**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 $\log(\text{PFR concentrations-distal/PFR concentrations-proximal})$ 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.

Keywords: Human hair; Serum; Organophosphate flame retardants; Segmental difference; Gender difference

■ **Information on funding sources and evidence of approval for human subjects**

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Application for ethical review

Ethics Committee in School of Life Sciences, Sun Yat-sen University,

Project Name: The feasibility study of using hair for POPs biomonitoring.

Project Leader: Zhong-Yi Yang, Jing Zheng

General Information of the Project: The project will focus on the feasibility of using hair for POPs biomonitoring, which will be conducted by the team of Dr. Zhong-Yi Yang in School of Life Sciences, Sun Yat-sen University and the team of Dr. Jing Zheng in South China Institute of Environmental Sciences, Ministry of Environmental Protection. The volunteer participants include 150 adults. Hair samples and 15 mL blood samples will be collected from each volunteer participant by medical professionals in local hospitals under consents from all participants after they are clearly informed of the study's objectives, contents, details, benefits and risks.

We promise that the data and samples acquired in this project will only be used for scientific research, including the data of POPs and heavy metals in hair and blood samples. The whole procedures will be conducted by professionals with no potential harm to the participants.

The research teams have good background and extensive experience in the related field. Though the academic achievements of this project will be published in international journals, we promise to protect the privacy of the participants and we will not report the personal information of participants in any papers or reports. All the original data and materials will be carefully recorded and well preserved.

We apply for the review and approval for this project by Ethics Committee in School of Life Sciences, Sun Yat-sen University.

The project leader: Zhong-Yi Yang, Jing Zheng

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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 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 $1620 \times g$ 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 μ L 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 Shimadzu 2010 gas chromatograph coupled to a mass spectrometer (GC/MS) with an electron ionization. A DB-5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, 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.

2.3 Quality control

Analyte recoveries were assessed by spiking 30 ng of the target compounds into 2 g of hair (n=5) and 5 mL of serum (n=5). The mean recoveries of the PFRs were in the range of $59.4 \pm 9\%$ to $128 \pm 20\%$ for hair and $77.5 \pm 12\%$ to $138 \pm 23\%$ for serum (The recoveries of each compound in the hair and serum are given in the Supporting Information, Table S1). The recoveries of the internal standard (TNBP- d_{27}) were $71.6 \pm 19\%$. Procedural blanks (n=5) were conducted in each batch of sample preparation, and the recoveries of the internal standard in the blanks were $110 \pm 5\%$ (mean $\pm$ SD). The blank concentrations of individual PFRs in hair and serum are given in the Supporting Information, Table S2. All target analytes were blank-corrected. The limits of quantification (LOQs) were calculated as the mean values plus three times the standard deviations of the analytes in the blanks. For the undetected compounds in the blanks, the LOQs were estimated as a signal to noise ratio of 10. The LOQs of the PFRs in the hair ranged from 0.05 to 6.53 ng/g dry weight (Table 1). The LOQs of the

PFRs in the serum ranged from 0.02 to 6.41 ng/mL for most PFRs and were 52.8 ng/mL for TEHP.

2.4 Statistical analysis

All statistical analyses were performed using SPSS Statistics 19.0 (SPSS, Inc., Chicago, IL). Statistical analysis was performed only for compounds with detection frequencies higher than 50% in the hair samples. The PFR concentrations in hair were log-transformed to obtain normal distributions, and concentrations less than the LOQs were defined as 1/2*LOQ values before analysis. The *t*-test was used to determine the gender and segmental differences in PFR concentrations in hair. Correlations between log K_{OA} and log (PFR concentrations-distal/PFR concentrations-proximal) were assessed using Pearson correlation analysis. The level of significance was set at $p = 0.05$ throughout the study.

3. Results and discussion

3.1 PFR concentrations in hair

The detection frequencies and concentrations of ten individual PFRs (TEP, TiPrP, TPP, TNBP, TCEP, TCIPP, TDCIPP, TPHP, EHDPP, and TEHP) are shown in Table 1. TMPP was not quantified due to chromatographic interference in some of the samples. TEP, TCEP, TDCIPP, TPHP and EHDPP were detected in 51-86% of the hair samples. TiPrP, TNBP, TCIPP, and TEHP were found in nearly all hair samples in the

present study (detection frequencies >90%). The total PFR concentrations varied from 10.1 to 604 ng/g, with a median of 148 ng/g. The concentrations of PFRs in our study were lower than those reported in previous studies from Belgium (2~5,032 ng/g) (Kucharska et al., 2014), Norway (<1~3,744 ng/g) (Kucharska et al., 2015) and the United States (1,100~3,900 ng/g) (Liu et al., 2015). Similarly, the PFR concentrations in dust from urban college dormitory and urban home in Guangzhou (He et al., 2015) were also lower than the reported PFR concentrations from the US and Europe. As PFRs are used as substitutes for banned BFRs, low PFR concentrations in the hair of Chinese people may imply that BFRs are still widely used in China. Our results suggested that the indoor contamination and relevant human exposure risks of PFRs were still lower in the urban cities of China than in cities in the US and Europe.

