Determination of Brominated Flame Retardants in Human Scalp Hair from Mothers Living in Payatas and Malate, the Philippines

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Introduction

Human monitoring of persistent organochlorine pollutants (POPs) has become increasingly important for exposure and risk assessment. Human scalp hair is a stable matrix that presents numerous advantages for human biomonitoring (Covaci et al., 2002). Due to its relatively high percentage of lipids (3.5 - 4%), scalp hair has been identified as a suitable indicator for short- and long-term exposure to organochlorines (Schramm et al., 1992). Because each hair follicle is surrounded by a system of capillary blood vessels at the root, the same information, as present in serum, is found in the hair (Spearman, 1997). Altshul et al. (2004) have reported strong and moderate correlations between the hair and blood levels of some of the organic pollutants. Significant relationship was reported between PBDE concentration in hair and endogenous tissues (Have et al., 2005), which indicate that hair, may be used in biomonitoring as an indicator of endogenous concentrations. There is little knowledge about the elimination kinetics in different body compartments. Because of easy collection, transport, storage and non-invasive sampling, scalp hair can be used an alternative matrix of human biomonitoring for environmental toxicants.

Because of their environmental stability, persistence and high production volume, polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) are among the most abundant brominated flame retardants (BFRs) detected in the environment and in wildlife and humans (Alaee et al., 2003). The data on human exposure to BFRs in the Philippines is rather scarce and comprehensive studies have not been made. The present study was therefore carried out to determine concentrations of two BFRs such as PBDEs and HBCDs in human scalp hair samples from mothers living in two locations in the Philippines, viz., one near a waste dumpsite, Payatas and a reference site Malate. In addition, the present study examined relationships between contaminant levels and age of the mothers to understand the incorporation of BFRs in scalp hair.

Materials and Methods

Sample collection

Human scalp hair samples (n=28) were collected at two different locations; Payatas a waste dump site and Malate a reference site in the Philippines during 2008 (Figure 1) and analyzed for two BFRs (PBDEs and HBCDs). Of these, twelve samples were from the reference site at Malate and sixteen samples were from the dumping site at Payatas. Scalp hair was cut with stainless scissors, and sealed in a plastic bag and shipped to the laboratory in Japan under frozen condition and stored at -20°C in the Environmental Specimen Bank (*es*-Bank) of Ehime University (Tanabe, 2006) until analysis. Almost all the randomly selected scalp hair donors were housewives in the age range of 17-40. Informed consents were obtained from all the donors and the answers for the questionnaire on dietary aspects were recorded.

Chemical analysis

Analysis of PBDEs and HBCDs was performed according to the method described elsewhere (Have et al., 2005), with slight modifications. To remove external contamination (e.g., fine soil particles, dust), individual hair samples were incubated in Milli-Q water in a shaking incubator along with shampoo (0.3% Polyoxyethylene lauryl ether - 1 h, 40 °C), and afterward rinsed with tape water followed by distilled water and Milli-Q water (ten times with each one). Samples were dried 24 hours at 40°C and cut into pieces of ~2 mm. Briefly, a 1.0 g sample of human scalp hair was accurately weighed, and spiked with 5 ng of internal standards of PBDEs ($^{13}C_{12}$ – labeled BDE-3, -15, -28, -47, -99, -100, -153, - 154, -183, -197, -207 and -209) and 10 ng of internal standards for HBCDs ($^{13}C_{12}$-labeled α-, β- and γ-HBCD) as surrogates, and incubated overnight at 40°C with 20 mL of hydrochloric acid (4 M) and 20 mL of hexane/dichloromethane (4:1,v/v). Extraction of analytes from the incubation medium was done by a liquid−liquid extraction (LLE) procedure with 2×4 mL of hexane/dichloromethane (4:1, v/v). The extract was then subjected to gel permeation chromatography (GPC) column for lipid removal and eluted with mixture of 50% hexane/dichloromethane (1:1). The GPC fraction containing BFRs was concentrated and passed through 4 g of activated silica gel (Wakogel DX). The first fraction of 5% dichloromethane in hexane contained PBDEs, while the second fraction of 25% dichloromethane in hexane contained HBCDs. Identification and quantification of PBDEs was done using gas chromatography combined with mass spectrometry (GC-MS), while HBCD isomers were identified and quantified using liquid chromatography combined with tandem mass spectrometry (LC-MS-MS) in a multiple reaction monitoring (MRM) mode. Procedural blanks were analyzed simultaneously with every batch of seven samples to check for interference or contamination from solvents and glassware. The concentration of PBDEs and HBCDs were expressed in ng/g hair basis unless otherwise specified.

