LEGACY AND EMERGING ORGANOHALOGENATED CONTAMINANTS IN MATCHED SERUM AND FLOOR DUST SAMPLES OF VARIOUS WORK PLACES

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Introduction

Organohalogenated contaminants (OHCs), including polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs), have been widely reported to occur in the environment and animal tissues[1,2,3]. Recent reports on the occurrence of new brominated flame retardants (NBFRs) and organophosphate flame retardants (PFRs) in different environmental compartments, including the indoor environment provided critical information about their current status and ecological risk[4,5,6]. The presence of OHCs in the environment is of particular concern due to their exposure to humans. Human are exposed to these contaminants through various routes such as food, air and dust intake[1,7]. In recent times, intake of indoor dust as potential human non-dietary exposure source to organic contaminants has attracted lot of attention[7,8]. Work places have always been a source of chemical exposure to workers, however due to the lack of technical facilities and awareness among public no literature is available on the occurrence of OHCs in occupational setting of Pakistan. The current study was aimed to determine the concentrations of various classes of OHCs and their metabolites in matched serum and dust samples collected from various occupational settings from Faisalabad, Pakistan. The city of Faisalabad is located in the province of Punjab, Pakistan and in past few decades was characterized by intensive industrial activities, such as textile industry, and urban development. To foresee impacts of various indoor chemicals on the environment, the differences in the levels and profiles of various OHCs in occupational settings and correlations between dust and serum levels were also investigated.

Materials and methods

Study participants, serum and indoor dust sampling: A total of 61 paired samples of blood and dust were collected from Faisalabad, Pakistan in December, 2011. Of these, 30 paired samples were collected from people (age ranged 17–55 years (mean 30 years)) working in old and new electronic (computer, home appliances and mobile) stores. The rest of the samples included people (age ranged 17–55 years (mean 29 years)) working in old and new clothing stores (N=15), and academics (age ranged 18–32 years (mean 25 years)) (N=16, young lecturers and post graduate students) from the University of Agriculture, Faisalabad, Pakistan. All individuals participating in this study were volunteers, who signed an informed consent. For blood samples, baseline data including age, gender and occupational history were collected from each participant. A volume of 7–8 mL blood was collected by venipuncture at a local clinic following overnight fasting. Blood was collected into sterile glass collection tubes without anticoagulant and after 1 hr at room temperature; the serum was separated by centrifugation @ 4000 g for 10 min, transferred to new tubes and kept at −20°C until analysis. The serum lipid content was determined from enzymatic measurements of cholesterol and triglycerides[9], which were done at the same clinic. The study was approved by the Quiad-i-Azam University-Ethical Review Committee (QAU–ERC).

Dust samples were collected by brushing the floor surface (4–8 m^2), and to avoid cross contamination brushes from the respective store/office/hostel room were used. After brushing, dust was swept onto the aluminum foil, rapped and sealed in polyethylene zip bags. General information about the indoor inventories were collected. Before transfer to the laboratory, samples were kept at dark to avoid photo-degradation. Before analysis to insure sample homogeneity, each dust sample was sieved through a 500 µm mesh sieve pre-cleaned with acetone.

Sample preparation: The procedure for extraction and clean-up of OHCs from serum was previously described was used with minor modifications[1,10,11]. Details about the serum sample preparation are given elsewhere[12]. The extraction and purification method of dust is described in detail elsewhere[13].

Instrumentation: Quantitative analysis of OHCs was perform using gas chromatography (GC) coupled with mass spectrometer (MS) techniques. Details about the instrumentation of serum and dust samples are given elsewhere[12,13,14].

Statistical analysis: Descriptive data was performed using Microsoft Excel 2003. Values below LOQ were replaced by DF*LOQ, where DF is the detection frequency, e.g. number of samples >LOQ. Mann-Whitney test was applied to study differences in the levels of OHCs among different occupational settings, while correlations between concentrations of serum and dust was tested using the Spearman rank-order correlation. Using Minitab 15, Pearson correlation was performed between age and serum concentration. The level of significance was set at $p < 0.05$, unless specified otherwise.

Results and discussion

Neutral OHCs in serum sample: Levels of ∑OCPs were noticeably higher than the rest, while ∑PCBs, ∑NBFRs and ∑PBDEs had comparably smaller contribution. Among all studied groups, *p,p'*-DDE was the major OCP (~75% of total OHCs), followed by pentachlorophenol (PCP) and Hexachlorocyclohexane (HCHs). The order of occurrence for OCPs is similar to those reported recently in the human serum from the Punjab, Pakistan[12], which might suggest similar OCPs usage pattern across the region in the past. Moreover, the dominance of OCPs among all OHCs might demonstrates the still on-going usage or unintentional leakage from pesticide obsolete storage places and historical application of these chemicals[15]. The higher OCP levels in the serum samples can also be attributed to the high lipophilicity (K_{OW}) of these contaminants, which ultimately lead to accumulation in humans *via* food chain transfer and dust ingestion for longer duration. Lower levels BDE 47, -99, and -153 were detected in >80% of the serum samples, but only two NBFRs, namely 1,2-bis(2,4,6-Tribromophenoxy)ethane (BTBPE) and bis(2-ethylhexyl) tetrabromophthalate (TBPH) were detected, albeit in <10% of the serum samples. The low levels of these compounds suggest lower application of NBFRs in the region.

