

PBDE levels in pooled serum samples of newborns, adolescents and adults from Flanders, Belgium

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Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants in polymer materials, textiles, electronic boards and other materials. Due to their widespread use and specific properties, such as high lipophilicity, persistency and bioaccumulative nature, these chemicals have already been detected in humans (Sjödin et al. 2003). They have been shown to possess toxicological potential (Darnerud 2003) and, therefore, their presence in high concentrations can have health consequences, especially for organisms at the top of the food chain, such as humans.

The aim of this study was to evaluate the actual contamination levels and profiles of PBDEs in human serum samples from Belgium and to investigate the relationship of PBDEs levels with age, residence area and other contaminants, such as polychlorinated biphenyls (PCBs).

Methods and materials

Sampling. Serum samples were collected from three different age groups: newborns (cord blood), adolescents and adults and 8 different regions (Table 1). Samples belonging to one age group and one region were pooled. The campaign was approved by the ethical committee of the University of Antwerp. Mothers were enrolled via 25 maternities and adolescents via 42 schools spread over the eight regions. Adults were selected out of community lists and contacted via letter followed by phone call. Inclusion criteria were living for at least 5 years in the area of interest, giving informed consent, being able to fill out Dutch questionnaires and belonging to the age group between 14 and 15 years for the youngsters and between 50 and 65 years old for the adults. The sampling period of the mothers was between October 2002 and December 2003. The adolescents were recruited between October 2003 and July 2004, while the adults were recruited between October 2004 and July 2005. The blood collection methods were tested on contamination and/or adhesion of the measured compounds. After blood sampling, serum was separated by centrifugation within one day in either the maternity, blood bank laboratories or by the field workers in the schools (adolescents) or at local sampling locations installed for the adults (Koppen et al. 2006). All serum samples were kept at +4 °C for maximum one week and, after pooling according to the above described criteria, they were kept at -20 °C until analysis.

In total 23 pooled samples (3 age groups x 8 regions, except for one sample not available), 4 procedural blank samples (5 ml water instead of serum) and 1 in-house laboratory reference material serum sample were processed in one batch. The following contaminants were targeted: PBDE

congeners (no. 28, 47, 49, 66, 85, 99, 100, 153, 154 (co-eluting with BB 153), 183 and 209, PCB congeners (no. 28, 52, 101, 118, 153, 138, 180 and 170), hexachlorobenzene (HCB) and p,p'-DDE.

Analysis. Serum samples were analysed using a previously reported procedure (Covaci and Voorspoels 2005). After addition of internal standards (PCB 143, BDE 104, BDE 140 and ¹³C-BDE 209) to 4.5 ml serum, the serum was mixed with 2 ml formic acid and 3 ml water. After sonication, the mixture was loaded onto a conditioned OASIS HLB solid-phase extraction (SPE) cartridge. The cartridges were washed with water, dried, eluted with 3 × 3 ml dichloromethane (DCM) and the eluate concentrated to ~ 1 ml under nitrogen. The extract was further cleaned-up on a 3 ml cartridge filled (from bottom to top) with acidified silica, activated silica and Na₂SO₄. Analytes were eluted with 3 ml hexane and 4 ml DCM. The final eluate was concentrated until dryness and resolubilised in 80 µl iso-octane.

All analyses were done using a GC-ECNI/MS equipped with a 10 m × 0.18 mm × 0.20 µm AT-5 column operated under instrumental conditions described by Covaci and Voorspoels (2005) with minor modifications. In order to achieve the analysis in the same run of PCBs and OCPs, the ion source temperature was set to 160 °C instead of 250 °C, normally used for PBDE analysis.

Quality assurance. Quantification was based on the sum of ions 79 and 81 for PBDEs, except for BDE 209 and ¹³C-BDE 209 for which ions *m/z* 487 and 495 were used, respectively. Five-points calibration curves were created for the quantification. The values for PBDE congeners obtained in the in-house laboratory material were in good agreement with previous results (sample Serum 11 from Covaci and Voorspoels 2005). Our laboratory participates successfully to the Arctic Monitoring and Assessment Programme ring test for PBDEs, PCBs and OCPs in human serum organised by the Toxicological Centre of Quebec (Canada).

