HUMAN EXPOSURE TO PCB AND PCB METABOLITES IN A HOT-SPOT AREA IN EASTERN SLOVAKIA

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Abstract
Polychlorinated biphenyls (PCBs) were commercially produced between 1959 and 1984 in the Michalovce district in eastern Slovakia. Contaminated waste water and improper storage has led to a significant PCB contamination of the local environment and high levels have been measured in humans and wildlife in the surrounding area. Data from serum sample analysis from two studies; adults, and pregnant women and paired cord blood samples, has been analysed to assess levels, patterns and transplacental transfer of PCB and PCB metabolites in eastern Slovakia. Liquid-liquid extraction together with separation of substance groups and further clean up on silica gel columns were applied prior to analysis by GC-MS. 4-OH-CB187 was the major hydroxylated PCB metabolite, followed by 4-OH-CB146, 3-OH-CB153 and 3'-OH-CB138. The dominating methyl sulfone metabolite was 3-$MeSO_2$-DDE, followed by a yet not identified $MeSO_2$-hexaCB, 4'-$MeSO_2$-CB101, 4'-$MeSO_2$-CB87 and 4-$MeSO_2$-CB149. This is the first time that PCB and DDE methyl sulfone metabolites have been analysed in human cord serum, showing that these metabolites are transported through the placenta. The adults and the pregnant women from the contaminated Michalovce area had two to three times higher concentrations of the PCB metabolites than women from the background districts.

Introduction
Polychlorinated biphenyls (PCBs) are due to their high chemical stability, lipophilicity, and ability to bioaccumulate one of the world's most widespread class of environmental contaminants[1]. 22,000 tons of PCBs were produced between 1959 and 1984 at the Chemko Inc., a chemical plant located in the district of Michalovce in eastern Slovakia. Discharges from the plant lead to PCB contamination of the surroundings and the nearby Laborec River and Zemplinska Sirava Lake. High concentrations of PCBs have been found in air, soil, water, fish, meat and human blood in the area[2-6] making this site one of the world's hot-spots for contamination of PCBs.

PCBs are metabolized to hydroxylated PCBs (OH-PCBs) via formation of an arene oxide mediated by cytochrome P-450 or via direct insertion of a hydroxyl group[7]. Some OH-PCBs have a high affinity to transthyretin (TTR), one of the transport proteins for thyroxine (T4) which is leading to competition with the hormone and retention of certain OH-PCBs in blood[8]. TTR is transported over the placenta and likely bring the OH-PCBs to the foetal side as confirmed by measuring OH-PCBs in cord serum[9-11]. The arene oxide can also react with glutathione and form methyl sulfones metabolites (e.g. $MeSO_2$-PCBs and 3-$MeSO_2$-DDE)[12]. These sulfones are formed via oxidation and the mercapturic acid pathway (MAP) in which the cysteine conjugate is cleaved at the cysteine C-S bond by C-S-lyase and the thiol formed is methylated and oxidized to the corresponding methyl sulfone metabolite[7]. Methylsulfonyl metabolites of PCBs and DDE have been analysed in human blood, milk, adipose tissue, lung, liver and brain[5,13-16]. The $MeSO_2$-PCBs and 3-$MeSO_2$-DDE are lipophilic and accumulate in adipose tissue. However they may also bind to specific proteins, leading to strong retention in certain tissues[17-19]. 3-$MeSO_2$-DDE binds irreversibly to the cell constituents in *zona fasciculata* in the adrenal cortex in mice and humans after metabolic activation of the 3-$MeSO_2$-DDE metabolite[20-22].

The present study is an integrated study of two cohort studies of PCBs and their metabolites in the contaminated Michalovce district and in Svidnik/Stropkov, a reference area with expected lower PCB contamination, that is located 70 km to the north. Data on PCB and PCB metabolites in serum samples from adults collected in 2001 and, in a second study, in maternal blood and cord blood from 2002-2004 have been evaluated. Patterns, concentrations and the transplacental transfer of PCB and its metabolites have been determined.

