DIOXINS, PCBs AND ORGANOCHLORINE PESTICIDES IN THE BLOOD SERUM OF SLOVAK RESIDENTS

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs, dioxins), polychlorinated dibenzofurans (PCDFs, furans), polychlorinated biphenyls (PCBs) and organochlorine pesticides belong to persistent organic pollutants (POPs), which are resistant to environmental decomposition, they accumulate in living organisms, their biological degradation is very slow and are toxic to animals and humans. POPs can promote cancer, and reproductive, neurobehavioral and endocrine disorders [1, 2, 3].

The aim of the study was to evaluate the dioxin, PCB and organochlorine pesticide exposure of general population living in a rural area with a suspected source of environmental contamination with dioxins and related compounds and in control areas where no such sources are present, and to compare the results with POP levels found in residents from other countries.

Materials and Methods

Study subject selection, blood collection

Blood samples were collected in the period of September – November 2006. Eighteen volunteers living close to the hazardous waste incinerator of Nemecká, and 15 volunteers living in four areas with no important industrial dioxin source (Bytča, Trenčín, Pezinok, and Dunajská Streda) were recruited. The participants were selected randomly from lists of insured subjects by care physicians from all the study areas. All the volunteers were asked to complete a structural questionnaire administered by trained interviewers, which included demographic and anthropometric information, occupation, and dietary and smoking habits. The questionnaire also contained questions about professional exposure to organochlorine compounds. Up to ten 9-mL vacutainers of whole blood were collected from each donor. A blood serum (25 – 35 ml) obtained after fast centrifugation (at 3 000 rpm for 15 minutes) of whole blood was stored in a screw glass vial with PTFE-sealed caps at -18 °C. The total lipid content in each serum sample was determined in a 0.5 mL aliquot of serum specimen using an enzymatic summation method [4].

Sample preparation and analysis

PCDDs/Fs and dl-PCBs

Each individual serum sample (25 – 30 ml) was spiked with a known amount of fifteen $^{13}C_{12}$-labelled PCDD/F and twelve $^{13}C_{12}$-labelled dl-PCB congeners before sample extraction. Solid phase extraction procedure (SPE, C_{18}) for the isolation of the compounds of interest was used. PCDDs/Fs and dl-PCBs were separated on a Power-Prep™ semi-automated cleanup system with pre-packed multi-layer silica, basic alumina and carbon columns (Fluid Management Systems, USA). Prior to GC injection, serum extracts were reconstituted with n-nonane containing method-recovery labelled standards. Each analytical batch consisted of a method blank and QC sample (in-house reference material: porcine serum spiked with native PCDDs/Fs and dl-PCBs). The measurements were performed by HRGC/HRMS using an MAT 95 XP high-resolution mass spectrometer (Thermo Finnigan, Germany) coupled to an HP 6890 gas chromatograph (Hewlett-Packard, USA). HRMS was operated in the positive ionization mode at 53 eV. For each substance two most abundant isotopes were monitored. The quantification was carried out by isotope dilution methods USEPA 1613, and 1668.

Di-ortho PCBs and organochlorine pesticides

Each individual serum sample (2 – 4 ml) was spiked with a PCB-174 congener before sample processing in order to check the recovery of a cleanup procedure. Di-ortho PCB congeners and organochlorine pesticides were isolated by solid phase extraction[5]. Serum extracts were reconstituted with recovery standard (PCB-103). An analysis batch consisted of 10 serum samples, one solvent blank and one spiked porcine serum in-house reference material.

The analysis of PCB congeners (28, 52, 101, 138^{+163}, 153 and 180), HCB, p,p'-DDE and p,p'-DDT were performed using a gas chromatograph 6890N (Agilent Technologies, USA) with a micro electron capture

detector. The quantification was based on a multilevel calibration curve constructed by five standard congener mixtures.

Results and Discussion

Distribution of age, gender, BMI (body mass index), resident areas, smoking status, PCDD/F, dl-PCB levels (expressed as $WHO_{98}TEQ$ values), PCB-153, HCB, p,p'-DDE and p,p'-DDT for the study participants are reported in Table 1. The blood samples taken from participants living in districts of Bytča, Trenčín, Pezinok and Dunajská Streda were considered as one control group. If some congener was present at a concentration lower than its limit of detection (LOD), half of the LOD was used for TEQ calculation.

The mean lipid content in the serum specimens from males and females was 8.7 mg/mL and 7.8 mg/mL, respectively. The serum mean $TEQ_{PCDDs/Fs/dl-PCBs}$ was 33.0 pg/g lipid for males and 30.2 pg/g lipid for females. 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF and 1,2,3,4,6,7,8-HpCDF congeners were positively detected in a range of 87-100% of all the samples. The most toxic congener 2,3,7,8-TCDD was detected in less than 40% of the samples. The congeners detected at the highest concentrations in all the samples were 1,2,3,4,6,7,8-HpCDD and OCDD. The profile of congeners was quite similar to those observed in human serum from Slovak residents living in non-polluted areas Stropkov-Svidník[6]. Nine out of twelve dl-PCB congeners (126, 169, 105, 114, 118, 167, 156, 157 and 189) were positively detected in all the serum samples. The incidence of increased values for PCB-77 was observed in the laboratory blanks. For this reason PCB-77 was not reported in 20 serum specimens and was excluded from statistical analysis. Thus, the most abundant non-ortho congener and also the major contributor to the TEQ levels was PCB-126.