For PBDE congeners consistently measured in the procedural blanks, the limit of quantification (LOQ) was calculated as 3 × SD of the blank values (Covaci and Voorspoels, 2005). Expressed on a lipid weight basis, LOQs ranged between 0.2 ng/g lw (for tri- to hepta-BDEs) and 20 ng/g lw (for BDE 209). The value of each PBDE congener in the procedural blank was subtracted from the corresponding value in the sample and the resulting value was compared to the LOQ calculated for each congener.

Data treatment. Values below the LOQ were assigned a value of p*LOQ, with 'p' the proportion of measurements with levels above the LOQ (Voorspoels et al. 2002). All statistical analyses were performed using Statistica for Windows and GraphPad InStat version 3.06 for Windows. One-way ANOVA with Tukey-Kramer post-hoc comparison was used to test differences in PBDE and PCB concentrations between the age groups. A Kruskal-Wallis test was used to investigate differences in the contaminant's levels between different regions.

Lipids. Total cholesterol (CHOL) and triglycerides (TG) were determined enzymatically using Roche tests in a separate aliquot of serum at a clinical laboratory. Total lipids (TL) were calculated as described by Covaci et al. (2006a) using the following formula: TL (g/l) = 1.12×CHOL + 1.33×TG + 1.48. Therefore, the concentrations of PBDEs in individual samples were reported as ng/g lipid weight (lw).

Results and discussion

BDEs 28, 49, 66 and 85 were below LOQ in all samples and were not taken therefore in the calculation of sum PBDEs. Although BDE 209 was present at very low and relatively constant levels, several serum samples presented very high values. The rigorous QA/QC protocol eliminates the possibility that these high values of BDE 209 are a result of contamination during sample processing. On the contrary, the most probable explanation is that some samples were contaminated during preparation of pools or during the repetitive cycles of thawing and aliquoting for other analyses. Excluding the samples with very high concentrations of BDE 209, the concentrations of BDE 209

ranged between < 20 and 104 ng/g lw for all age groups, with a detection frequency of 80%. Similar concentrations of BDE 209 were already reported by Thomas et al. (2006), but at a lower detection frequency.

PBDEs were detected in samples from all regions and the concentrations of PBDEs (sum of congeners 47, 99, 100, 153, 154 and 183) found in the serum samples are summarised in Table 1, The average total concentrations (sum of 6 PBDEs) and standard deviation across each age group were 2.21 ± 0.45 , 3.95 ± 0.39 and 4.55 ± 0.82 ng/g lw for newborns, adolescents and adults, respectively.

PBDE concentrations in the pooled serum samples agreed with previous concentrations reported in Belgian samples (Covaci and Voorspoels 2005; Covaci et al. 2002; Van Wouwe et al., 2004; Covaci et al. 2006b) and were also in the range of values reported in Europe (Hites 2004; Thomas et al. 2006).

Table 1: Average concentrations (ng/g lw) of PBDEs and PCBs in pooled serum samples from newborns (cord blood), adolescents and adults collected in 8 regions of Flanders, Belgium. Values in brackets represent the number of individual samples used to constitute one pool.

Code	Residence area	Sum PBDEs ^a (ng/g lw)			Sum PCBs ^b (ng/g lw)		
		Newborns ^c	Adolescents	Adults	Newborns ^c	Adolescents	Adults
1	Antwerp conurbation	2.04 (162)	3.62 (151)	4.65 (182)	106	110	423
2	Port areas	2.17 (89)	4.47 (170)	4.41 (193)	91	125	455
3	Fruit-farming area	1.55 (158)	3.59 (158)	4.05 (182)	75	95	436
4	Non-ferrous ind. region	2.09 (93)	4.53 (186)	4.57 (189)	80	94	475
5	Ghent conurbation	2.50 (56)	3.80 (155)	4.33 (197)	102	117	456
6	Incinerator	na	3.57 (167)	4.03 (185)	na	93	500
7	Rural area	2.13 (129)	4.17 (149)	6.47 (196)	111	107	511
8	Albert channel	3.01 (48)	3.87 (150)	3.92 (191)	85	85	439

