# PBDE serum concentration and pre-school maturity of children from Slovakia

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**Introduction**

Polybrominated diphenyl ethers (PBDEs) are a group of 209 congeners used as flame retardants (BFRs) in plastics and textiles first produced commercially in the 1970s. Humans are exposed to PBDEs from dietary sources, including fish, fatty food, and mother's milk. However, oral ingestion from dust and leachates may be a larger source[1], particularly for children[2]. The presence of PBDEs in human samples (e.g. blood serum, breast milk, etc.) is of particular concern due to their potential health risks including endocrine-disrupting effects and developmental neurotoxicity in laboratory mammals as well as their presence in locations far from where they were produced or used[3,4,5].

As result, the production and use of the commercial mixtures Penta-BDE and Octa-BDE have been restricted in European Union in 2004[6,7]. Use of commercial product Deca-BDE in electronic and electrical applications was banned in Europe in 2008. In 2009 tetra-, penta-, hexa-, and hepta-BDE have been included in the list of persistent organic pollutants (POPs) under the Stockholm Convention[8]. They have been added in Annex A (chemicals to be eliminated). Despite regulations and restrictions in the use and production of PBDEs, exposure to these compounds will continue because PBDEs are persistent and lipophilic, which gives these chemicals a relatively high potential for bioaccumulation and biomagnification[9].

A Californian study determined the body burden of PBDEs in children, and evaluated associations with sociodemographic, household, and dietary factors. Higher levels in children suggest exposure pathways depend upon age[10]. Hand-to-mouth activity may be a significant source of exposure to PBDEs in case of toddler. Furthermore, age, socioeconomic status, and breast-feeding were significant predictors of exposure, but associations varied by congener[11]. Peak concentrations of PBDEs were found at 2.6–3 years of age in Australian children. The observed peak concentration is later than the period when breast-feeding is typically ceased. This suggests that in addition to the exposure via human milk, young children have higher exposure to these chemicals and/or a lower capacity to eliminate them[12]. PBDEs at 8 years were significantly associated with poorer emotional and impulse control. No associations were noted between childhood PBDEs and metacognition or global executive function[13]. Finally, 6 year old children from Slovakia are the aim of interest of our study from the point of view of PBDE serum concentrations and pre-school maturity. These results represent the first child serum PBDE data from Slovakia.

**Material and methods**

Blood serum was collected from 91 children of age of 72 month coming from a PCB contaminated region of Eastern Slovakia in 2010-2011. Mothers of children were informed about the purpose and procedures of the study and subsequently signed an informed consent. The study protocol was approved by institutional review boards at the Slovak Medical University. Details of serum collection and PCB analysis are published elsewhere[14].

*Lipid determination in blood serum*

Total cholesterol, free cholesterol, phospholipids, and triglycerides were determined in each serum sample and total lipid content was calculated according to Akins et al.[15] for purpose of evaluation of PBDE concentration on lipid basis.

*Sample preparation, extraction and clean-up*

Serum samples were treated by modified solid-phase extraction (SPE) using a previously published method[16]. Each of the serum samples was thawed, then spiked with a known amount of 13C-labelled compounds and kept overnight at +6 °C. Serum mixed with an equivalent amount of water : 1-propanol (85 : 15, v/v) mixture was applied to a conditioned SPE column (2 g C18, endcapped; Alltech, Deerfield, Illinois, USA). The analytes were eluted with an n-hexane : dichloromethane (1 : 1, v/v) mixture, and the eluate was concentrated. The extract was cleaned-up on a multi-layer florisil–silica/$H_2SO_4$ column and eluted with hexane : dichloromethane (9 : 1, v/v). The eluate was concentrated under a gentle nitrogen stream just to dryness. 13C-labelled recovery standard solution was added immediately to the extract prior to GC injection.

