ORGANOCHLORINE CONTAMINANTS IN HAIR OF ADOLESCENTS FROM IASSY, ROMANIA

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Abstract

Hair samples of adolescents (n=42) from Iassy, Romania collected in 2002-2003 were analyzed for five PCB congeners and selected organochlorine pesticides (OCPs), such as hexachlorocyclohexane isomers (HCHs), p,p'-DDT and its metabolites, hexachlorobenzene, oxychlordane and *trans*-nonachlor. A high detection frequency was observed for all contaminants. Furthermore, the high levels of α- and γ-HCH and also of *p,p'*-DDT suggest an ongoing exposure to technical HCH and lindane formulations, but also to "fresh" DDT. With few exceptions, the concentrations of contaminants were higher levels in girl adolescents than in boys. Compared to other studies, the results of the present study were significantly higher for OCPs, but not for PCBs.

Introduction

Hair has been identified as an alternative matrix for the assessment of human exposure to persistent organic pollutants (POPs)[1,2]. It has several advantages compared to "classical" matrices used for biological monitoring, such as blood or milk. Blood is not always available in sufficient amounts for reliable analysis, whereas tissues need to be obtained by invasive procedures (e.g. surgery). Contrarily, hair collection is simple, inexpensive, non-invasive, involves minimal stress to individuals and the subject compliance is high.[1] Hair analyses can be performed in any population group, unlike human milk which is restricted to women of lactating period, and allow repeated sampling of the same individual. Relatively high lipid content (1-4 %) makes hair a suitable matrix for the analysis of POPs in humans, but also in animals.[1-6] Nevertheless, there are several drawbacks of hair analysis, such as the issue of whether the analytes measured in hair are biologically incorporated or environmental deposited from air or the missing information about the eventual correlations between POP levels in hair and in other tissues. Yet, the use of hair as a reliable analytical matrix is a very promising issue.

The aim of this study was to evaluate the contents of selected organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in hair samples collected from Romanian adolescents (15 - 18 years old). Secondly, relationships between gender of adolescents sampled and concentration of various pollutants measured was also investigated.

Materials and Methods

Sample description and materials. Hair samples were obtained in 2002-2003 period from a total of 42 adolescents of which 22 were boys, aged between 15 and 18 years, living in Iassy County, Eastern Romania, who voluntarily participate in the study.

The OCPs under investigation were α-, β-, γ- and δ-HCH (expressed as HCHs), *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *o,p'*-DDT, *p,p'*-DDD and *p,p'*-DDT (expressed here as DDTs), hexachlorobenzene (HCB), oxychlordane (OxC) and *trans*-nonachlor (TN). The following PCB congeners (IUPAC numbers) were targeted: 118, 153, 138, 180 and 170. Internal standards used were PCB 46, PCB 143 and ε-HCH. All individual standards of PCBs and OCPs were obtained from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). Hexane (Hex) and dichloromethane (DCM) were of pesticide-grade purity from Merck (Darmstadt, Germany). Anhydrous sodium sulphate (Merck) for residue analysis and silica gel, 60-200 mesh, (Merck) were washed with hexane and used after heating overnight at 120°C.

Sample preparation and analysis of organochlorine contaminants. The analytical procedure for the determination of POPs in human hair was based on the method described in Covaci and Schepens[3]. Hair samples were cut into 1 mm portions, washed with 5 ml MilliQ water and ultrasonicated for 5 min. Approximately 500

mg of washed and cut hair samples were accurately weighted, spiked with internal standards 7.5 ng of (PCB 46 and PCB 143) and 5 ng of ε-HCH and incubated overnight at 40°C with 4 mL HCl 4M and 3 mL Hex:DCM (4:1, *v/v*). Extraction of the analytes from the incubation medium was done by a liquid-liquid procedure (LLE) with 2 x 4 mL Hex:DCM (4:1, *v/v*). The organic layer (from LLE) was purified on a cartridge filled with approximately 500 mg of acidified silica (45% H_2SO_4, *w/w*) and 250 mg anhydrous Na_2SO_4. The cartridge was pre-washed with 3 mL Hex:DCM (4:1, *v/v*). After loading, the cartridges were eluted with 4 mL Hex:DCM (1:1, *v/v*) and the final eluate was concentrated to approximately 75 µl under a gentle nitrogen stream and transferred to an injection vial.

An Agilent (Palo Alto, CA, USA) 6890 GC-µECD was equipped with a 50m x 0.22mm x 0.25µm, HT-8 (SGE, Zulte, Belgium). Helium was used as carrier gas at a constant flow of 1.0 mL/min and Ar/CH_4 (95:5) as make-up gas (40 mL/min). One µl was injected in the pulsed splitless mode (pulse pressure = 40 psi, pulse time = 1.5 min) with the split outlet opened after 1.5 min. Injector and detector temperatures were set at 280°C and 320°C, respectively. The temperature program of the HT-8 column was set to 90°C for 1.2 min, then a rate of 20°C/min was applied to 180°C, kept for 1 min, then to 275°C by 3°C/min and further by 25°C/min to 290°C and kept for 18 min.