***Phenolic OHCs in serum samples*:** Hydroxylated metabolites of PBDEs and PCBs were detected at lower levels and DF than their respective parent compounds. Among selected HO-PBDE only 6'HO-BDE 99 was detected and quantified in >20% serum samples. 4HO-CB 79 (median 0.8 ng/g lw), 4HO-CB 107 (median 0.6 ng/g lw), and 4HO-CB 187 (median 0.2 ng/g lw) were the major HO-PCBs detected in >70% of the samples. ∑BPs were the 2nd largest contributing group in the serum profile, 2,4,5- tribromophenol (2,4,5-TBP) and 2,4,6-TBP were the major BPs. The presence of BPs in human blood might indicates the ether bond cleavage of PBDE congeners (BDE 47, -99, -100 and -154), which is an important metabolic pathway for PBDEs[16]. Higher levels of 2,4,6-TBP in the serum samples (median 20 ng/g lw) compare to PBDEs indicates other sources, such as its usage as reactive FR intermediate and/or as wood preservative[17].

***OHCs in dust samples*:** ∑PFRs was the major OHCs in dust, followed by ∑NBFRs, ∑PBDEs, ∑OCPs and ∑PCBs for all occupational settings. These findings are similar with our earlier studies in Pakistan, where these OHCs were reported in household indoor dust[14]. The higher levels of FRs (PFRs, NBFRs and PBDEs) than OCPs and PCBs are not surprising, since these chemicals are used as additive materials in large number of household products and the use of selected OCPs and PCBs has been banned in Pakistan[18]. The higher levels of PFRs in dust compared to PBDEs and NBFRs are also consistent with the literature[8,13,14,19, 20], which suggests their application in a large range of polymers as a replacement to regulated PBDEs.

Differences of OHCs among three groups: Most OCPs were present at higher levels in the serum samples of university volunteers compare to other two groups. Information from the questionnaire could not explain any specific reason for these differences, since the volunteers lived in the same city and are in the age group. Our data revealed higher OCPs levels in dust from the student hostel rooms compared to dust from clothing and electronic stores, which might be a contributing factor for higher OCP levels in the serum. However, no statistically significant correlation between the levels of OCPs in dust and serum suggested the existence of additional factors/sources besides indoor dust ingestion. Most of the student donors who live in the hostel rooms are the residents of other geographic areas (urban and rural), where these OCPs might have been used historically and contributed significantly towards total OCP body burdens. All other OHCs in serum samples were detected in similar pattern from each of three studied groups and no significant difference ($p < 0.05$) was observed. Significantly higher ($p > 0.05$) levels of ∑HCHs and ∑DDTs were present in dust from university hostels rooms. The dust brought in the room *via* ventilation and tracked in soil from outside could be a reason for these higher levels. The hostel is situated

in an agricultural university with large surrounding area covered by agricultural fields in the close proximity; a possible use of these chemicals in the past could be an argument for such higher levels. Expectedly, levels of all FR classes were significantly higher (p >0.05) in dust from electronic stores compare to clothing stores. Interestingly, most of the FRs, except for BTBPE and TCPP, were present at similar levels in dust from electronic stores and hostel rooms. An argument could be the more regular dusting of floor surface in electronic stores (on average daily) compares to the hostel rooms (on average once a week). A closer look at the data revealed that higher levels of FRs were present in stores which were not vacuumed during the last 5 days and had bulk of old repairable electronics.

Table 1 Concentration (ng/g lw) of selected OHCs in serum samples.

