounds detected in pooled samples of human milk samples collected in 2002/03.

Values given are expressed on a lipid basis (ng g^{-1} lipid).

PBDE congener	Mean	Min	Max	Median	No. of positive detects
BDE 17	0.01*	n.d. (0.01)	0.02	0.01*	11
BDE 28 + BDE 33	0.56	0.3	0.78	0.54	17
BDE 47	5.6	2.8	9.6	5.6	17
BDE 49	0.12*	n.d. (0.07)	0.2	0.12*	11
BDE 66	0.07	0.03	0.17	0.07	17
BDE 77	n.c.	n.d. (0.001)	0.002	n.c.	3
BDE 85	0.14	0.05	0.26	0.12	17
BDE 99	1.9	1	3.5	1.8	17
BDE 100	1.3	0.69	2.3	1.2	17
BDE 138 + BDE 166	0.02*	n.d. (0.01)	0.04	0.02*	15
BDE 153	1.1	0.59	1.6	1.0	17
BDE 154	0.14	0.09	0.23	0.14	17
BDE 183	0.11*	n.d. (0.05)	0.23	0.1*	16
Sum PBDE	**11**				

BDE-71 and BDE-126 were not detected and BDE-119 was only detectable in one sample hence data not reported here.

* incl. LOD values; n.d. ( ) – not detected (limit of detection)

Table 2. Minimum, maximum, mean and median for PBDE compounds detected in pooled samples of human milk samples collected in 1993.

Values given are expressed on a lipid basis (ng g^{-1} lipid).

	Mean	Min	Max	Median	No. of positives detected
BDE 17	0.008	0.006	0.01	0.008	3
BDE 28 + BDE 33	0.8	0.6	1.0	0.8	3
BDE 47	5.4	4.1	7.0	5.2	3
BDE 49	n.c.	n.d. (0.09)	n.d. (0.2)	n.c.	0
BDE 66	0.04*	n.d. (0.04)	0.06	0.04*	2
BDE 85	0.1	1.3	1.7	0.1	3
BDE 99	3.3	0.04	0.04	3.2	3
BDE 100	1.5	0.9	1.2	1.5	3
BDE 138 + BDE 166	0.04			0.04	3
BDE 153	1.0			1	3
BDE 154	0.3			0.3	3
BDE 183	0.1*			0.2*	2
Sum PBDE excl. LOD	**13**				

BDE-71 and BDE-126 were not detected and BDE-119 was only detectable in one sample hence data not reported here.

* - including LOD values; n.d. ( ) – not detected (limit of detection)

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