ANALYSIS OF PCB AND PCB METABOLITES IN HUMANS FROM EASTERN SLOVAKIA

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Introduction

Polychlorinated biphenyls (PCBs) are still major environmental contaminants threatening human health. A chemical plant in Eastern Slovakia manufactured 22,000 tonnes of PCBs between 1959 and 1984. The water in the effluent canal drained into a nearby river and contaminated the adjacent area. Over the past decade, PCB contamination in part of the district has been well documented. In 1997 and 1998, samples of soil, air, water, sediments and wildlife were collected and PCBs were analysed [1].

Since PCBs form hydroxylated metabolites (OH-PCBs) that may show endocrine modulating properties and are, depending on their structures, retained in the blood, it is of interest to determine the OH-PCBs levels[2]. PCB also form methylsulfonyl substituted metabolites ($MeSO_2$-PCBs) that may be retained in the body[2]. In human plasma samples, the level of CB-153 show a good correlation with the total PCB level (around 25%) and could be used as a marker for total PCB[3]. The aim of the present study was to determine the levels of OH-PCBs and $MeSO_2$-PCBs and also CB-153 in plasma from humans living in the contaminated area and in background areas.

Materials and Methods

Samples: Plasma samples from 141 humans living in a PCB contaminated area, the Michalovce district and 178 plasma samples from humans living in background areas, the Stropkow and Svidnik districts were analysed for three major OH-PCBs, three major $MeSO_2$-PCBs and CB-153.

Clean up: The extraction method for the plasma samples is described elsewhere[4]. Plasma samples (5g) were extracted and partitioned into a neutral and a phenolic fraction. The phenolic fraction, i.e. OH-PCBs, was subjected to diazomethane for methylation of the halogenated phenols. The MeO-PCBs were further cleaned with sulfuric acid, and on a sulfuric acid silica gel column. The neutral fraction with $MeSO_2$-PCBs and PCBs were isolated with DMSO partitioning. The clean up schedule for the neutrals is shown in Figure 1.

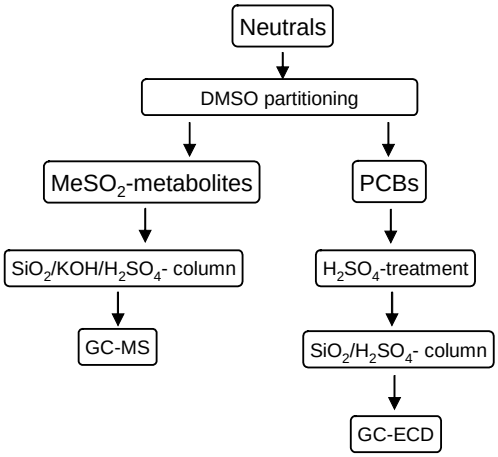

Figure 1. Work-up scheme for analysis of $MeSO_2$-PCBs and PCBs in blood plasma.

The PCB and the MeO-PCBs fraction is analysed by GC-ECD. $MeSO_2$-PCBs are present at very low concentrations only and it is therefore necessary to quantify the most abundant congeners by GC-MS in SIM mode. All analyses were performed in comparison to authentic reference standards that have been synthesised in house.

Gas chromatography electron capture detection (GC-ECD) was performed on a Varian 3400 GC equipped with a Varian 8200 auto sampler and a split/splitless injector operated in the splitless mode. A non-polar column, CP-SIL 8CB (25 m x 0.15 mm x 0.12 µm), from Chrompack (EA Middelburg, The Netherlands) was used. For the MeO-PCB the oven was programmed as follows: 80°C (1 min) - 50°C/min - 200°C (1 min) -1°C/min - 230°C -50°C/min - 330°C (2 min). For the PCBs the GC oven was programmed: 80°C (1 min) - 20°C/min - 330°C (5 min). The injector temperature was 280°C and the detector temperature 360°C.