*Instrumental method*

The PBDE congeners (IUPAC No. 28, 47, 99, 100, 153, 154, 183 and 209) were analysed by isotopic-dilution method using a Trace GC Ultra gas chromatograph coupled to a high-resolution mass spectrometer DFS (Thermo Fisher Scientific, Bremen, Germany). The compounds were separated using a Rtx®-1614 capillary column (Restek Corporation, Bellefonte, USA), 30 m x 0.25 mm I.D., 0.1 μm film thickness. Helium was used as a carrier gas at a constant flow rate of 1 ml·min$^{-1}$. Injector temperature of 280 °C and splitless/surge mode (surge pressure 150 kPa for 2 min) was used. The oven temperature was programmed from initial 120 °C held for 2 min, increased at 20 °C·min$^{-1}$ to 230 °C and finally increased at 6 °C·min$^{-1}$ to 325 °C and held for 20 min. The ion source and transfer line temperature were set at 270 °C and 280 °C, respectively. The mass spectrometer was operated in electron ionization mode with an electron energy of 45 eV at a resolution of 10 000. The mass spectrometer was operated in multiple ion detection mode (MID). Perfluorokerosene (PFK) was used as internal mass reference during analysis. For all native PBDE congeners as well as for all 13C-labelled internal PBDE standards, one quantitation mass and one ratio mass were monitored.

*Pre-school maturity test of children*

The Wechsler Preschool and Primary Scale of Intelligence, the third Ed. (WPPSI-III)[17] was used to evaluate neuropsychic development in the coming-of school age period (6 years of age) of children. It provides subtest and composite scores that represent intellectual functioning in verbal and performance cognitive domains, as well as providing a composite score that represents a child's general intellectual ability. The coming-of school age period is characterized by the maturity of higher cognitive functions (abstract thinking, decision making and logical memory). Social and emotional background were investigated, too.

**Results and discussion**

PBDEs similarly as polychlorinated biphenyls (PCBs) are endocrine disruptors whose effects on children health are not yet fully investigated. In this study, we focused on children of school age and pre-school maturity. As shown in previous, though not large studies in adults[18] and in breast milk[19], PBDE levels are not very high in Slovakia compared to the world. In this study, the first PBDE blood serum concentrations of children were measured in Slovakia. The results are presented in Table 1. PBDE concentrations from this study are markedly lower than those stated in studies of Vuong et al.[13], Toms et al.[12] and Darrow et al.[20]. As can be seen in Table 1, high percent of data especially BDE-28$^{+33}$, BDE-154, BDE-183 and BDE-209 concentrations were below limit of detection (LOD) or not quantified mainly because of blank concentrations. The congener specific differences between boys and girls were interesting. Median and mean concentrations of BDE-99, -100 and -153 seemed to be higher in serum of boys comparing to girls, however not significantly. BDE-47, -99, -100, -153 were positively detected in 90, 67, 66 and 96% of cases, respectively. The most abundant congeners were BDE-47 and BDE-153.

As it is obvious, exposure data e.g. concentrations of BDE congeners in serum did not have Gaussian distribution. The distribution of data was right-skewed with maximum levels two orders of magnitude higher than median exposures, which caused the differences between mean and median concentrations. PBDE congeners BDE-47, -99, -100 and BDE-153 are correlated to each other with correlation coefficients from 0,59 to 0,912 (Spearman´s non-parametric test). The strongest correlation was revealed between BDE-47 and BDE-100. Investigated PBDE

congeners did not correlate significantly with PCB-153, the most abundant non-dioxin-like PCB and with PCB-118, the most abundant dioxin-like PCB.

Table 1 Descriptive statistics of PBDE concentrations in serum of 6 year old children from Slovakia.

