PHENOLIC ORGANOHALOGENATED CONTAMINANTS IN HUMAN SERUM FROM BELGIUM AND ROMANIA

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Introduction

Polychlorinated biphenyls (PCBs) are one of the most studied compounds classes since they were discovered as environmental pollutants over 30 years ago[1]. Even if they were banned in most countries in late 1970s, PCBs usually dominate the burden of organohalogen contamination in humans[2]. PCBs are biotransformed by cytochrome P-450 monoxygenases and the major metabolic pathways lead to the formation of hydroxylated PCBs (HO-PCBs) and methylsulphone PCBs[3]. Even the most persistent PCBs in the environment, such as CB 153, are biotransformed both *in vitro* and *in vivo* forming a number of hydroxylated metabolites[3]. Compared to studies investigating the presence of PCBs in humans and the environment, less attention has been given to the HO-PCB metabolites.

The present study aims at investigating the presence of HO-PCBs in human serum samples collected from two locations with different degrees of pollution: a) the Eastern part of Romania, an area known to be highly contaminated with organohalogenated contaminants, especially pesticides[4] and b) Belgium with relatively low reported levels of organohalogenated contaminants in human serum[5]. The differences in capacity to metabolize such contaminants by humans living into different areas and having different life styles were therefore investigated. Additionally, differences between the levels of other phenolic contaminants, such as tetrabromobisphenol A (TBBP-A) in collected samples, were also assessed.

Materials and methods

Samples collection. In total, 53 persons (22 females) were sampled in December 2007 from Iassy, Eastern-Romania and 20 individuals (4 females) were sampled in 2000 from Belgium. Detailed information regarding the lipid content (cholesterol and triglycerides) of the samples together with gender, age and area living of the sampled persons are known for each sample.

Investigated analytes. The following analytes were measured in each of the collected samples: pentachlorophenol (PCP), 3-HO-CB118, 4'-HO-CB107, 3-HO-CB153, 4-HO-CB146, 4'-HO-CB127, 3'-HO-CB138, 4'-HO-CB130, 4-HO-CB163, 4-HO-CB187, 3'-HO-CB180, 4'-HO-CB172, 4-HO-CB193 and TBBP-A. Parental PCBs (CB 101, CB 99, CB 118, CB 146, CB 153, CB 105, CB 138/163, CB 187, CB 183, CB 156, CB 180 and CB 170) were also measured in samples in order to find correlations between levels of phenolic and neutral components concentrations.

Analysis methodology. The analysis of the phenolic contaminants from serum samples was adapted from the method described by Weiss et al[6]. A volume of serum accurately measured (between 2 and 3.5 mL) was spiked with internal standard, 4'-HO-CB 159, diluted 1:1 with Milli Q water and mixed with formic acid. The resulting mixture was sonicated for 20 min and then extracted using solid-phase extraction cartridges (6 mL/ 500 mg Oasis® HLB). Elution was done by 10 mL of MeOH:DCM (1:1, *v/v*) and the eluate was evaporated to near dryness. The analytes were reconstituted in 500 µl Hex:DCM and subjected to fractionation on a silica SPE cartridges (3mL/500 mg). A first fraction containing PBDEs was eluted with 5 mL Hex, while the second fraction containing phenolic compounds was eluted with 6 mL MeOH:DCM (1:1, *v/v*). The phenolic fraction was derivatized with freshly prepared diazomethane in diethyl ether (30 min, room temperature) when MeO-PCBs were formed. The solvent was then evaporated, the dried residue was reconstituted in 200 µl DCM and further cleaned on 500 mg acid silica impregnated with concentrated sulfuric acid (25%, *w/w*). Methoxylated compounds were eluted with 10 mL

Hex:DCM (1:1, *v/v*). Further, the cleaned extract was evaporated to dryness under a gentle nitrogen stream, reconstituted in 2ng BDE 77 used as syringe standard and transferred to an injection vial.
For the analysis of methoxylated derivatives of phenolic compounds, an Agilent 6890-5973 GC-MS operated in electron capture negative ionisation mode was equipped with a 20 m x 0.18 mm x 0.20 µm AT-5 capillary column.

