Measurement of Hydroxylated PCB Metabolites for Slovakia Maternal Blood Serums

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Introduction

Adverse human health and developmental effects of PCBs have been reported but were sometimes not clear whether they were caused by PCBs themselves or related compounds such as OH-PCB metabolites. OH-PCB metabolites of para- and meta-substituted OH with adjacent chlorine atom have relatively high affinity with TTR [1-3] and are largely responsible for hypothyroidism in brain and blood, especially of fetal compartment [1,4,5]. From 1959 to 1984, Chemko chemical plant located in Michalovce district, eastern Slovakia, intensively produced PCBs and resulted in environmental PCB contamination due to improper disposal of PCB waste [6]. Thus mothers exposed to high levels of PCBs and subsequently OH-PCB metabolites may result in some residual problems in children after birth (e.g., deficit in the physical growth and impaired development of neurosensory and immune systems). To our knowledge, the measurement of OH-PCB metabolites in maternal blood is the first time ever here although there have been a few studies elsewhere [7-11]. In this study, we attempted to characterize and quantify the levels of specific OH-PCB metabolites in Slovakia maternal serum exposed to the high environmental PCB levels.

Materials and Methods

From 1959 to 1984, Chemko chemical plant located in Michalovce district, eastern Slovakia, intensively produced PCBs and resulted in environmental PCB contamination due to improper disposal of PCB waste. About 1100 maternal serum specimens were collected from two eastern Slovakia districts, Michalovce district as a high PCB exposure area and Svidnik/Stropkov district with low exposures. All specimens were analyzed for PCBs. A subset of the samples (N=166) were analyzed for OH-PCB metabolites. Each batch for the OH-PCB analysis was consisted of one reagent blank (water), 1~2 control samples, 10 maternal serum specimens, and five calibration standards for quantification. 4-OH-CB159 as a recovery internal standard was added to all samples before extraction. Wallenberg blood extraction method was adopted to separate the OH-PCBs from maternal serum and described in detail elsewhere [8,12]. The OH-PCBs in the sample extracts and calibration standards were methylated by adding diazomethane. The extracts were cleaned up by using concentrated H_2SO_4 (98%) and then 0.5 g of H_2SO_4/silica gel (22% H_2SO_4, w/w) column eluted with 1:1 dichloromethane:hexane (10 mL). The final eluates and calibration standards were spiked with PCB209 as an injection standard before GC analysis. The OH-PCBs in the final extracts were determined as methyl derivatives (MeO-PCBs) by using a GC-MS (HP 6890N and 5973N) equipped with DB-5MS capillary column (30 m×0.25 mm i.d., 0.25 mm film thickness, J&W Scientific, USA). The MS was operated in electron capture negative ionization with an electron voltage of 130 eV. Helium and methane were used as a carrier gas and a reagent gas, respectively. The final concentrations of OH-PCB congeners from each sample were corrected by the recoveries of internal standard and subtracted by the concentrations of reagent blank.

Results and Discussion

The recoveries of OH-PCBs from control samples ranged from 75±9% (4'-OH-CB130) to 101±11% (4-OH-CB146) (Figure 1). Average recovery of internal standard (4'-OH-CB159) was 84±16%. Median concentrations of OH-PCB metabolites of Michalovce mothers were >2 times higher than Svidnik mothers ($p<0.001$). Concentrations of total OH-PCB metabolites varied from 0.12 to 6.94 ng/g fresh wt with a median of 0.84 in Michalovce mothers and from 0.08 to 1.57 with a median of 0.34 in Svidnik mothers. 4-OH-CB187 was a primary metabolite and followed by 4-OH-CB146. And 4-OH-CB107, 3-OH-CB153, 3'-OH-CB138, and 4'-OH-CB172 were also detected. Concentrations of total OH-PCB metabolites correlated with total PCBs ($R^2=0.71$, $p<0.001$), indicating the blood levels of OH-PCB metabolites were dependent on PCB levels. Ratio of total OH-PCBs to total PCBs ranged from 0.03 to 0.47 with an average of 0.11±0.06. Concentrations of OH-PCBs of Michalovce mothers were comparable to Faroe Island mothers [7] andnorthern Canadian female Inuit [9] that were reportedly among the highest in human blood (Figure 1). Although a temporal decreasing trend of PCBs was observed, the population residing in Michalovce district was still highly exposed to the environmental PCBs and particularly OH-PCB metabolites. Further study is focused on placental transfer of OH-PCBs to fetus and subsequently epidemiologic approaches to determine the relationship between the exposure of OH-PCB metabolites and child development. Future research is needed to

investigate the detailed dietary and other pathways of human exposures to PCBs to alert the people living in this area, so they can minimize their future exposures.