GC-MS analysis were performed on a Varian GC 3400 equipped with a J&W DB5 MS capillary column (30m x 0.25 mm x 0.25µm), a split/splitless injector and a CTC A200S autosampler coupled to a Finnigan MAT SSQ710 mass spectrometer. The injector was operated in splitless mode (1 min) at a temperature of 280°C with helium as carrier gas. The transfer line was held at 280°C and the GC temperature program was 80°C (2 min) - 20°C/min - 230°C -3°C/min - 300C° (5 min). The ion source at 150 °C was operated in the electron capture negative ionization (ECNI) mode with methane (5.0 AGA) as buffer gas with an electron energy of 70eV. The instrument was set to scan in selected ion monitoring (SIM) mode measuring the masses corresponding to M^- and $(M+2)^-$ of the analytes of interest over three different time frames ranging from 4 to 7 chlorine atoms.

Results and Discussion

An improved method for cleanup of $MeSO_2$-PCBs was developed. Quantitative data of CB-153, three major OH-PCBs and $MeSO_2$-PCBs, respectively. The concentration of 3-$MeSO_2$-DDE was also determined as shown in Table 1. The levels of OH-PCBs are twice as high in plasma from persons living in the PCB contaminated area compared to the background area. Notably high concentrations were determined of 3-$MeSO_2$-DDE, particularly when compared to the $MeSO_2$-PCBs.

Table 1. Concentrations (ng/g lipid weight) of CB-153, OH-PCBs, $MeSO_2$-PCBs and 3-$MeSO_2$-DDE from the Michalovche, Stropkov and Svidnik districts.

	Michalovche district, PCB contaminated area		Stropkov and Svidnik districts, Background areas	
Analyte	median	range	median	range
CB-153	590	110-7200	240	51-1200
4-OH-CB107	63	3,7-1100	26	0,9-84
4-OH-CB146	74	6,7-1000	26	6,6-92
4-OH-CB187	140	11-2000	58	15-260
4-$MeSO_2$-CB101	0,80	0,16-19	0,29	0,13-1,4
4-$MeSO_2$-CB87	1,1	0,14-22	0,32	0,12-1,4
4-$MeSO_2$-CB149	2,9	0,25-72	1,0	0,2-9,2
3-$MeSO_2$-DDE	7,3	0,40-81	3,5	0,3-73

Two chromatograms are shown; Figure 2 shows the pattern of PCB methyl sulfones in plasma from the Michalovce district and in Figure 3, a chromatogram of methylated hydroxyl-PCBs is shown. The chromatograms are given to show that there are a number of other isomers and homologues of the $MeSO_2$-PCBs and OH-PCBs, respectively, in the plasma analysed.

The major OH-PCB congener is 4-OH-CB-187, a metabolite known to be the dominating congener in human blood[5]. The peak patterns are similar to previous reports on OH-PCBs in human blood (plasma or serum)[3,5-8]. The most abundant OH-PCB, 4-OH-CB187, is present in such a high concentration as 28% of the CB-153 level with a range of (1,7-110%). Hence 4-OH-CB187 is present in the blood at a higher concentration than many of the individual PCB congeners. The 4-OH-CB187/CB-153 ratio is identical for the humans from the Michalovche area and the Stropkov/Svidnik area. The 4-OH-CB187 is a metabolite of CB-183 and CB-187, the former going through a 1,2-shift to form 4-OH-CB187. This metabolite was recently shown to have a half-life in rats of 15 days[9] that indicate a rather long half-life in humans.

It is possible to detect and quantify PCB methyl sulfones in all subjects from the cohorts. It is notable that the major congener is 4-$MeSO_2$-CB149, which is a chiral compound. This needs some further and future attention. PCB methyl sulfones have rarely been analysed and reported in humans which makes this study particularly interesting. In a Swedish study mean $MeSO_2$-PCB concentrations were reported at concentrations of 0.8-5.6 ng/g l.w.[10] which is rather similar to what is reported in the blood from Stropkov/Svidnik individuals, while there is a larger range for $MeSO_2$-PCB in the human plasma sampled in Michalovche subjects. A temporal trend study for $MeSO_2$-PCBs in human milk show that the concentrations have decreased with time, but again the concentration range is similar to what is reported herein[11]. The most abundant PCB methyl sulfone in this study, 4-$MeSO_2$-CB149, is present at 0.5% of the CB-153 level making this type of PCB metabolites less dominant in humans than in e.g. grey seals in which the $MeSO_2$-PCB/PCB ratio is one order of magnitude higher than in humans[12]. The reason behind this is not yet understood but may have something to do with the intestinal microflora. The 3-$MeSO_2$-DDE was the most abundant sulfone and present in levels exceeding those determined in the early 1970s in Swedish mother's milk[11]. This is agreement with high DDE concentrations in the human subjects from the Slovak Republic.