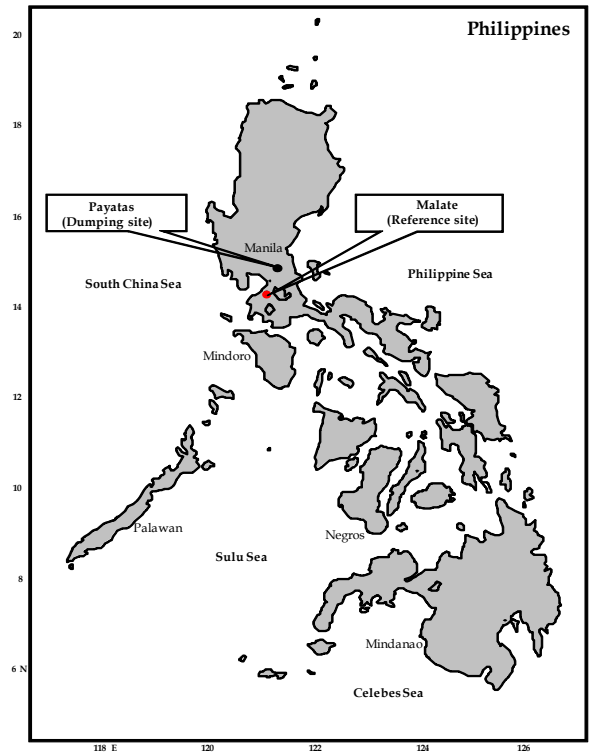

Figure 1. Map showing the sampling locations.

Results and Discussion

Residue levels and contamination status of BFRs

BFRs were detected in variable quantities in all the human scalp hair samples collected from Payatas and Malate, suggesting that the mothers living in two locations in the Philippines have been widely exposed to these contaminants. Twelve PBDE congeners from tri- to deca-BDE were identified. BDE-3 and -15 were not found above the detection limit of 0.05 ng/g hair in all the samples. The concentrations of the sum of the 12 congeners (∑PBDEs) found in human scalp hair samples varied widely, from 21 to 646 ng/g hair, with an overall mean of 99 ng/g hair (Table 1). Overall mean concentrations of PBDEs found in our study were higher than those in Spain (Tadeo et al., 2009), and China (Zhao et al., 2008). Interestingly, it seems that PBDEs may become a major environmental concern in the Philippines as they

were found higher in the present study and also in human breast milk (Malarvannan et al., 2009a) and house dust samples (Malarvannan et al., 2009b) in the same locations, when compared to other Asian countries. In the case of HBCDs, this is the first comprehensive study on human scalp hair samples from the world. The concentrations of the sum of the 3 isomers (∑HBCDs) found in human scalp hair samples varied widely, from ND to 5.3 ng/g hair, with an overall mean of 1.5 ng/g hair (Table 1). The observed levels of the PBDE congeners and HBCD isomers from the two different locations (Payatas and Malate) were not statistically significant and probably due to the corresponding background residue levels in the environment. The variations in the BFR profiles in human scalp hair among individuals may be suggestive of multiple sources and pathways of exposure and also possibly different commercial mixtures.

Table 1. Concentrations of PBDEs and HBCDs (ng/g hair) in human scalp hair from the Philippines.