Quality assurance. Multi-level calibration curves were created for the quantification and good linearity (r^2 > 0.999) was achieved for tested intervals that included the whole concentration range found in samples. The identification of POPs was based on their relative retention times to the internal standard used for quantification. Peak area ratios (analyte response/internal standard response) were plotted against the concentration ratios (analyte concentration/internal standard concentration). Reagent blanks were run to check for interferences which can occur. Using the above described procedure, analyte recoveries were found acceptable (> 85%) with a relative standard deviation of less than 12%.[3] Relatively low detection limits were obtained and they ranged between 0.1 and 0.2 ng/g hair for individual compounds. The external quality control was assessed through participation to an interlaboratory exercise in which measured values for PCBs and p,p'-DDE were within a relative standard deviation of 15%.[7]

Results and Discussion

Levels of OCPs and PCBs in hair samples.

With few exceptions (HCB and OxC which were quantified in 48 and 50% of the samples, respectively), the investigated OCPs had a high detection frequency (> 88%). The contaminants measured in higher concentrations were γ-HCH (median 79 ng/g) and β-HCH (55 ng/g), TN (89 ng/g), *p,p'*-DDE (127 ng/g), *o,p'*-DDT (73.5 ng/g) and *p,p'*-DDT (192 ng/g) (Figure 1).

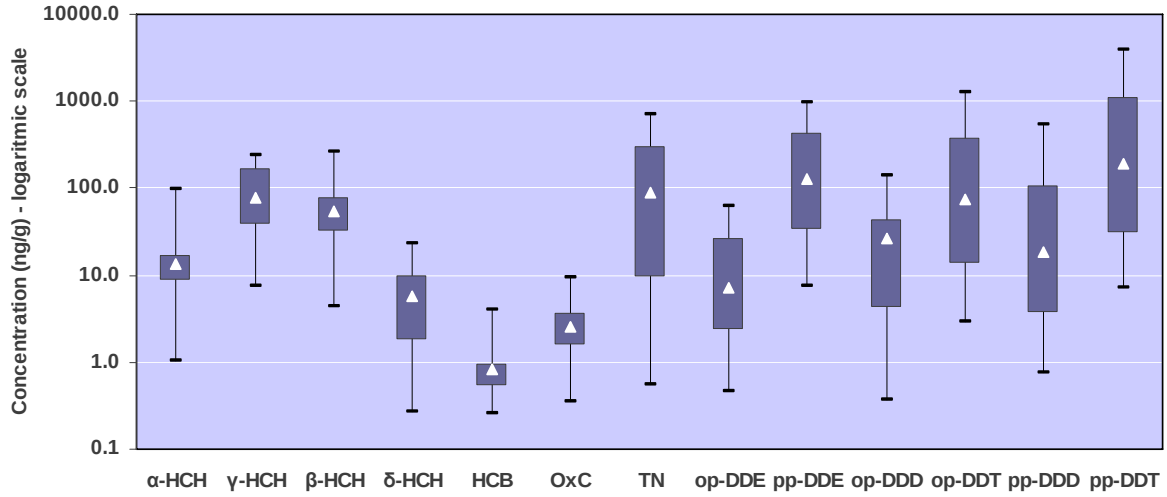

Figure 1. Distribution of OCPs (ng/g) in hair samples from adolescents (n=42) (median values, 1st and 3rd quartile and range)

The γ-HCH isomer was measured at higher concentrations compared to β- isomer, which generally is the most prevalent HCH isomer in human tissues, indicating exposure to lindane formulation. Additionally, measurable amounts of α-HCH suggest the parallel use of technical HCH. Very low levels found for HCB and OxC (0.8 and 2.5 ng/g, respectively) are in agreement with previous reported results from samples from the same area with adolescents sampled for this study, indicating a low usage of these pesticides.[8,9]

With exception of *o,p'*-DDD, all other DDT analogs were measured with 100% detection frequency. *p,p'*-DDE and *p,p'*-DDT were the main contributors to DDTs and represented 81% of sum DDTs. A recent exposure of sampled individuals to "fresh" DDT resulted from the observation that *p,p'*-DDT was found at the highest level compared to other DDT analogs measured in samples and that the concentrations of *p,p'*-DDT were 50% higher compared to *p,p'*-DDE. Also, the median ratio values found for *p,p'*-DDT/*p,p'*-DDE was of 2.3, which can additionally sustain this hypothesis.

PCBs were present in samples with a high detection frequency. CB-118 and -153 were the dominant congeners (1.7 and 2.1 ng/g, respectively) making up to 61% to the ΣPCBs and were detected in all hair samples. The distribution of PCB concentrations in hair samples is presented in Figure 2. The total PCB values ranged from 0.9 to 46 ng/g with a median value of 6.3 ng/g and the 25th and 75th percentiles of 3.7 and 10.0 ng/g, respectively. The less abundant PCB congener was CB-170 with a median value of 0.4 ng/g.