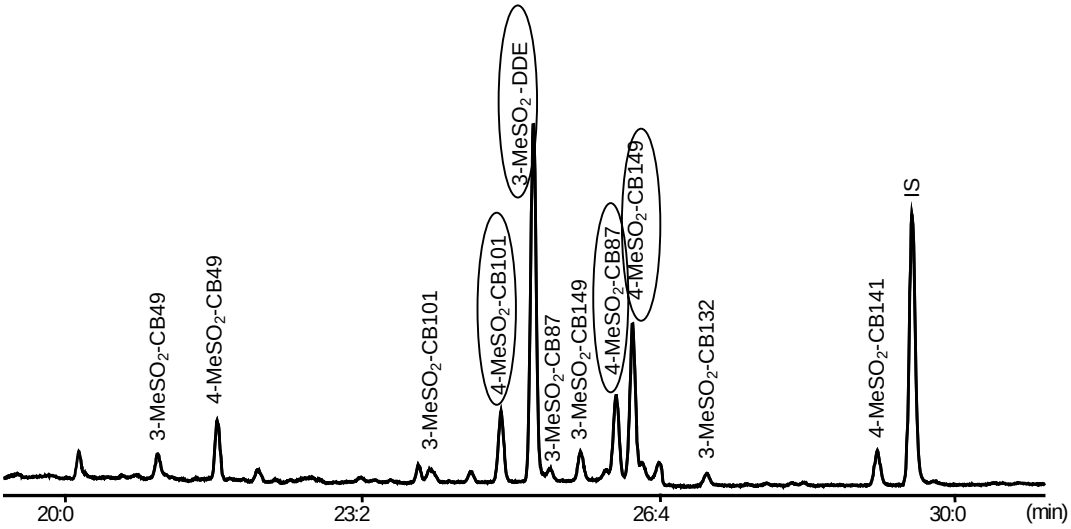

Figure 2. Chromatogram, GC-MS (SIM), of the $MeSO_2$-PCB congeners in human plasma from a subject from the Michalovche district. Note the abundant 3-$MeSO_2$-DDE peak.

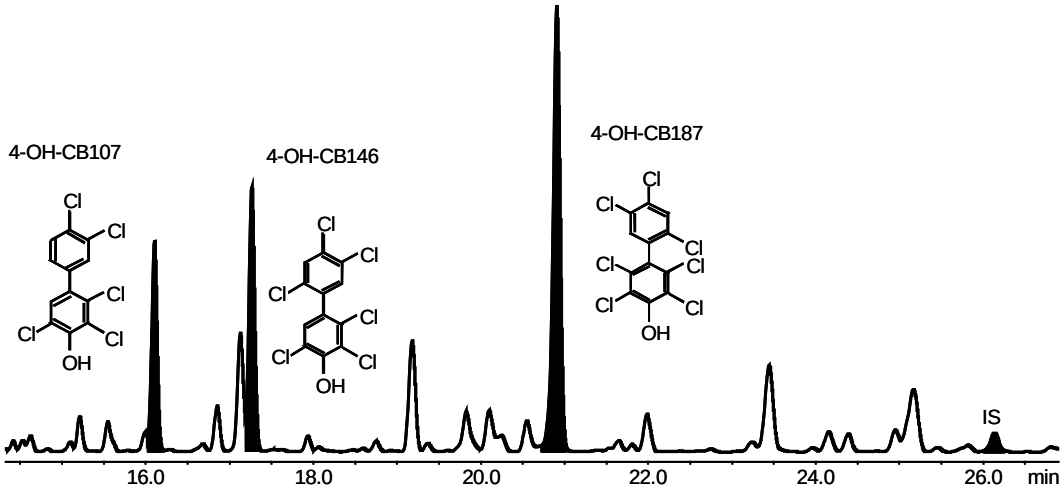

Figure 3. Chromatogram, (GC-ECD) of the MeO-PCBs in a human plasma sample from the Michalovche district, the PCB contaminated area.