Materials and methods

Samples: Approximately 2000 serum samples from adults living in the contaminated Michalovce district and the background district Svidnik/Stropkov were collected in 2001. 122 samples from Michalovce and 175 samples from Svidnik/Stropkov (age 20-59) were analysed for PCBs, OH-PCBs and $MeSO_2$-PCBs. During 2002-2004 maternal and cord serum samples were collected from pregnant women living in Michalovce (n=762) and Svidnik/Stropkov (n=341) for a PCB and early childhood development study[23]. Two hundred maternal samples were selected for OH-PCB analysis and 50 samples were selected for methyl sulfone metabolite analysis. The selection of samples for the maternal analyses was based on a stratified sample designed to represent the full range of PCB exposures, but with greater representation of the higher PCB concentrations. Cord serum samples corresponding to the 10 maternal serum samples with the highest concentrations of PCB methyl sulfones were analysed for $MeSO_2$-PCB and 3-$MeSO_2$-DDE metabolites.

Clean-up and analysis: The methods used for the extraction, clean-up and instrumental analyses of PCB and PCB metabolites are described elsewhere[6,24,25]. Shortly, hydrochloric acid and 2-propanol and the analytes were extracted with a mixture of n-hexane:MTBE. After re-extraction of the serum, the combined organic phases were washed with a 1% potassium chloride solution. A neutral and a phenolic fraction were received through partitioning with potassium hydroxide. The neutral fraction was partitioned with dried DMSO to isolate methylsulfonyl metabolites of PCBs and DDE from their parent compounds since aryl methyl sulfones partition into the DMSO phase as previously described[5,26,27]. The methylsulfonyl substituted analytes were extracted from the DMSO by addition of water and n-hexane and the methyl sulfone phase was further cleaned up on a multilayered silica gel column. The phenolic fraction was derivatized with diazomethane and further cleaned up with sulfuric acid and a sulfuric acid:silica gel column. The samples were analysed with GC-MS or GC-ECD.

Results and Discussion

The median concentrations of PCB, OH-PCBs and $MeSO_2$-PCBs in adults, maternal and cord samples are given in Table 1. The mean lipid content was 0.7% in adults, 1.1% in the maternal samples and 0.2% in the cord blood samples. There were two to three times higher levels of PCB and the metabolites in Michalovce compared to the background district Svidnik/Stropkov. The major hydroxylated PCB metabolite in most samples was 4-OH-CB187, followed by 4-OH-CB146, 3-OH-CB153 and 3'-OH-CB138. The ratio between ΣOH-PCBs and CB153 was 0.17 in the pregnant women, 0.60 in women and 0.53 in men sampled 2001. One reason for the low ratio in maternal serum compared to the women and men might be the relatively higher transfer of OH-PCBs compared to PCBs to the foetus, but this is only speculations which can not be confirmed at this stage. A comparison of the OH-PCB concentrations and patterns in the pregnant women in Slovakia and in women from other areas is demonstrated in Figure 1. The levels in eastern Slovakia are among the highest reported levels to date[6,9,10,28,29]. 3-$MeSO_2$-DDE was the major sulfone metabolite in serum , the dominating $MeSO_2$-PCB metabolite was a hitherto unidentified $MeSO_2$-hexaCB, with the next most abundant ones being 4'-$MeSO_2$-CB101, 4'-$MeSO_2$-CB87 and 4-$MeSO_2$-CB149, respectively. The unidentified compound was quantified using the response factor of 4-$MeSO_2$-CB149. The ratio between Σ$MeSO_2$-PCBs and CB153 was about 0.01 in serum from both adults and maternal samples, the 3-$MeSO_2$-DDE/DDE ratio was 0.004. *Para*-$MeSO_2$-PCBs were more abundant compared to *meta*-$MeSO_2$-PCBs, similarly as in a Swedish study of exposed workers[30].

