PROFILING OF ORGANOHALOGENATED CONTAMINANTS IN THE SERUM OF MOTHERS AND THEIR CHILDREN FROM PAKISTAN WITH DIFFERENT RESIDENTIAL SETTINGS

Ali N[1,5], Ali LN[2], Eqani SAMAS[3], Malik RN[4], Neels H[1], Covaci A[1]

[1] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium; [2] Environmental Sciences Department, Kuwait Institute for Scientific Research, Kuwait; [3] Department of BioSciences, COMSATS Institute of Information Technology, Park Road Islamabad, Pakistan; [4] Environmental Biology and Ecotoxicology Laboratory, Department of Environmental Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan; [5] Center of Excellence in Environmental Studies, King Abdulaziz University, P.O Box: 80216 Jeddah 21589, Saudi Arabia

Introduction

Organohalogenated contaminants (OHCs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and their metabolites, owing to high persistence in the environment, more lipophilicity, and bioaccumulative nature are reported to pose negative health impacts to both humans and wildlife[1,2].Due to their persistence together with damaging effects to the environment, PCBs and many of the OCPs were listed as restricted/ banned chemicals in 2001 under the Stockholm Convention, followed in 2009 by two commercial formulations of Penta- and Octa- brominated diphenyl ethers[3]. Being signatory of the Stockholm convention, the use of PCBs and listed OCPs were banned in Pakistan in 2001[4]. However, the existence of large stockpiles, along with the possible illegal use of regulated OCPs due to poor law enforcement and cheaper cost may have been associated with possible contamination of various environmental compartments[4,5]. None of the study in the country reported the baseline data on these chemicals in human either due to be short of technical facilities, lack of awareness and no commitment from government to priorities environmental contamination related issues and their impacts on the human health. In the present study, we measured the levels of different OHCs and their metabolites in serum samples from Pakistan. We aimed to provide useful information on the serum concentrations of OHCs, which will help to provide the baseline levels of these chemicals in people of Pakistan. The difference of OHCs levels between mothers and their children, and different residential settings were investigated.

Materials and methods

SamplingHuman serum samples, paired mothers and children from the same household, were collected from rural area of Gujrat (N=17) and city of Islamabad (N=17), Pakistan. A selection criterion was that donors were among the general population who lived in rural or urban regions for most of their life and were not accidentally or occupationally exposed to OHCs. Serum samples were collected in March 2012 by venipuncture at local clinics following overnight fasting. The serum was separated by centrifugation and transferred to contamination free tubes and were kept frozen at -20°C until analysis. General information regarding age, height, weight, gender, place of residence, and occupation were collected from each participant. The study was approved by the Quaid-i-Azam University–Ethical Review Committee (QAU-ERC). The donors were invited to volunteer for the study, and samples were collected with the consent of donors and children were compensated with gift packs. Total cholesterol (CHOL) and triglycerides (TG) were determined in a separate serum aliquot at the collection clinics using routine laboratory analysis. Total lipids (TL) were calculated using the formula TL=2.27×TG+1.12×CHOL+1.48 (in g/L) as described elsewhere[6]. Consequently, concentrations of OHCs were expressed per lipid weight (lw) basis.

Sample preparation and instrumentation Detailed information on sample preparation and instrumentation are given elsewhere[7]. Briefly, the procedure for extraction using OASIS™ HLB cartridge and clean-up using Silica Bond Elut (3 mL) cartridge topped with 100 mg of 10% freshly prepared acid silica was used with minor modifications from the methods described elsewhere[8,9]. The mixture of internal standards: BDE 77, BDE 128, $_{13}C^{12}$-BDE 209, 4HO-PCB 159, PCB 143 and ε-hexachlorocyclohexane (HCH) were used to measure different OHCs. Recoveries ranged between 65% and 105% (relative standard deviation (RSD)<15%) for OCPs, PCBs,

HO-PCBs and PBDEs in serum[8,9], while for NBFRs, except TBPH, ranged 84–117%(RSD <15%).The analysis of OHCs was performed by 6890 Agilent (Palo Alto, CA, USA) gas chromatography (GC) coupled to a 5973 mass spectrometer (MS) operated in electron capture negative ionization (ECNI).

