PBDEs, PBB-153, PCB-153 AND SELECTED ORGANOCHLORINE PESTICIDES IN BREAST MILK OF SLOVAK POPULATION: DAILY INTAKE AND TRENDS

Škarba J[1,2*], Chovancová J[1], Čonka K[1], Drobná B[1], Stachová Sejáková Z[1]

[1]Department of Toxic Organic Pollutants, Faculty of Medicine, Slovak Medical University, Limbová 12, 833 03 Bratislava, Slovak Republic

[2]Department of Chemistry, Faculty of Natural Sciences, University of Ss. Cyril and Methodius, Nám. J. Herdu 2, 917 01 Trnava, Slovak Republic

Introduction

Halogenated organic compounds such as flame retardants, organochlorine pesticides and polychlorinated biphenyls (PCBs) are known as persistent organic pollutants (POPs). Even though the production and usage of these compounds is restricted or banned for several years, human population is still exposed to them through environment and food chain. Due to their hydrophobic and lipophilic character they can easily accumulate in human adipose tissue or breast milk. Breast-fed infants in prenatal age are the most vulnerable group, since their only source of nutrition is mother milk. This study investigated and determined concentration as well as daily intake of seven polybrominated biphenyl ethers (PBDEs), PCB-153, three congeners from polybrominated biphenyls (PBBs), hexachlorobenzene (HCB), pentachlorobenzene (PeCBz) and hexachlorocyclohexane (HCH) isomers in eight regions of Slovakia. Recruited primiparas participating in this study were also asked to fill out the questionnaires about their lifestyle and dietary habits. Although there have been two studies investigating residue levels of PBDEs, HCHs and PCBs in human milk in Slovak population[1,2], there are no information about the levels of PBBs in milk samples so far. Estimated levels of obtained pollutants were statistically processed and compared to those reported in world studies as well as to those reported in two previous Slovak studies.

Material and method

Sampling

In total 53 samples of human breast milk from 8 regions of Slovakia were collected in addition to the WHO-coordinated study in 2005. 98 % participating women were having their first child. Questionnaires included basic information about participant such as pre/post/present pregnancy weight, length, age, date of sampling, county of living, etc. The participating woman also answered several questions about consumption of chosen food groups which significantly contributes to dietary exposure of toxic organic pollutants. Food frequency intake of cheese, eggs, poultry, fish and fish mammals was performed by 5 predefined answers ranging from "never" to "more than twice a week/day". Addition information about origin and kind of meat/fish, daily intake of milk was also obtained.

Extraction and clean up

The extraction of lipids from breast milk samples was performed according to previous study[3]. Isolated lipid fraction ranged from 1.21 to 6.61% with the mean of 3.21% and the median of 3.14% respectively. After extraction, dissolved lipid fraction in n-hexane was fortified by $^{13}C_{12}$ labelled PBDEs, PCBs, PBB-153 and selected organochlorine pesticides (OCPs). Clean up of samples was performed on column filled with florisil (0.5 g), H_2SO_4/silica (1 g), florisil (0.5 g) and anhydrous Na_2SO_4. Elution of pollutants was provided using 10% dichloromethane in n-hexane (10 ml). Prior to GC injection, milk extracts were dissolved in 20 µl of n-nonane containing method-recovery $^{13}C_{12}$ labelled PCB-80.

Analysis of PBDEs, PBB-153

Seven PBDE congeners: 2,4,4´-triBDE (BDE-28[+33]), 2,2´,4,4´-tetraBDE (BDE-47), 2,2´,4,4´,5-pentaBDE (BDE-99), 2,2´,4,4´,6-pentaBDE (BDE-100), 2,2´,4,4´,5,5´-hexaBDE (BDE-153), 2,2´,4,4´,5´,6-hexaBDE (BDE-154), 2,2´,3, 4,4´,5´,6-heptaBDE (BDE-183), three congeners from polybrominated biphenyls 2,2',4,4',5,5'-hexabromobiphenyl (PBB-153), 2,2´,4,4´,5,6´-hexabromobiphenyl (PBB-154), 2,2´,4,4´,6,6´-hexabromobiphenyl (PBB-155) were measured using a DFS high resolution magnetic sector mass spectrometer coupled to two Trace GC Ultra gas chromatographs (Thermo Scientific, Bremen, Germany). For chromatographic separation of the target compounds was used Rtx®-1614 capillary column (Restek, Bellefonte, USA)