The PCB congeners 28, 52, 101 were detected in more than 50% of the analysed samples. The sum of dominant PCB congeners 138, 153 and 180 ranged between 154 to 2006 ng/g lipid in both areas. HCB was detected in 31 samples and the participants had levels between <0.24 to 1485 ng/g lipid.

Table 1: Distribution of demographic characteristics, PCDD/F, dl-PCB (expressed as $WHO_{98}TEQ$), PCB-153, HCB, p,p'-DDE and p,p'-DDT levels in human blood samples

Area	Control	Nemecká
N	15	18
Age (average; min-max)	39; 24-54	41; 26-56
Gender		
Male	7	10
Female	8	8
BMI (average; min-max)	25.3; 19.4-36.6	25.8; 21.2-31.4
Residential area		
Urban	9	0
Rural	6	18
Smoking status		
Non-smokers	5	12
Active smokers	10	6
Past smokers	1	5
Passive smokers	2	8
$WHO_{98}TEQ_{PCDDs}$; *pg/g lipid (average; median; min-max)*	3.6; 3.4; 0.92-9.8	3.7; 3.3; 0.59-8.5
$WHO_{98}TEQ_{PCDFs}$; *pg/g lipid (average; median; min-max)*	6.2; 6.5; 3.3-9.7	8.8; 7.5; 1.9-16.2
$WHO_{98}TEQ_{PCDDs+PCDFs}$; *pg/g lipid (average; median; min-max)*	9.9; 9.9; 4.6-17.5	12.5; 12.9; 2.4-20.7
$WHO_{98}TEQ_{dl-PCBs}$; *pg/g lipid (average; median; min-max)*	14.4; 13.7; 4.4-26.7	26.0; 24.4; 4.9-47.2
PCBs-153; ng/g lipid (average; median; min-max)	233; 184; 54.4-856	322; 282; 95.4-714
HCB; ng/g lipid (average; median; min-max)	135; 53.1; <0.24-727	269; 187; 21.2-1485
p,p'-DDE; ng/g lipid (average; median; min-max)	875; 577; 59.7-2118	918; 812; 96.4-2290
p,p'-DDT; ng/g lipid (average; median; min-max)	22.6; 22.8; 5.5-52.6	24.3; 23.2; 4.4-65.2

As can be seen in Table 1, the mean concentrations of PCDDs/Fs and PCB-153 in the donors living close to the waste incinerator were 1.3 and the concentrations of dl-PCBs and HCB almost twice higher than in the donors from the control area. None of the donors from the control area was occupationally exposed according to questionnaire data. It means that only contaminated food can be considered in long-term ingestion of persistent organochlorine compounds. The results indicate that higher levels of PCDDs/Fs, PCBs and HCB observed in the donors from Nemecká could have been caused by higher environmental contamination due to long-term emissions from the waste incinerator situated in a rural mountainous area. The mean ratio of TEQ_{PCDDs} and TEQ_{PCDFs} was 0.58 and 0.42 in control and potentially polluted area, respectively. Our findings are comparable with those found in our previous study[6]. Dioxin-like PCBs are comparable with levels in France, significantly higher than in Greece, Germany, and Japan (Table 2)[7, 8, 9, 10]. Overall, the PCDD/F levels are significantly higher than those found in Greece, little lower than levels in Germany, Portugal, Japan, and considerably lower than in France[7, 8, 9, 10, 11]. The study results also showed that di-ortho PCBs are higher than those in Greece, Japan, and France[7, 8, 10].

Table 2: Mean TEQ (pg/g lipid) in blood serum from various countries

Country	PCDDs/Fs	Dioxin-like PCBs
Slovakia (this study)	11.3	20.9
Germany	16.8	11.6
Greece	6.8	3.2
France	23.1	20.8
Portugal	15.3	
Japan	15.8	7.8

No correlation was observed between serum TEQ levels and smoking habits. Little difference in the median $TEQ_{PCDD/F}$ levels was found between non-smokers (11.1 pg/g lipid) and active smokers (9.2 pg/g lipid). Median $TEQ_{dl\text{-}PCBs}$ was higher in non-smokers (19.8 pg/g lipid) in comparison with active smokers (13.7 pg/g lipid). The highest TEQ levels of PCDDs/Fs (15.0 pg/g lipid) and dl-PCBs (21.8 pg/g lipid) were determined in serum of passive smokers.