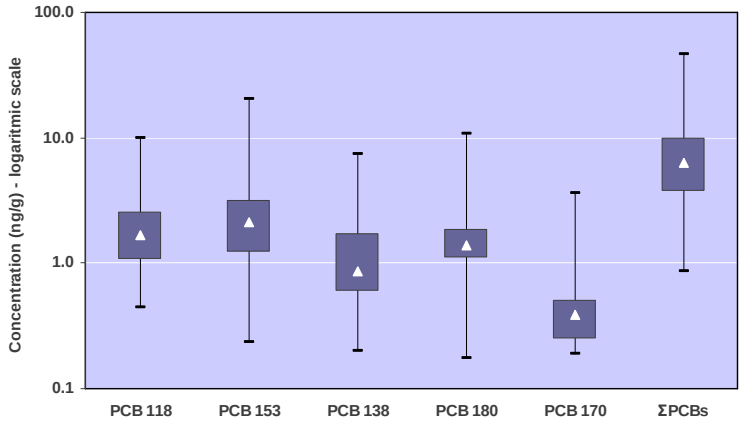

Figure 2. Distribution of PCBs (ng/g) in hair samples from adolescents (n=42) (median values, 1st and 3rd quartile and range)

Gender correlations.

When gender was considered, significant differences between girls and boys were found for most of the investigated contaminants (one-way ANOVA and Scheffe's post hoc test performed with SPSS 11.0 for Windows) (Figure 3). With few exceptions (α- and β-HCH, CB 138 and CB 170), the concentrations of OCPs and PCBs were significantly higher in sampled hair from girls compared to boys.

No particular factors associated with samples could explain the observed differences, especially since such difference in exposure between boys and girls was not expected. However, similar results were previously found for serum samples of general population from the same region.[9]

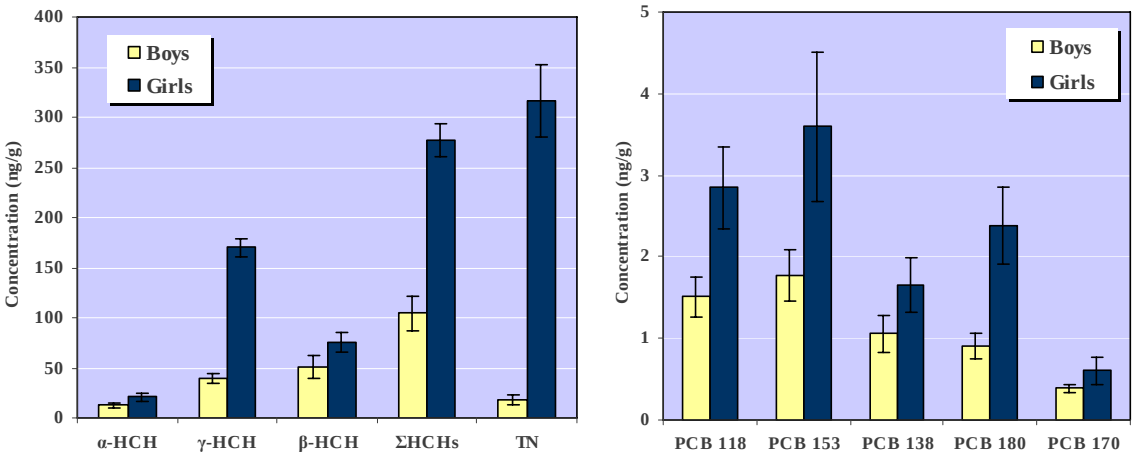

Figure 3. Gender comparison of mean concentrations of POPs found in hair samples. Error bars represent standard errors.

Comparison to other studies on human hair

Compared to other previous studies, the results of the present study were significantly different only in case of the OCPs content of the samples (Table 1). High levels were found in the present study for HCHs and DDTs compared to samples from Greece and Western Europe. In case of PCBs, the levels found in Romanian samples were found to be in the same range compared to previously published data.

Table 1. Mean concentrations (ng/g) of OCPs and PCBs in human hair samples from other previous studies.

Country/ Region	Nr. of samples	Year	γ-HCH	β-HCH	*p,p'*-DDE	*p,p'*-DDT	PCB 118	PCB 138	PCB 153	PCB 180	ΣPCB	References
USA[a]	10	2004	-	-	4.6	2.3	1.4	3.9	4.4	1.4	52.8[b]	4
Greece	35	2002	33.8	6.1	37.6	22.0	-	2.1	1.6	0.9	5.2[c]	3
Romania	2	2002	16.3	12	37.5	7.9	1.4	2.0	3.3	1.1	10.2[c]	3
Belgium	10	2002	9.6	3.5	10.5	5.9	1.6	2.8	4.4	2.1	13.7[c]	3
Western Europe	3	1998	ND	ND	1.3	-	-	1.9	3.1	1.0	-	10
Romania	**42**	**2002 2003**	**102**	**62**	**238**	**779**	**2.1**	**1.4**	**2.6**	**1.7**	**7.7**	**present study**

[a] results recalculated in ng/g of hair (for a typically fat content of 2%). [b] Sum of 57 individual PCB congeners. [c] Sum of PCB 99, 118, 138, 149, 153, 170 and 180.

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