The composition profiles of PFRs in the hair samples are shown in Figure 1. The most abundant compounds were TCIPP, TEHP and TPHP for both females and males, which were different from the PFR compositions in hair in previous studies (Kucharska et al., 2014; Kucharska et al., 2015). The predominant PFRs in Belgian and Norwegian hair samples were TDCIPP and TCEP (Kucharska et al., 2014), and TBOEP, TNBP, and TPHP (Kucharska et al., 2015), respectively. Liu et al. (2015) found that TCEP, TDCIPP, and TPHP dominated PFRs in five hair samples from the United States. The distinct composition profiles are likely due to the diverse usage of PFR-containing household products. For example, TCIPP is present in furniture and plastic components (Reemtsma et al., 2008), and TPHP is widely used in foam

furniture, general plastics, and electronic equipment, such as video display units
cables (Kim et al., 2014; Van der Veen and de Boer., 2012), which may cause different
PFR patterns in indoor environments and also in human tissue.

No PFRs were detected in serum samples (<LOQs). Unlike most brominated
flame retardants, PFRs are recognized as non-persistent chemicals because they can
be efficiently metabolized and excreted by the human body (Van de Eede et al., 2015;
Cooper et al., 2011; Meeker et al., 2013; Van de Eede et al., 2013). In *in vitro* studies,
TBOEP can be quickly metabolized in both human serum and the liver (Van de Eede
et al., 2015). The half-lives of PFRs in the human body were estimated to be only
approximately several hours (Cooper et al., 2011; Meeker et al., 2013). In the only
study investigating PFRs in human serum, the authors could detect only TPHP (Shah
et al., 2006), but the results were questioned because the bag used to store the blood is
an important source of TPHP (Reemtsma et al., 2008). Our results confirmed that hair
is a more suitable indicator for biomonitoring retrospective human exposure to PFRs.

3.2 Segmental differences in PFRs in hair

The paired segmental PFR data for individuals are provided in the Supporting
Information, Table S3. The differences in PFR concentrations between the proximal
segment and the distal segment were analysed in paired samples from 15 females
(Table 2). Aside from TEP (detection frequency: 46.7%), TPP (detection frequency:
26.7%) and TCEP (detection frequency: 60.0%), other individual PFRs were detected

in almost all of the distal segment samples. In the proximal segments, TEP, TPP, TCEP, TDCIPP, TPHP, and EHDPP were detected in less than 50% of the samples (TEP: 26.7%, TPP: 20.0%, TCEP: 6.67%; TDCIPP: 46.7%, TPHP: 26.7%; EHDPP: 6.67%). Total PFRs had significantly higher concentrations in the distal segments than in the proximal segments (t -test, $p < 0.01$) (Figure 2). The median concentrations of most of the PFRs in the distal segments were 1.5~8.6 times (TiPrP: 1.5 times; TNBP: 1.9 times; TCIPP: 3.6 times; TEHP: 8.6 times) higher than those in the proximal segments, with TEHP increasing most along the hair shaft. In the limited number of studies that conducted a segmental analysis of contaminants in hair, PBDE (Carnevale et al., 2014) and FAEE (Fatty acid ethyl ester) (Auwärter et al., 2001) concentrations increased along the hair shaft. The greater accumulation of contaminants in the distal segments may be affected by many factors (Auwärter et al., 2001): (a) hair is continuously bathed in sebum, leading to an accumulation of contaminants along with sebum deposition from the bloodstream in the more distal segments of the hair; (b) the sebaceous layer in hair near the root may be removed due to intensive shampooing of the hair closest to the scalp; (c) hair distant from the scalp is older and has been exposed to air/dust for a longer period of time; (d) aged hair is more damaged, which allows better diffusion of external compounds into the hair matrix. As PFRs have relatively short half-lives in organisms (Meeker et al., 2013) and none of the PFRs were detected in serum samples in our study, we suggest that the last two factors (the distal segments are more damaged and has longer exposure time) were more

important, which would explain the higher concentrations in the distal segments than in the proximal segments of the hair in our study.

The composition profiles of PFRs also differed between the proximal and distal segments (Figure S3, Supporting Information). TCIPP (29.2%), TEHP (23.2%) and TPHP (21.4%) were the most abundant compounds in the distal segments of the hair. Whereas, TCIPP contributed 45.1% of the total PFRs in the proximal segments, followed by TEHP (25.3%). EHDPP and TPHP were much more abundant in the distal segments (EHDPP: 15.1%, TPHP: 21.4%) than in the proximal segments (EHDPP: 2.78%, TPHP: 5.45%). As discussed above, PFRs in hair are most likely from external exposure to environmental media rather than deposition from the bloodstream. The PFRs analysed in the present study span a wide range of physical-chemical properties, which may govern their deposition fates in the hair samples (proximal segments and distal segments). To further evaluate the accumulation of PFRs in hair segments, we performed a correlation analysis between $\log K_{OA}$ of PFRs (US EPA, 2012) and $\log (\text{PFR concentrations-distal/PFR concentrations-proximal})$. As shown in Figure 3 and Figure S4 (Supporting Information), the medians of the $\log (\text{PFR concentrations-distal/PFR concentrations-proximal})$ values were positively and significantly correlated with $\log K_{OA}$ values ($p < 0.05$, $r = 0.68$). The results indicated that individual PFRs do not accumulate at the same rate in hair and that the PFR accumulation in hair is mediated by physico-chemical properties. Given that PFRs easily undergo hydrolysis and