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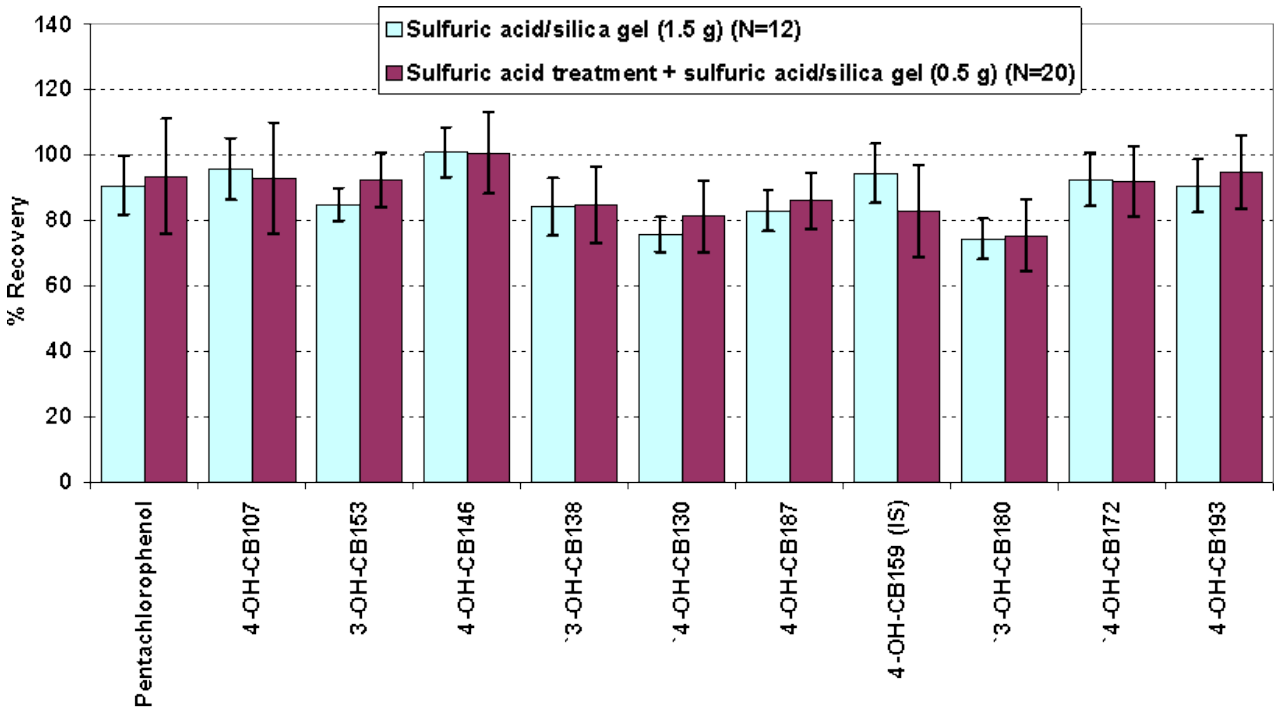

Figure 1. %Recovery of OH-PCBs from control matrix spike serums from two different clean up procedures.

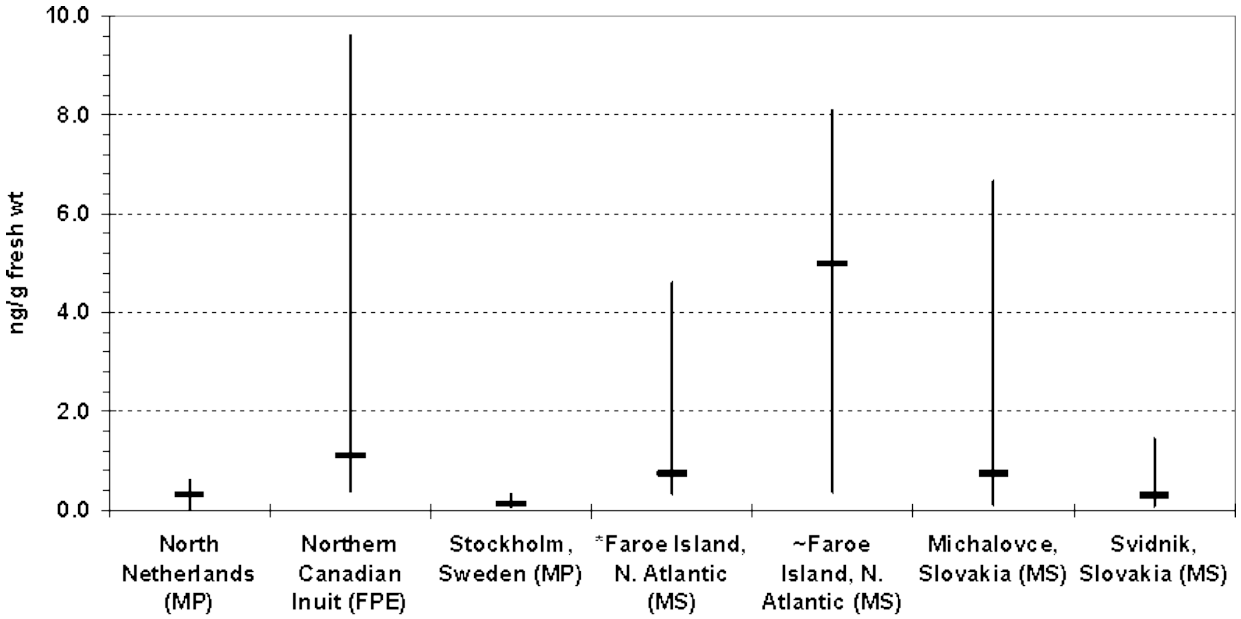

Figure 2. Maternal blood concentrations of major OH-PCB metabolites measured from other areas (median and range). *Low fish consumption. ~High fish consumption. MP: maternal plasma. FPE: female plasma equivalent. MS: maternal serum. Data for Netherlands, Canadian Inuit, Sweden, Faroe Island, and Slovakia were available in Soechitram et al. (2004 & personal communication), Sandau et al. (2000), Guvenius et al. (2003), Fangstrom et al. (2002), and this study, respectively.