Statistical analysisDescriptive analysis was performed using Minitab 15. Non-detects were replaced by *df* x LOQ, where *df* is the detection frequency of samples above LOQ[10]. Keeping in mind the small sized datasets in this study, non-parametric tests were employed. Spearman rank-order correlation coefficient was performed to study correlations among respective mothers and children, while Wilcoxon signed-rank test was employed to study if the levels of OHCs were significantly different among children and their mothers. Similarly, Mann-Whitney test was applied to study the difference in the levels of OHCs among urban and rural mothers and children.

Results and Discussion

Brominated flame retardantsThe concentrations of different OHCs detected in serum are shown in the Table 1. OCPs were the major OHCs in all studied groups, while HO-PBDEs were present <LOQs. BDE 47, 153 and 99 were the most abundant PBDEs. The levels of PBDEs were similar to lower than those reported in the literature. One Novel BFRs 1,2-bis(2,4,6-tribromophenoxy)-ethane (BTBPE) was detected that ranged between <1 to 8.20 ng/g lw. To the best of our knowledge, this is the first study reporting occurrence of BTBPE in human serum. However, median levels of <0.2 ng/g lw and the detection frequency of <25% implied to a low exposure to BTBPE among the studied population. BPs were detected with different detection frequencies, but most of them in <50% of the all samples. The most frequently detected BPs were 2,4,5-tribromophenol and 2,4,6-tribromophenol, recorded in 60% and 72% of the samples, respectively. The average levels of ∑BPs were 11 ng/g lw, while for comparison HO-PBDEs were <0.2 ng/g lw. This indicates that BPs have other sources, such as its usage as reactive FR intermediate or as wood preservative[11], than the cleavage of the ether bond, which is considered as an important metabolic pathway for PBDEs[12].

Organochlorinated pesticidesThe major OCPs found in the serum samples were hexachlorobenzene (HCB), pentachlorophenol (PCP), Hexachlorocyclohexane (HCH), and 1,1-Bis-(4-chlorophenyl)-2,2-dichloroethene (*p,p'*-DDE), which were above LOQ in >80% of all samples. The major contributor (>90%) to the ∑DDTs was *p,p'*-DDE. The ratio *p,p'*-DDT/ ∑DDTs (range between 0.00 and 0.15) suggests various past degree of exposure to DDT. This indicates that fresh DDT exposure may have ceased now and that DDT residues (mainly *p,p'*-DDE) probably derive from food as a secondary source of exposure. This difference in the range might be due to exposure at different times for different individuals. Other arguments could be the differences in the metabolic efficiency for *p,p'*-DDT, together with differences in the sources of *p,p'*-DDT and *p,p'*-DDE within the volunteers.

Polychlorinated biphenylsThe total PCB values ranged from 2–105 ng/g lw. The profile of PCBs was dominated by heavier chlorinated congeners and CB 153 which is known to make up to ~25% of the ∑PCBs burden, in our study its contribution ranged between 0.1 to 0.40 (10–40%) with a mean value of 0.22 (22%). Various HO-PCBs were investigated, but in most cases, their levels were close to LOQ. The ∑HO-PCBs ranged from<0.20 to 20 ng/g lw. The major HO-PCB metabolites were 4HO-CB 107 and 4HO-CB 187.

