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Analysis of human hair to assess exposure to organophosphate flame retardants: Influence of hair segments and gender differences

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ABSTRACT

Hair is a promising, non-invasive, human biomonitoring matrix that can provide insight into retrospective and integral exposure to organic pollutants. In the present study, we measured the concentrations of organophosphate flame retardants (PFRs) in hair and serum samples from university students in Guangzhou, China, and compared the PFR concentrations in the female hair segments using paired distal (5∼10 cm from the root) and proximal (0∼5 cm from the root) samples. PFRs were not detected in the serum samples. All PFRs except tricresyl phosphate (TMPP) and tri-n-propyl phosphate (TPP) were detected in more than half of all hair samples. The concentrations of total PFRs varied from 10.1 to 604 ng/g, with a median of 148 ng/g. Tris(chloroisopropyl) phosphate (TCIPP) and tri(2-ethylexyl) phosphate (TEHP) were the predominant PFRs in hair. The concentrations of most PFRs in the distal segments were 1.5∼8.6 times higher than those in the proximal segments of the hair (*t*-test, $p < 0.05$), which may be due to the longer exposure time of the distal segments to external sources. The values of log (PFR concentrations-distal/PFR concentrations-proximal) were positively and significantly correlated with log K_{OA} of PFRs ($p < 0.05$, r = 0.68), indicating that PFRs with a higher log K_{OA} tend to accumulate in hair at a higher rate than PFRs with a lower log K_{OA}. Using combined segments of female hair, significantly higher PFR concentrations were observed in female hair than in male hair. In contrast, female hair exhibited significantly lower PFR concentrations than male hair when using the same hair position for both genders (0–5 cm from the scalp). The controversial results regarding gender differences in PFRs in hair highlight the importance of segmental analysis when using hair as an indicator of human exposure to PFRs.

1. Introduction

Currently, human exposure to organic pollutants is an increasingly important issue and has been of great concern in scientific research. Human biomonitoring can provide accurate data for assessing human exposure risks by determining contaminants and their metabolites in human samples. For many years, blood has been considered the ideal matrix for human biomonitoring as it comes into contact with all tissues and is in equilibrium with organs and tissues (Smolders et al., 2009), but blood sampling may have ethical and practical limitations due to its invasive nature. Human milk is a non-invasively collected matrix, but it is available only in lactating women. Hair can be non-invasively collected and has the additional advantages of low cost, sample stability, and convenient transportation and storage. In addition, both external (transportation from air and dust) and internal exposure (through deposition from blood at the hair follicle/root) contribute to the accumulation of contaminants in hair. Thus, hair samples can provide valuable information for retrospective exposure to pollutants, especially for the non-persistent chemicals. To date, many studies have used hair to assess human exposure to various

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organic pollutants, such as polychlorinated biphenyls (PCBs) (Covaci et al., 2002), polybrominated diphenyl ethers (PBDEs) (Zheng et al., 2014), polycyclic aromatic hydrocarbons (PAHs) (Toriba et al., 2003), and Dechlorane Plus (DPs) (Zheng et al., 2010; Chen et al., 2015).

Organophosphate flame retardants (PFRs) are a class of compounds that are mainly utilized as plasticizers, stabilizers in plastic polymers, and additives in commercial products (Van der Veen and de Boer., 2012). Due to the phase-out of PBDEs, PFRs have been increasingly used as alternatives for legacy flame retardants (Marklund et al., 2003). Many PFRs are proven to have toxic effects on animals and humans. Studies on laboratory animals have shown that PFRs can exert adverse effects on DNA synthesis, mRNA expression, thyroid hormones, and embryonic development (Dishaw et al., 2011; Egloff et al., 2014; Farhat et al., 2013; Fu et al., 2013; Wang et al., 2013). In humans, tri-n-butyl phosphate (TNBP) has been associated with Sick Building Syndrome (Kanazawa et al., 2010). Concentrations of tris(1,3-dichloroisopropyl) phosphate (TDCIPP) and triphenyl phosphate (TPHP) in house dust were associated with altered hormone concentrations and decreased semen quality in men (Meeker and Stapleton, 2010). TDCIPP and TPHP can disrupt sex steroid hormone balance in the human adrenal cell line (Liu et al., 2012). As PFRs are not chemically bonded to the polymer matrix, they are likely to leach out from household products by abrasion and/or volatilization into the environment like other additive flame retardants (Reemtsma et al., 2008). Consequently, a growing number of researches have detected PFRs in various environmental media and biota (Gentes et al., 2012; Sundkvist et al., 2010). However, few studies reported the occurrence of PFRs in human samples (Sundkvist et al., 2010; Kim et al., 2014). As non-persistent chemicals, PFRs are always measured as metabolites in urine, which represent only short-term exposure to PFRs. Kucharska et al. (2014; 2015) found that PFRs existed in all hair samples from Belgian and Norwegian individuals. Liu et al. (2015) developed an efficient method for determining PFRs in human hair and nails. All of the above studies suggested that hair may be used as a suitable human biomonitoring matrix for retrospective and integral exposure to PFRs (Kucharska et al., 2014).