Information on PCB metabolites in maternal and cord serum samples is scarce. By studying a relatively high PCB exposed population, the placental transfer of PCB and DDE methyl sulfones have been confirmed for the fist time. It is not likely that this metabolism takes place in the foetus itself since it is quite complicated involving intestinal micro flora[7]. PCBs are known to pass the placenta and OH-PCBs have also been found in cord serum[10,11,31]. The PCB and Σ$MeSO_2$-PCB ratio in 10 paired cord and maternal samples were 0.9 and 0.6 on a lipid weight basis. But there seems to be individual differences in the maternal/cord ratio of the methyl sulfones, in six out of ten cases the Σ$MeSO_2$-PCBs are higher in the maternal serum compared to the cord serum but in four cases the concentration is the same or even higher in the cord sample. $MeSO_2$-PCBs and 3-$MeSO_2$-DDE are, to a higher extent than the parent PCB and DDE, associated with the protein rich fraction of the blood rather than with the lipoproteins[14].

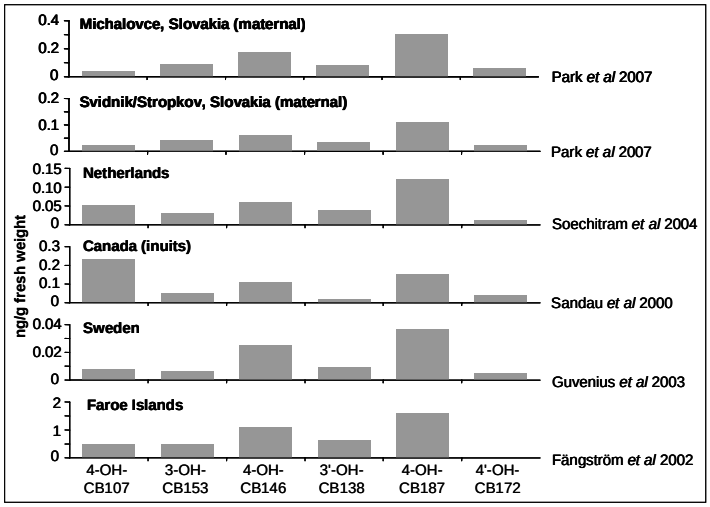
Table 1. Median levels of PCB, OH-PCB and $MeSO_2$-PCB in men, women, maternal and cord samples (ng/g fat).

	n	CB153		Σ(3)OH-PCB[a]		Σ(4)$MeSO_2$-PCB[b]		3-$MeSO_2$-DDE	
District		Michalov.	Svi/Str.	Michalov.	Svi/Str.	Michalov.	Svi/Str.	Michalov.	Svi/Str.
Men[c]	78/104	650	250	270	120	5.0	1.5	6.2	4.0
Women[c]	44/71	480	200	260	110	3.8	1.3	8.3	3.1
Maternal[d]	43/7	250	100	34	19	2.6	1.2	1.8	1.3
Maternal[e]	10	630		-		17		1.7	
Cord[e]	10	600		-		10		1.7	

[a]Σ(3)OH-PCB= sum of: 4-OH-CB107; 4-OH-CB153; 4-OH-CB187. [b]Σ(4)$MeSO_2$-PCB= sum of $MeSO_2$-hexaCB; 4'-$MeSO_2$-CB101; 4'-$MeSO_2$-CB87; 4-$MeSO_2$-CB149. [c]Hovander *et al.* 2006. [d]Park *et al.* 2007. [e]Linderholm *et al*. 2007.

The concentration of PCB and its metabolites in the second study (maternal samples) were lower than in the first study containing samples from adults from 2001. But the second study consisted of a more homogenous group of young, pregnant women (18-44 years) whilst the first study consisted of both men and women in a wider age range (20-59 years). The PCB levels were found to be higher in men compared to women whereas the levels of metabolites were about the same in men and women. Also, PCB and $MeSO_2$-PCB correlated with age. Since the population in eastern Slovakia are exposed to relatively high levels of PCB it can be used to locate adverse effects of PCB. Some effects have been correlated with PCB in this population already, such as neurobehavioral changes, hearing loss[32] and dental effects[33] in children and an increased frequency of diabetes[34] and levels of thyroxine (T4) in adults[35].

Figure 1. A comparison of pattern and concentration OH-PCBs in women from different countries.



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