PCBs and OCPs, particularly DDTs, were lower than those reported in human serum from Bangladesh[13]. Background information revealed that all Bangladeshi donors eat fish in their everyday meals and several times in a week dry fish which may contain high levels of DDT and its metabolites [13]. Eating contaminated food, e.g. fatty fish, and living closer proximities to a contamination source, have been established as major exposure pathways for OCPs and PCBs[14]. In the present study, the Pakistani donors do not live close to industrialized regions, nor is fish part of their regular diet. Pakistan is characterized of diverse and extreme climatic conditions; keeping in view high temperature in different parts of the country (including Gujrat and Islamabad) may induce the mobilizations of the POPs across the country's border and towards the colder parts of the country including Himalayas and associated areas. Agricultural activities are very limited in Islamabad region and being a capital city regulations are generally followed in its industrial sector. Agriculture in the main activity in rural areas of Gujrat, but in past heavy use of pesticides was never been a common practice. These can be possible

explanations for the lower levels of OHCs in serum samples from Pakistan. Pearson's correlation revealed significantly positive correlations between ∑PCBs and HCB, *p,p'*-DDE and HCHs ($p<0.01$). Our findings were in agreement with other studies, where strong correlations between the serum levels of PCBs and OCPs have been found, indicating that these two groups of pollutants have common routes of exposure (e.g. fatty foods etc) in humans[15,16,17.]

Table 1OHCs in human serum from Pakistan, all concentrations are expressed in ng/g lw.

Group		∑PCBs	∑HO-PCBs	*p,p'*-DDE	∑OCPs	∑PBDEs	∑BPs
Rural	Detection %	100	71	94	100	94	88
mothers	Median	5.8	0.3	142	163	1.6	6
(N=17)	Range	1.5–14.5	<0.2–1.4	<1–1,290	3–1,330	0.2–4.2	<5–15
	Mean ± SD	6.8 ± 4.2	0.4 ± 0.4	255 ± 350	285 ± 365	1.7 ± 0.9	6.5 ± 2.8
Rural	Detection %	100	71	100	100	94	94
children	Median	19.5	1.6	525	575	3	18
(N=17)	Range	2–30	<0.2–4	75–2,700	95–2,885	<0.2–5	<5–45
	Mean ± SD	18 ± 7.5	1.6 ± 1.3	815 ± 765	900 ± 855	3.2 ± 1.5	21.5 ± 13
Urban	Detection %	100	35	100	100	100	94
mothers	Median	12	<0.2	155	265	1.7	5.5
(N=17)	Range	6–25	<0.2–20	35–943	60–1,190	1–3	<5–15
	Mean ± SD	12 ± 3.7	3.5 ± 7	255 ± 280	365 ± 335	1.9 ± 0.7	6.5 ± 3.9
Urban	Detection %	100	71	100	100	100	94
children	Median	18	0.7	235	310	2.3	8
(N=17)	Range	8–25	<0.2–18	32–1,075	125–1,215	0.5–7	<5–25
	Mean ± SD	17 ± 5.5	3.0 ± 5.5	330 ± 260	455 ± 350	2.8 ± 1.6	9.0 ± 5.5

Comparison between children and their mothers and with their residential settingsSpearman's rank–order correlation coefficients showed a significant correlation between rural children and their mothers for *p,p'*-DDE ($r=0.461$, $p<0.05$), and ∑BPs ($r=0.420$, $p<0.05$). While, for urban children and their mothers ∑PBDEs ($r=0.592$, $p<0.05$) showed significant correlation. No significant correlation between urban children and their mothers was observed for *p,p'*-DDE which indicates a difference in the exposure between these two residential settings. Wilcoxon-rank tests revealed that the exposure burden of major OHCs was significantly higher ($p<0.05$) in children than in their mothers.This might be primarily due to the differences in the feeding habits and to the specific activities such as indoor and outdoor sports that children are daily involved. Involuntary dust ingestion during these activities and more hand-to-mouth habits are possible source of exposure for the children.

Mann–Whitney U test revealed that the levels of *p,p'*-DDE and ∑BPs were significantly higher ($p<0.05$) in rural children compared to urban children. Outdoor activities close to the agricultural areas might contribute to the higher *p,p'*-DDE levels in the rural children. Such differences may, at least partly, be explained by the specific activities and the dietary habits of urban and rural populations from the selected region.

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