^a – sum of BDE 47, 99, 100, 153, 154 and 183; ^b – sum of CB 118, 138, 153, 180 and 170; ^c – cord blood

In newborns and adolescents, BDE 153 was the highest contributor to the sum PBDEs (41 and 36 %, respectively), followed by BDE 47 (19 and 31 %, respectively). BDE 153 is a constituent of both Penta-BDE and Octa-BDE technical products which are both banned from use in the European Union. The relatively high contribution of BDE 153 has already been evidenced in several other studies (Covaci et al. 2002; Thomas et al. 2006) and could be explained by the high persistency of BDE 153 in combination with a possible lower half-life of BDE 47, the most important BDE congener observed in the environment. However, in 50-65 years old adults, BDE 154/BB 153 was the most important contributor to sum PBDEs with an average of 31 %. It is worth to note that in other Belgian samples (Covaci and Voorspoels 2005; Van Wouwe et al. 2004), BDE 154 was present at much lower concentrations than BB 153.

Influence of age on contaminant levels. PBDE concentrations in newborns differed significantly from those in adolescents and adults. On the other hand, the average PBDE concentration in 14-15 years old adolescents and 50-65 years old adults show no significant difference (one-way ANOVA) (Figure 1). For PCBs and p,p'-DDE, a significant difference in the concentrations was observed between the age groups. However, for the organochlorine contaminants, no difference in concentrations could be observed between newborns and adolescents, while significant differences were seen between adolescents and adults (Figure 1). Concentrations of HCB showed a significant variation between every age groups (data not presented). Therefore, we can conclude PBDEs

show different trends and behave differently than POPs (PCBs, p,p'-DDE and HCB). The reason for this is not yet clear, but it is possible that the metabolism rate is very different between PBDEs and PCBs, on one hand and between different age groups on the other hand. Differences in exposure through different time periods could also be involved.

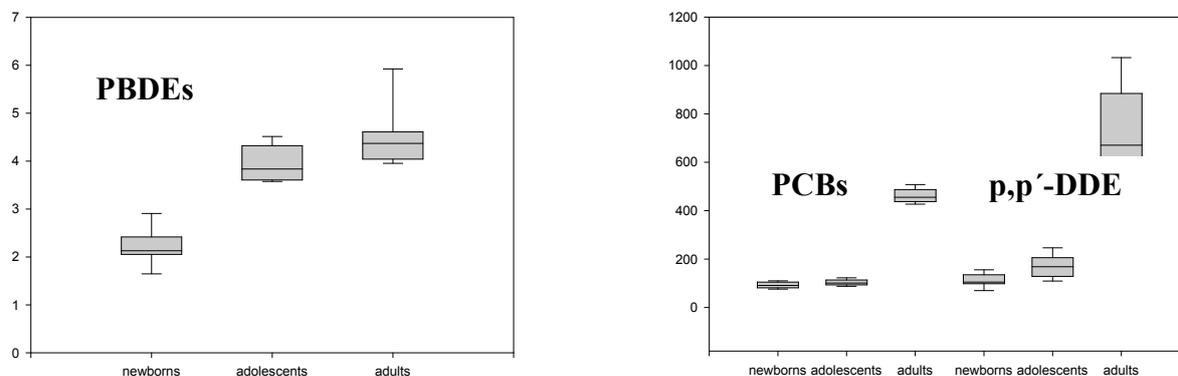


Figure 1: Distribution of PBDE, PCB and p,p'-DDE levels between different age groups.

Influence of region on contaminant levels. To investigate this, we grouped PBDE concentrations by area and calculated if a significant difference was present between the average PBDE values of all groups. Due to the low amount of data (only three pooled samples per area) a non parametric ANOVA (Kruskal-Wallis) test was used. There were no significant differences between the PBDE concentrations through all residence areas, meaning that the no major differences in internal exposure to PBDEs were detected

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