Quality assurance/Quality control. Multi-level calibration curves in the linear response interval of the detector were created for the quantification, and good correlation ($r^2 > 0.999$) was achieved. The identification of target analytes was based on the relative retention times (RRTs) to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions. A deviation of the ion intensity ratios within 20% of the mean values obtained for calibration standards was considered acceptable. The MS was used in selected ion monitoring mode with minimum two specific ions monitored for each HO-PCB congener and PCP in specific windows, while ions *m/z* = 79 and 81 were monitored for TBBP-A during the entire run. For each analyte, the mean procedural blank value was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank considering the amount of sample taken into analysis. For analytes that were not detected in procedural blanks (most HO-PCBs), LOQs were calculated for a signal-to-noise ratio equal to 10. For internal standard 4'-HO-CB 159, the recovery values were also assessed for the analysis of each serum sample compared to PCB 143.

The quality control was performed by regular analyses of procedural blanks, by random injection of standards, spiked samples and solvent blanks. The quality control scheme is also assessed through regular participation to inter-laboratory comparison exercises organized by AMAP (organohalogenated pollutants in serum). Obtained values were deviating with less than 10% from the certified values.

Results and discussion

Phenolic and neutral contaminants. Levels and profiles. The main chlorinated phenolic compound found in Belgium samples was PCP (detection frequency of 100%) which contributed up to 85% to the total chlorinated phenolic compounds quantitated, compared to only 35% contribution recorded for the Romanian samples (detection frequency of 20%). Such a high contribution of PCP to the total phenolic fraction is not singular in the literature[7]. To some extent, PCP may be formed through HCB metabolism[8], but this does not explain the high levels of PCP found in Belgium samples (correlation between HCB and PCP levels: r=0.022). The source of PCP is most presumably the historical use of PCP for wood protection. The difference recorded between the two batches of samples analyzed, might be of a high importance since PCP binds to TTR with twice the affinity of the native hormone[9]. Considering the significance of PCP in the chlorinated phenolic fraction of the Belgium samples, further investigation of PCP and its possible effects on thyroid hormone transport is needed.

In general, the detection frequency recorded for the phenolic contaminants in Belgium samples was much higher compared to Romanian samples, except 4HO-CB193 and TBBPA which were detected in less than 50% in both batches of samples.

Significant differences were obtained between median concentrations of sum HO-PCBs in Belgium samples (312 pg/mL) and in Romanian samples (175 pg/mL). This is an interesting result since PCBs were comparable between both countries (3100 pg/mL for sum PCBs in Romanian samples compared to 3380 pg/mL for Belgium samples). The levels of phenolic contaminants in the present study are in the same range compared to previously published papers[10] or lower than in highly contaminated population to PCBs[11].

The main contributors to the sum HO-PCBs in Romanian samples were 4HO-CB187 > 4HO-CB146 > 3HO-CB138 (in total constituting up to 66 % from the sum of HO-BCBs) and in Belgian samples were 4HO-CB107 > 4HO-CB146 > 4HO-CB187 (constituting up to 76 % from the sum HO-PCBs). The profile of phenolic contaminants measured in samples shown that the higher chlorinated compounds tend to have a higher contribution in the Romanian samples compared to Belgium ones (Figure 1) and inverse, the lower chlorinated HO-CBs tend to have a higher contribution in Belgium samples. Interestingly, but 3HO-CB153 was not as important in contribution as it was found to be by other authors. The profile recorded for Romanian samples, with 4HO-CB187 being the primary HO-CB metabolite is in agreement with many other previously published studies[12], while the results found for

Belgium samples which show 4HO-CB107 as a primary HO-CB metabolite is reported here for the first time. 4HO-CB146 has been found to be the second most abundant metabolite for samples from both locations, these results being consistent for most of previously published studies[13].

Table 1. Median concentrations, range (pg/mL) and detection frequencies-DF (%) of neutral and phenolic organic compounds in human serum samples from Romania and Belgium (values for method LOQs for each analyte are also presented).

Compound	Method LOQ (pg/mL)	Romanian samples (N=53)				Belgium samples (N=20)			
		DF (%)	range (pg/mL)		Median (pg/mL)	DF (%)	range (pg/mL)		Median (pg/mL)
			min	max			min	max	
T4Cl-P	50	42	20	400	21	100	150	2145	300
T3BrP	50	9	4.4	80	4.4	100	130	816	260
PCP	500	20	<LOQ	1200	<LOQ	100	1285	20250	6290
HO-T4ClB	3	87	<LOQ	210	7	100	9.5	150	29
3HO-CB118	3	33	<LOQ	30	1	89	<LOQ	40	7.7
4HO-CB107	3	96	<LOQ	130	12	100	36	190	78
3HO-CB153	3	84	<LOQ	85	8.3	100	4.2	57	15
4HO-CB146	3	100	12	125	37	100	33	145	92
4HO-CB127	3	47	<LOQ	7	1.4	100	3	12.5	4.5
3HO-CB138	3	98	<LOQ	130	14	100	6	100	22
4HO-CB130	3	9	<LOQ	5	0.2	100	5.2	27	16
4HO-CB163	2	67	<LOQ	11	2.8	100	4.4	16.5	10.5
4HO-CB187	2	100	13	300	51.5	100	28	105	57
3HO-CB180	2	87	<LOQ	80	6.5	89	<LOQ	18	4.5
4HO-CB172	2	100	2.3	75	12	100	3.8	24.5	11
4HO-CB193	2	51	<LOQ	8	1.6	44	<LOQ	3	0.9
sum HO-CBs			50	790	175		153	675	312
TBBP-A	2	36	<LOQ	13	0.7	6	<LOQ	2.3	0.1
sum PCBs			3745	19400	3110		765	6975	3385
pp-DDE	50	100	1940	38850	12200	100	255	9910	2485
pp-DDT	40	100	125	5200	590	50	20	93	33
sum DDTs			2100	41500	1325		275	9970	2550