The concentrations of contaminants in hair are affected by many factors, such as age, occupation, and gender. However, previous studies have yielded conflicting results regarding gender differences in pollutant concentrations in human hair. Altshul et al. (2004) found that PCB concentrations in female hair were 1.7 times those in male hair, which is consistent with the results from Romania (Covaci et al., 2008). In our recent study, PBDE concentrations in female hair were 3 times those in male hair (Zheng et al., 2014), but Poon et al. (2014) did not find any gender differences in PBDE concentrations in hair from a Canadian cohort. The gender differences for contaminant concentrations in hair are likely due to the longer hair of females than males because longer hair may undergo a longer exposure time. Different hair washing frequencies may also explain the gender difference. Although hair has been widely used for the analysis of metals, drugs of abuse, and other organic pollutants for decades, the hair samples collected in previous studies varied by segment and the position of the hair relative to the scalp. Some researchers cut only the distal portion of the hair (Covaci et al., 2008), whereas others cut hair samples within 1 cm from the scalp and collected the entire length of the hair (Poon et al., 2014). In other cases, hair collection occurred during participant haircuts (Chen et al., 2015). These different hair sampling procedures may cause great uncertainty with regards to human biomonitoring results. In a recent study focused on different segments of hair, Carnevale et al. (2014) observed a significant increase in PBDE concentrations from the proximal to distal segments along the hair shaft. Segmental analysis of pollutants in hair is essential to obtain accurate human biomonitoring data and to better understand the accumulation of pollutants in hair.

In the present study, we collected hair and serum samples from volunteer participants (27 males and 22 females) at a university in Guangzhou, China. Fifteen female hair samples were sectioned into segments to examine the influence of hair segment and position on PFR concentrations and profiles in human hair. All participants were students aged 21～25, and it is expected that they have similar exposure routes to PFRs from dietary and indoor sources, which facilitates the analysis of gender differences and hair segmental differences in PFR concentrations. The overall aims of the present study were (1) to investigate the concentrations and composition profiles of PFRs in hair and serum samples and to evaluate the feasibility of hair as an indicator for PFR monitoring; and (2) to perform an analysis of gender differences and segmental differences to explore the accumulation features of PFRs in hair segments.

2. Materials and methods

2.1. Sample collection

Human hair and serum samples were collected from volunteer participants at Sun Yat-sen University, located in Guangzhou, China in April 2014. This research was launched with the authorization of the Ethics Committee of the School of Life Science, Sun Yat-sen University. Samples were collected by medical professionals in a hospital with consent from all participants after they were clearly informed of the study's objectives. The participants included 27 males and 22 females (21～25 years old), and none of them had used any colouring agents in last 2 years. All samples were cut on the posterior vertex as close to the scalp as possible. Male hair lengths were 0–5 cm from the hair root. To perform an analysis of the segmental differences in hair and to facilitate comparisons between genders, we cut 15 female hairs (greater than 5 cm) into two segments: the proximal segment (0～5 cm from the root) and the distal segment (5～10 cm from the root). The hair samples were wrapped in aluminium foil, sealed in polyethylene zip bags, and kept at −80 °C prior to chemical analysis. Paired 10～15 mL venous blood samples of the participants were collected in anticoagulant-free tubes. The serum was isolated from the blood by centrifugation at 1620 × *g* for 5 min and kept at −80 °C prior to analysis.

2.2. Sample clean-up and analysis

Hair samples were purified by rinsing with Milli-Q water, freeze-dried and cut into small pieces (2～3 mm). Approximately 2 g of hair was spiked with 150 ng of TNBP-d_{27} and Soxhlet-extracted with a mixture of acetone and hexane (1:3, v/v) for 48 h. The extracts were concentrated to 1 mL, solvent-exchanged with hexane, and purified with a florisil cartridge (Supelclean ENVI-Florisil, 3 mL 500 mg, Supelco, USA). The cartridge was pre-cleaned with 8 mL of ethyl acetate and 6 mL of hexane and then eluted with 10 mL of hexane and 8 mL of ethyl acetate. The latter fraction was collected and further purified on a multilayer Florisil-silica gel column (length, 30 cm; inner diameter, 10 mm) packed from bottom to top with Florisil (14 g, 3% deactivated), neutral silica (8 g, 3% deactivated), and anhydrous sodium sulfate (2 g). The extracts were eluted with 40 mL of ethyl acetate and concentrated to near dryness under a gentle nitrogen stream and finally dissolved in 300 μL of isooctane. Prior to instrumental analysis, the extract was spiked with known amounts of a surrogate standard (TPHP-d_{15}). For serum analysis, approximately 5 mL of each sample was extracted using diatomite-Soxhlet methods and then purified using the same procedure as for the hair samples.

PFRs were quantified using a Shimazu 2010 gas chromatograph coupled to a mass spectrometer (GC/MS) with an electron ionization. A DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Agilent, USA) was used, and the MS was operated in SIM mode with 2 characteristic ions acquired for each compound. The injection temperature was set at 70 °C and ramped up to 300 °C, with a sampling time of 1 min. Helium was the carrier gas with a flow rate of 1.0 mL min^{-1}. Dwell times ranged from 20 to 30 ms. The ion source and interface temperatures were set at 200 °C and 290 °C, respectively (He et al., 2015). A total of eleven PFRs were determined, including triethyl phosphate (TEP), triisopropyl phosphate (TiPrP), tri-n-propyl phosphate (TPP), TNBP, tri(2-Chloroethyl) phosphate (TCEP), Tris(chloroisopropyl) phosphate (TCIPP), TDCIPP, TPHP, 2-ethylhexyl diphenyl phosphate (EHDPP), tri(2-ethylexyl) phosphate (TEHP), and tricresyl phosphate (TMPP). Hair PFR concentrations were normalized by dry weight, and serum PFR concentrations were normalized by wet weight.