Analyte	**University (N = 16)**		**Clothing stores (N = 15)**		**Electronics stores (N = 30)**	
	Mean ±SD	*Median (Range)*	*Mean ±SD*	*Median (Range)*	*Mean ±SD*	*Median (Range)*
∑PCBs	9±5	7.5 (3–23)	9.5±3.5	8.0 (5–18)	11±11	7 (0–50)
∑HO-PCBs	5.5±7.5	2.2 (0.6–27)	4±5	2.0 (0.8–17)	5.5±4.5	3.5 (1–18)
PCP	30±18	24 (10–82)	57±31	53 (17–140)	60±70	40 (<10–275)
p,p'-DDE	115±70	105 (30–275)	200±170	185 (37–690)	130±120	92 (0–580)
∑OCPs	130±75	120 (40–295)	230±180	210 (50–725)	155±130	105 (15–620)
Penta-BDE	4.5±6	3 (1–25)	2.3±1.2	2.5 (0.5–5)	3.0±2.2	2.5 (0–11)
2,4,6-TBP	25±12	22 (7–50)	20±6	19 (11–33)	41±60	21 (8–320)
∑BPs	32±12	30 (10–60)	25±6	26 (15–40)	53±65	32 (13–340)

Table 2 Concentration (ng/g dust) of selected OHCs in dust samples.

Analyte	**University (N = 16)**		**Clothing stores (N = 15)**		**Electronics stores (N = 30)**	
	Mean ±SD	*Median (Range)*	*Mean ±SD*	*Median (Range)*	*Mean ±SD*	*Median (Range)*
∑PCBs	4.8 ±2.8	4.6 (1.0–10)	5.5±3.5	5.5 (1–12.5)	4±5.5	1.3 (0.7–27)
p,p'-DDT	35 ±50	18 (1.0–190)	4.5±3	4 (0.5–11)	7±13	3.5 (0.5–70)
∑OCPs	113 ±190	35 (2–730)	10±14	6.5 (1–60)	31±73	6 (1–325)
Penta-BDE	12 ±12	7.5 (2.5–50)	6.5±5.5	5 (0.8–19)	59±210	10 (1–1150)
Octa-BDE	5 ±7	3.0 (1–30)	2±1.5	2 (<0.5–6)	57±145	4 (0.7–650)
BDE 209	75 ±50	65 (12–205)	65±60	45 (<2–195)	3200±10900	155(<2–51500)
TBB	2 ±3.5	1.3 (<0.2–16)	0.6±0.4	0.7 (<0.2–1.2)	3±4	1 (<0.2–15)
TBPH	35 ±50	19 (3–225)	11±10	9 (<0.2–35)	100±200	20 (0.6–950)
BTBPE	15 ±27	9.5 (2–125)	7.5±6	6.5 (0.5–20)	1000±3450	17 (0.5–17150)
DBDPE	62 ±35	60 (10–120)	36±37	31 (<2–155)	7100±15300	140 (2–52150)
TPhP	1850±5550	170 (10–23450)	88±70	62 (<2–220)	750±1200	195 (10–5000)
TDCPP	40±45	20 (<5–185)	11±10	7 (<5–30)	235±465	29 (<5–1475)
∑OPFRs	3290±6100	1060(120–24600)	345±200	395 (60–650)	1830±2400	710 (75–9150)

Associations between serum and dust levels of OHCs: Levels of Penta-BDE congeners in dust and serum were significantly correlated (r = 0.64, p <0.01) for the university group. A significant correlation (r = 0.54, p <0.01) was observed for BDE 99 in the electronics group, but no such correlation was observed for other Penta-BDE congeners in both electronics and clothing groups. A possible explanation for this difference in the profile patterns among studied groups could be the number of hours/day spent by each individual in their respective indoor environment. Students are more closely associated with their belongings compared to the people working (8–10 hours/day) in the stores. These findings are similar with the handful of studies who demonstrated various relationships between the levels of PBDEs in human serum and dust collected from the donor's homes[21,22,23]. No significant (p>0.05) correlation were observed for other OHCs between serum and dust samples. In the literature, strong correlations

between serum concentrations of organochlorine pollutants are reported [24,25,26] which explains that humans are exposed to OCPs and PCBs *via* similar exposure routes; this seems logical as both groups of chemicals were widely used in past. We also computed significant correlation among OHCs, especially PCBs with other OHCs. However, levels of PCBs were 2–3 orders of magnitude lower than OCPs, and the scenario indicates the wider past application of OCPs in the region for malaria control and other industrial or agricultural purposes[15]. The situation addressed in current study is similar to other studies conducted in Asia, e.g. China. Here too the authors have reported relatively higher concentrations of OCPs contrasted with low concentrations of PCBs [24,27].

Conclusions

This is the first study reporting OHCs in matched samples of human serum and dust from different working environments of Pakistan. ∑PFRs in dust and ∑OCPs in serum samples were the major OHCs in the three investigated occupational settings. Levels of Penta-BDEs were positively correlated in serum and dust samples from the university group, suggesting that dust is an important exposure pathway to the PBDE present in consumer articles for this group. There were minimal differences in the levels and profile of OHCs among three groups. The occurrence of OHCs in human serum and indoor dust suggests on-going exposure to various pollutants. This study provides an important benchmark for bio-monitoring and exposure assessment of OHCs in Pakistan.

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