An increase of PCDD/F levels with age is evident from Figure 1. The correlation with age was confirmed also for dl-PCBs except for the age group of 31 – 40 years.

Figure 1: Median WHO_{98}TEQ (pg/g lipid) in blood serum vs age groups

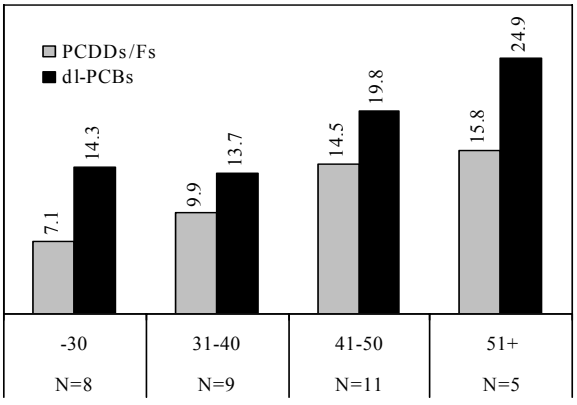


Figure 2: Median WHO_{98}TEQ (pg/g lipid) in blood serum by gender and area

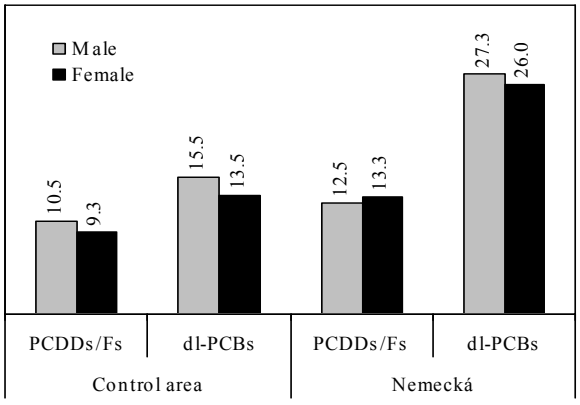


Figure 2 shows PCDD/F and dl-PCB levels in combination of gender and area. The concentrations of dioxin and dl-PCBs in participants from Nemecká were higher in both males and females than in the control area. The median TEQ_{PCDFs} in females were approximately twice higher in both studied areas in comparison with

TEQ_{PCDDs}, and were 1.9 times higher in males from the control area and 2.8 times higher from an area near the waste incinerator.
Age was positively associated with serum levels also for di-ortho PCBs (Figure 3). However, for HCB and DDE concentrations linear association with age was not clearly confirmed in all age groups. That might have been caused by the small number of participants in each age group. Substantial elevated DDE concentrations were determined at age 41 and more. As observed for dioxins and dl-PCBs, higher levels of di-ortho PCBs and HCB in males and females were found in adults living close to the waste incinerator (Figure 4). Although a declining trend of HCB concentrations has been observed in blood in comparison with our previous study, the Slovak population still shows elevated exposure to HCB in comparison to other European countries[6, 12]. Median DDE was markedly higher in females from Nemecká in comparison with the control group, on the contrary higher concentration was observed in control area in males.

Figure 3: Median HCB, DDE, PCBs (ng/g lipid) in blood serum vs age groups

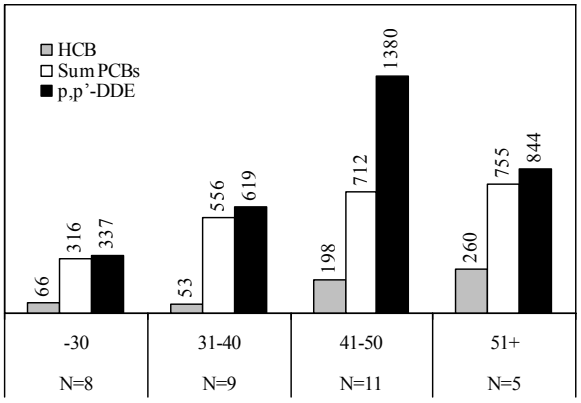
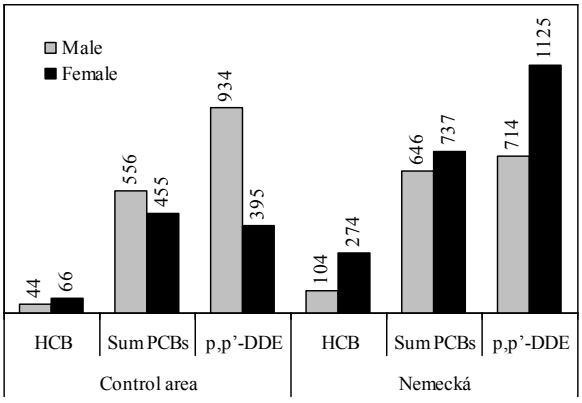


Figure 4: Median HCB, DDE, PCBs (ng/g lipid) in blood serum by gender and area



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