| | | BDE-28$^{+33}$ | BDE-47 | BDE-99 | BDE-100 | BDE-153 | BDE-154 | BDE-183 | BDE-209 |
|---|---|---|---|---|---|---|---|---|---|
| **All** | %<LOD | 27,5 | 4,4 | 23,1 | 33,0 | 4,4 | 62,6 | 52,7 | 37,4 |
| *N=91* | %>LOD | 44,0 | 90,1 | 67,0 | 65,9 | 95,6 | 35,2 | 46,2 | 18,7 |
| | % nq | 28,6 | 5,5 | 9,9 | 1,1 | 0,0 | 2,2 | 1,1 | 44,0 |
| *PBDE concentration (ng.g$^{-1}$ lipid)* | | | | | | | | | |
| | min | <0,004 | <0,005 | <0,019 | <0,012 | 0,034 | <0,006 | <0,037 | <0,49 |
| | P5 | 0,005 | 0,029 | 0,022 | 0,016 | 0,050 | 0,007 | 0,041 | 0,68 |
| | median | 0,015 | 0,184 | 0,079 | 0,046 | 0,176 | 0,014 | 0,097 | 2,45 |
| | P95 | 0,229 | 3,32 | 1,51 | 1,01 | 1,91 | 0,200 | 0,562 | 22,5 |
| | max | 0,82 | 14,0 | 3,19 | 6,95 | 16,9 | 0,97 | 3,52 | 31,8 |
| | mean | 0,052 | 0,720 | 0,272 | 0,235 | 0,535 | 0,050 | 0,218 | 4,77 |
| **Boys** | %<LOD | 23,1 | 5,8 | 25,0 | 28,8 | 3,8 | 67,3 | 50,0 | 34,6 |
| *N=52* | %>LOD | 48,1 | 90,4 | 67,3 | 71,2 | 96,2 | 30,8 | 48,1 | 21,2 |
| | % nq | 28,8 | 3,8 | 7,7 | 0,0 | 0,0 | 1,9 | 1,9 | 44,2 |
| *PBDE concentration (ng.g$^{-1}$ lipid)* | | | | | | | | | |
| | min | <0,004 | <0,005 | <0,019 | <0,015 | 0,034 | <0,006 | <0,038 | <0,486 |
| | P5 | 0,005 | 0,021 | 0,022 | 0,016 | 0,056 | 0,007 | 0,040 | 0,542 |
| | median | 0,024 | 0,184 | 0,096 | 0,050 | 0,207 | 0,014 | 0,101 | 3,10 |
| | P95 | 0,305 | 6,56 | 2,34 | 1,21 | 2,59 | 0,278 | 0,558 | 28,9 |
| | max | 0,82 | 14,04 | 3,19 | 6,95 | 16,9 | 0,68 | 3,52 | 31,8 |
| | mean | 0,064 | 0,965 | 0,327 | 0,289 | 0,723 | 0,049 | 0,211 | 6,41 |
| **Girls** | %<LOD | 33,3 | 2,6 | 20,5 | 38,5 | 5,1 | 56,4 | 56,4 | 41,0 |
| *N=39* | %>LOD | 38,5 | 89,7 | 66,7 | 59,0 | 94,9 | 41,0 | 43,6 | 15,4 |
| | % nq | 28,2 | 7,7 | 12,8 | 2,6 | 0,0 | 2,6 | 0,0 | 43,6 |
| *PBDE concentration (ng.g$^{-1}$ lipid)* | | | | | | | | | |
| | min | <0,004 | 0,029 | <0,022 | <0,012 | 0,035 | <0,006 | <0,037 | <0,777 |
| | P5 | 0,005 | 0,029 | 0,022 | 0,015 | 0,046 | 0,007 | 0,047 | 0,786 |
| | median | 0,013 | 0,184 | 0,065 | 0,042 | 0,162 | 0,015 | 0,096 | 2,17 |
| | P95 | 0,291 | 1,62 | 0,922 | 1,02 | 1,64 | 0,169 | 1,07 | 8,55 |
| | max | 0,45 | 3,12 | 1,19 | 1,57 | 1,86 | 0,97 | 3,41 | 8,99 |
| | mean | 0,035 | 0,381 | 0,195 | 0,161 | 0,284 | 0,052 | 0,226 | 2,60 |

LOD = limit of detection; nq = not quantified; P5 = 5th percentile; Med = median; P95 = 95th percentile.

Multivariate analysis of individual PBDE congeners (BDE-47, -99, -100, -153), indicator non-dioxin-like PCB-153 and dioxin-like PCB-118 with WPPSI-III score in 90 children were performed. In spite of lower PBDE concentrations comparing to other studies, the results showed significant association of higher BDE-153 serum concentration of children with lower WPPSI-III score (p=0.010, B=-23.1) also using sex as confounder. Multivariate analysis with predictor BDE-153 and confounders PCB-153 and sex showed significant association

of BDE-153 (p=0.011, B=-23.6), and no association of PCB-153 with WPPSI-III score. Similar association was found in case of BDE-153 (p=0.002, B=-29.8) with WPPSI-III, if another confounder PCB-118 was used. Sex had significant effect on WPPSI-III score in this model (p=0.045), too. Negative association was found also for BDE-100 with WPPSI-III composite score (p=0.034, B=-14.5) in model with confounder PCB-118, which is not associated with WPPSI-III significantly. Detailed testing using subtest scores of WPPSI-III brought more significant associations in this group of children, which point to a possible adverse effect of PBDEs on children's intellectual abilities in the coming-of school age period. Especially BDE-153, -100 and -47 indicate possible negative effects on children's performance in WPPSI-III subtests for example block design, information, matrix reasoning, picture concepts and word reasoning. No associations of PCBs and WPPSI-III composite score were found.

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