	Dumping site (Payatas)				Reference site (Malate)				All samples			
	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
Number of samples	16				12				28			
Compounds												
PBDEs												
BDE-28	0.63	0.51	0.21	1.5	0.48	0.34	0.11	1.8	0.57	0.45	0.11	1.8
BDE-47	12	6.8	2.8	52	18	4.3	1.8	125	14	6.0	1.8	125
BDE-99	11	5.6	2.0	39	37	3.5	1.1	385	22	5.4	1.1	385
BDE-100	2.0	1.1	0.31	8.1	4.0	0.81	0.25	35	2.9	1.1	0.25	35
BDE-153	1.3	0.93	0.36	3.2	3.1	0.68	<0.05	29	2.1	0.73	<0.05	29
BDE-154	0.74	0.40	<0.05	2.8	2.2	0.28	0.10	22	1.4	0.36	<0.05	22
BDE-183	1.1	0.59	0.25	5.0	0.82	0.67	0.21	2.7	1.0	0.60	0.21	5.0
BDE-196	0.66	0.46	<0.05	1.8	0.83	0.60	0.37	2.9	0.73	0.55	<0.05	2.9
BDE-197	1.0	0.53	0.20	3.0	0.73	0.60	0.37	2.1	0.84	0.60	0.20	3.0
BDE-206	4.2	3.4	1.1	15	6.3	4.3	2.3	25	5.1	3.5	1.1	25
BDE-207	3.3	2.7	0.87	11	4.6	3.0	1.7	19	3.8	2.9	0.87	19
BDE-209	37	29	13	92	56	41	12	175	45	39	12	175
Sum PBDEs (mono to hepta)	28	18	6.5	107	66	12	4.24	598	44	17	4.2	598
Sum PBDEs (mono to deca)	73	72	29	145	134	74	21	646	99	72	21	646
HBCDs												
α-HBCD	1.3	0.96	0.48	4.6	1.7	1.4	0.30	4.2	1.5	0.96	0.30	4.6
β-HBCD	0.27	0.27	0.27	0.27	<0.01	<0.01	<0.01	<0.01	0.27	0.27	0.27	0.27
γ-HBCD	0.50	0.50	0.50	0.50	1.4	1.4	1.4	1.4	0.94	0.94	0.50	1.4
Sum HBCDs	1.3	0.94	< 0.01	5.3	1.8	1.4	0.30	4.2	1.5	0.95	<0.01	5.3

Congener profiles of BFRs

Among the PBDE homologues analyzed, BDE -209, -47, -99, -206 and -207 had the highest abundance at both the sites (Table 1). Deca-BDE mixture is the most widely used commercial mixture, and comprises 97% BDE-209 in Asia. Studies on PBDEs in human scalp hair are limited, but increasing worldwide. The scalp hair data was dominated by BDE-209. Perhaps due to its larger size, or due to higher protein binding affinities, BDE 209 is hindered from diffusing into the muscle tissue at rates that are comparable to the lighter congeners. Future studies are needed to examine the binding affinity of BDE 209 to scalp hair proteins. However, if we discard BDE-209 and express the congener contribution as the percentage the remaining congeners (-47 and -99) it becomes apparent that the penta-formulation is also ubiquitous in all the samples. In general, BDE 209 has been determined in a few other studies in human samples (Hites, 2004; Sudaryanto et al., 2008), probably because of its difficult determination due to its low

volatility and high molecular weight. Currently very limited information is available regarding the use of PBDEs, especially that of deca- BDE congener, in the Philippines environment. Therefore more investigations into the use and impacts of PBDEs in the Philippine environment are needed in order to further substantiate the above notion. There are no reports available regarding stereoisomer-specific concentrations of HBCDs in human scalp hair samples in the world. In this study, the stable isomer, α-HBCD was only predominant in both locations (Table 1). Isomer ratios of HBCD observed in this study are consistent with the results observed in human breast milk in the same locations (Malarvannan et al., 2009a), indicating that α-HBCD is more persistent and bioaccumulative.

Specific accumulation according to age

No significant correlation was observed between BFR levels and age of mothers in this study, as also seen in other studies (Covaci et al., 2002) of some of the organic pollutants. This might be an evidence for the contemporary exposure to these chemicals in the general population. Thus, the general population would have the same level of exposure, regardless of age, provided there is a common pathway of exposure. Based only on kinetics, the level of the chemical would be similar across age groups when the net balance for absorption versus clearance is zero.

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