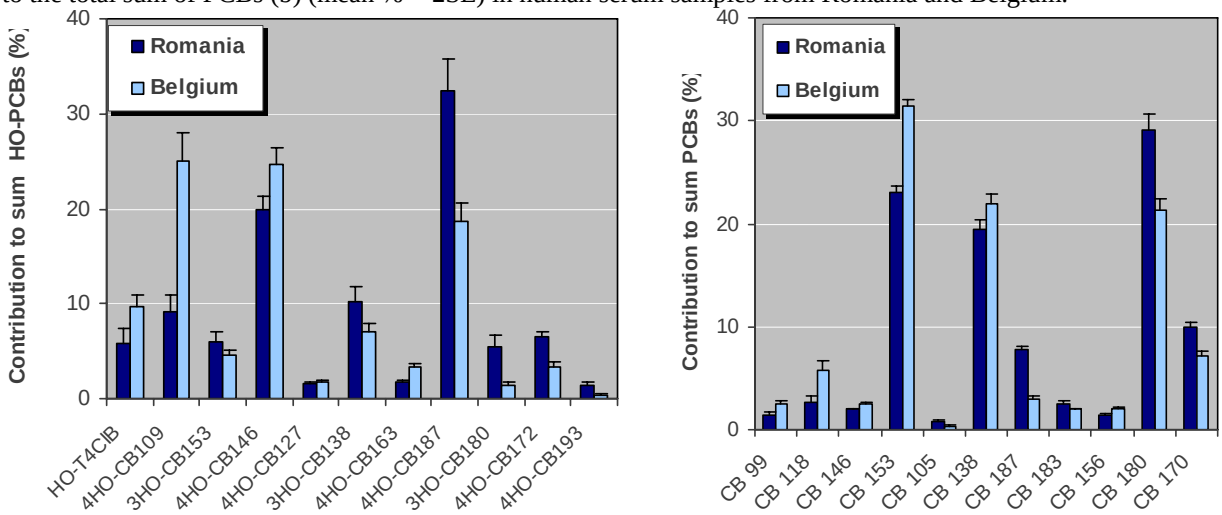
When gender of individuals was considered as variable, the results showed that significant differences ($p<0.05$) were recorded for neutral contaminants (CB146, CB187, CB183, CB156, CB180, CB170) and non-significant differences ($p>0.05$) were found for all phenolic contaminants.

For other neutral contaminants measured in samples from both countries, the most important are the differences recorded for the levels of *pp*-DDT and *pp*-DDE: up to 5 times higher for *pp*-DDE and up to 15 times higher levels of *pp*-DDT recorded for the Romanian samples ($p<0.001$). As other previous studies conclusions, this results shows that the regulation of the use of DDTs in Romania is only official, in reality such contaminants being still illegally in use.

Relationships between phenolic and neutral contaminants. Pearson correlations applied on *log* transformed data were performed in order to check whether there are relations between the parental PCBs and HO-PCB metabolites. Therefore, significant correlations ($p<0.05$) were found for most of the HO-PCB metabolites and their parental

compounds in case of samples from both countries. For Romanian samples all correlations were stronger between the hydroxyl-compounds and their parental PCBs which correspond to the metabolism via direct insertion of HO-group compared to Belgium samples where the correlations were in some cases higher between the HO-PCB metabolites and their parental PCBs which correspond to a metabolism via NIH-shift.

Figure 1. Contribution of single HO-PCB congeners to the total sum of HO-PCBs (a) and of single PCB congeners to the total sum of PCBs (b) (mean % + 2SE) in human serum samples from Romania and Belgium.



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