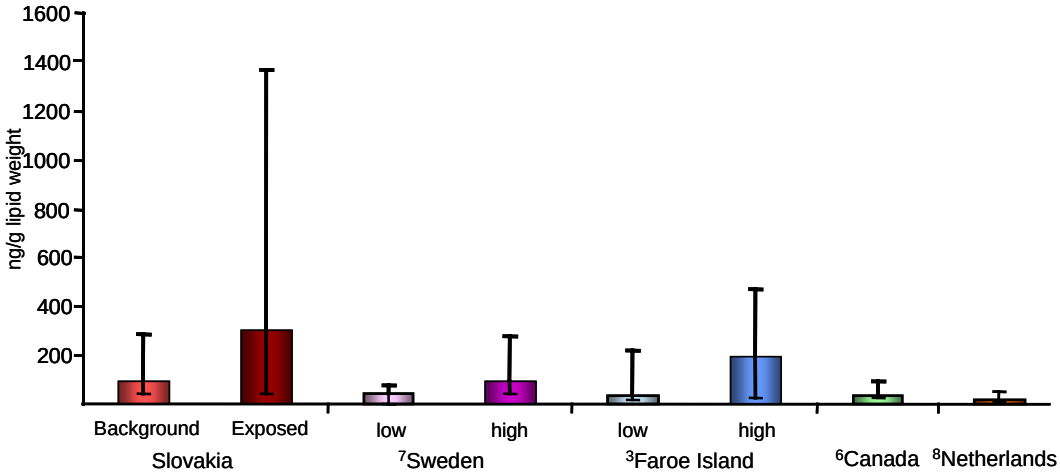

Figure 4. Sum of OH-PCB concentrations in blood plasma or serum from humans with different level of PCB exposures. The Slovakian data are compared to previously published data on OH-PCBs in human blood.[3,6-8].

As visualised in Figure 4 the cohort living in the PCB contaminated area in Eastern Slovakia have the hitherto highest concentrations also of OH-PCBs. Interestingly the levels are not very different from OH-PCBs in PCB exposed women from the Faroe Islands. It seems relevant to report the OH-PCB concentrations but it is probably time to see if it is possible to determine factors between OH-PCB congener concentrations to parent PCB congeners.

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References

1 Kocan, A., Petrik, J., Jursa, S., Chovancova, J. and Drobna, B. (2001) *Chemosphere*, **43**, 595-600.
2 Letcher, R. J., Klasson Wehler, E. and Bergman, Å. (2000) New Types of Persistent Halogenated Compounds. 1, 315-359, Springer-Verlag, Berlin.
3 Fängström, B., Athanasiadou, M., Grandjean, P., Weihe, P. and Bergman, Å. (2002) *Environ. Health Perspect.*, **110**, 895-899.
4 Hovander, L., Athanasiadou, M., Asplund, L., Jensen, S. and Klasson Wehler, E. (2000) *J. Anal. Toxicol.*, **24**, 696-703.

5 Hovander, L., Malmberg, T., Athanasiadou, M., Athanassiadis, I. , Rahm, S., Bergman, Å. and Klasson Wehler, E. (2002) *Arch. Environ. Contam. Toxicol.*, **42**, 105-117.
6 Sandau, C. D., Ayotte, P., Dewailly, E., Duffe, J. and Norstrom, R. J. (2000) *Environ. Health Perspect.*, **108**, 611- 616.
7 Sjödin, A., Hagmar, L., Klasson Wehler, E., Björk, J. and Bergman, Å. (2000) *Environ. Health Perspect.*, **108**, 1035-1041.
8 Soechitram, S. D., Athanasiadou, M., Hovander, L., Bergman, A. and Sauer, P. (2004) *Environ. Health Perspect.*, In press
9 Malmberg, T., Hoogstraate, J. , Bergman, Å. and Klasson-Wehler, E. (2004) *Xenobiotica*, In press.
10 Weistrand, C., Norén, K. and Nilsson, A. (1997) *Environ. Sci. Pollut. Res. Int.*, **4**, 2-9.
11 Norén, K., Lundén, Å., Pettersson, E. and Bergman, Å. (1996) *Environ. Health Perspect.*, **104**, 766-772.
12 Haraguchi, K., Athanasiadou, M., Bergman, Å., Hovander, L. and Jensen, S. (1992) *Ambio*, **21**, 546-549.