Analysis of PCB-153, HCB, PeCBz, α-HCH, β-HCH, γ-HCH
An HP 6890 Plus GC (Hewlett-Packard, Palo Alto, CA, USA) coupled to a MAT 95XP (ThermoFinnigan, Bremen, Germany) operating at resolution of 10000 in the selected ion monitoring mode was used for PCB-153 and organochlorine pesticides analysis. PCB-153 as well as all organochlorine pesticides were separated on the DB-5MS column (60 m, 0.25 mm i.d., 0.25 mm film thickness). Detailed information about the analysis is described elsewhere[4].

Results and Discussion
Sampling of mother milk was provided in eight Slovak regions Bratislava (n=12), Banská Bystrica (n=7), Žilina (n=7), Trnava (n=8), Trenčín (n=6), Prešov (n=3), Nitra (n=5) and from Košice (n=5)) with 53 participating mothers. Volunteers characteristic such as body mass weight, age, height and weight before pregnancy were collected by questionnaires. The statistic evaluation of obtained data was accomplished using the statistical software IBM SPSS version 22.

Organochlorine pesticides
Total mean concentration ∑HCHs (α-, β-, γ-HCH) was 19.4 ng g^{-1} lw. The most abundant β-HCH isomer was obtained in all breast milks. The median concentrations of γ -HCH (ng g^{-1} lw) were similar in all region ranged from 0.132 in Bratislava to 35.6 in Žilina. As it can be seen in Tab. 1 our results indicate high level of HCB in mother breast milks especially in regions Banská Bystrica, Nitra and Prešov. The comparison of our results with those reported in previous Slovak study[2] (Tab. 1) is giving clear evidence of decline trend of these compounds in Slovak population by at least one magnitude in period 1993-2005.

Tab.1: Comparison of mean concentrations of selected organochlorine pesticides in Slovakia (ng g^{-1} lw).

	n	α –HCH	β-HCH	γ-HCH	HCB	PeCBz
This study	53	0.206	17.9	1.36	80.3	0.275
Slovakia 1993 [2]	26	7	136	15	339	2

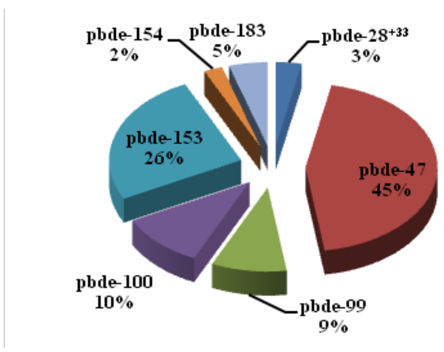
Flame retardants and PCB-153
Total concentration of ∑PBDEs in the mother's milk samples varied widely from 0.051 to 2.56 ng g^{-1} lw with the median of 0.497 ng g^{-1} lw for all samples. The highest mean concentration of PBDEs according to region was in order: Bratislava (0.744 ng g^{-1} lw), Banská Bystrica (0.525 ng g^{-1} lw), Prešov (0.501 ng g^{-1} lw), Žilina (0.497 ng g^{-1} lw), Nitra (0.491 ng g^{-1} lw) , Trenčín (0.399 ng g^{-1} lw), Košice (0.342 ng g^{-1} lw) and Trnava (0.297 ng g^{-1} lw). Slightly higher levels of PBDEs in human breast milk samples in the biggest and most populous city in Slovakia is probably due to the higher amount of dust and products contained PBDEs mixtures (electronics, plastic, building and insulating materials). Fig. 1 shows PBDEs profile in Slovak population with predominant congeners BDE-47 and BDE-153. Concentration of PBDEs in this study (Tab. 2) was of the same order of magnitude than in samples collected in year 2005 (n=34) in the Slovakia[1]. Spearman analysis proved strong negative correlation between BDE-153 (r=-0.6) and BMI. Furthermore positive relation was found between PCB-153, (r=0.478) and age. This study also determined the level of PBB-153 in human milk for first time in Slovakia. Total PBB-153 mean concentration was 0.022 ng g^{-1} lw. PBB-154 and PBB-155 were not statistically processed since 98% of samples were below limit of detection. In present, there are just a few studies reporting information about levels of PBB-153 in breast milk. Nevertheless, PBB-153 median concentration of 0.08 ng g^{-1} lw was determined in the breast milk sampled in UK[5], while recent Chinese study[6] obtained median of 0.024 ng g^{-1} lw in breast milk samples. The highest

median concentration of 3.3 ng g^{-1} lw and 1.2 ng g^{-1} lw was found in Northwest American and Greenland human breast milk[7,8], respectively.

Table 2: Concentration of all observed brominated flame retardants (ng g^{-1} lw)

			Total concentration					
	BDE-28	BDE -47	BDE -99	BDE -100	BDE -153	BDE -154	BDE -183	BB-153
Median	0.016	0.219	0.045	0.052	0.125	0.008	0.023	0.011
Min	0.004	0.029	0.008	0.006	0.067	0.001	0.003	0.0004
Max	0.064	1.62	0.497	0.330	0.490	0.045	0.046	0.302

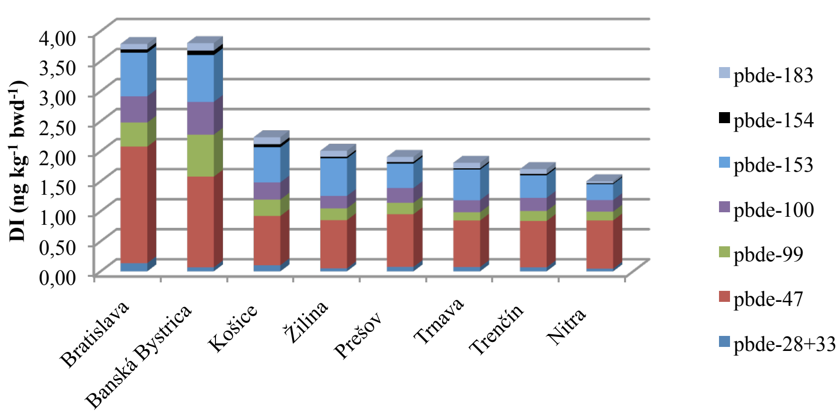
Figure 1: The profile of seven congeners of PBDEs in Slovak population



Estimation of daily infant intake (DI)

We estimated that the Slovak breast-fed infant exposure averaged 2.50 ng kg^{-1} bw d^{-1} for ΣPBDEs, assuming individual percentage of lipid in milk, 0.6 L/day consumption and 5 kg of body weight. The DI profile of individual PBDE congeners in each Slovak region is shown in Fig. 2. The results in this study are very similar to the most recent Slovak study[1], where estimated mean value of daily infant intake of ΣPBDEs in region Košice was 3.0 ng kg^{-1} bw d^{-1}, while in this study estimated mean value for the same region was 2.3 ng kg^{-1} bw d^{-1}.

Figure 2: The estimated DI of seven individual PBDE congeners in eight regions of Slovakia



The mean daily exposure to BDE-47, BDE-99 and BDE-153 for breast-fed infants with average human milk consumption ranges across EU countries[9] (24 EU studies) from 0.64-13.8, <0.14-5.05 and 0.46-11.0 ng kg^{-1} bw d^{-1}.

In current study the mean of daily exposure of BDE-47, BDE-99 and BDE-153 was 1.18, 0.31, 0.59 ng kg^{-1} bw d^{-1}, respectively.

Table 3 Estimated DI (ng kg^{-1} bw d^{-1})

	Daily intake									
	BDE-47	BDE -99	BDE -100	BDE -153	PBB-153	PCB-153	HCB	PeCBz	β-HCH	γ -HCH
Mean	1.18	0.307	0.309	0.588	0.087	0.411	340	1.05	68.4	4.80
Min	0.099	0.028	0.021	0.097	0.001	0.058	62	0.261	15.9	0.748
Max	6.64	3.94	2.35	2.38	1.23	1.47	4182	9.18	208	122

Furthermore, this study presents historically first results concerning the DI of PBB-153 with median of 0.05 ng kg^{-1} bw d^{-1}. Estimated DI values of β, γ-HCH, HCB, PeCBz were similar in all regions of Slovakia and total mean DI are reported in Tab. 3. In comparison with other countries, median concentration of HCB in this study (212 ng kg^{-1} bw d^{-1}) was almost the same than it was observed in China (210 ng kg^{-1} bw d^{-1})[10], but much higher than in Turkey (30 ng kg^{-1} bw d^{-1})[11].

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