hydroxylation processes by human serum and the liver (Van den Eede et al., 2013), extraneous PFRs from air or dust seem to be the main source of PFRs in hair. In a biomonitoring study over a period of 24 h, no relevant associations between metabolites of PFRs in urine and food consumption data could be obtained (Cequier et al., 2015). The authors suggested that the residential environment is a more important exposure pathway for PFRs than the diet (Cequier et al., 2015). Our results further indicated that the partition of PFRs between the air and hair is an important source of PFRs in the hair. Overall, the differences between the proximal and distal segments indicate that the accumulation of PFRs in hair does not occur at the same rate for individual chemicals. Certain PFRs with higher log K_{OA} values tend to accumulate more in hair. The results indicate that hair may be used as a “personal passive sampler” for airborne PFRs.

3.3 Gender differences in PFRs in hair

The PFR concentrations were 62.7-604 ng/g (median: 202 ng/g) and 10.1-331 (median: 111 ng/g) in female hair (data of combined segments) and male hair, respectively. A *t*-test was used to determine the gender differences in the PFR concentrations. Using the PFR concentrations of the combined female hair segments, females had significantly higher hair PFR concentrations ($p < 0.01$) than males (Figure S1, Supporting Information). For individual PFRs, the hair concentrations of TPHP, EHDPP and TEHP in females were also significantly higher than those in

males (EHDPP: $p < 0.01$; TPHP and TEHP: $p < 0.05$). Other PFRs, except TCEP, all exhibited higher median concentrations in female hair than in male hair (not significant). However, when we compared the PFR concentrations in the same hair segments (0-5 cm from the root) between females and males, the results were very divergent (Figure S2, Supporting Information). The PFR concentrations in male hair (median: 111 ng/g) were significantly higher than those in female hair (median: 49.1 ng/g). Male hair also showed significantly higher concentrations of TCEP, TCIPP, and TPHP than female hair. The composition profiles of the PFRs in hair samples (0-5 cm from the root) also differed between genders (the number of samples from females and males were 15 and 27, respectively). Female hair exhibited slightly lower proportions of TCEP (0.93%), TPHP (5.45%) and EHDPP (2.78%) and higher proportions of TiPrP (7.34%) (Figure S3, Supporting Information) than male hair.

Previous studies have frequently reported higher concentrations of organic pollutants in female hair than in male hair (Zheng et al., 2014; Altshul et al., 2004; Covaci et al., 2008). Covaci et al. (2008) found that the concentrations of most organochlorine pesticides (OCPs) and PCBs were significantly higher in hair from females than in hair from males. Zheng et al. (2014) also reported that PBDE concentrations in female hair were 3 times those in male hair, whereas Poon et al. (2014) did not find any gender difference in PBDEs in Canadian hair samples. In the above studies, hair samples were collected by different methods. The distal portion of the hair was used by Covaci et al. (2008), whereas Poon et al. (2014) collected hair

within 1 cm of the scalp at the posterior vertex. Altshul et al. (2004) collected hair samples during participant haircuts. In the previous studies, hair samples were collected without standardized methods (Zheng et al., 2014; Altshul et al., 2004; Covaci et al., 2008; Poon et al., 2014), such as using the same hair segment or the same distance from the scalp, which would greatly influence the results of the gender differences. In the present study, we found that the PFR concentrations varied in different segments because the distal segments of hair have longer exposure time than the proximal segments. Additionally, as females have longer hair, the proximal segments of female hair are mostly covered by distal hair, leading to less extraneous exposure than the distal hair. Thus, it is reasonable to observe lower PFR concentrations in the proximal segments of the hair (0-5 cm from scalp) from females than in those of the hair from males. However, female hair had higher fractions of TiPrP and lower fractions of TCEP, EHDPP, and TPHP than male hair, which seems not to be relevant for the log K_{OA} values. The reason of the different PFR profiles between genders remains unclear and may be attributed to personal living habits, such as hair washing frequencies, and different metabolic capacities of PFRs in human serum and the liver.

3.4 Limitations

The present study consisted of limited hair samples for convenience in segmental analysis and gender differences. Our data provided preliminary results for the

occurrence of PFRs in hair segments from university students but was not representative of urban residents in China. To facilitate the comparison of PFR concentrations between genders, female hair was separated into two segments: 0~5 cm and 5~10 cm (male hair: 0~5 cm). More segments would be useful to trace the retrospective exposure of PFRs in a future study.

4. Conclusions

The concentrations and composition profiles of 11 PFRs were studied in 49 paired hair and serum samples from university students in South China. All PFRs except TMPP were detected in the hair samples, whereas PFRs were not detected in serum samples. Hair has proven to be a suitable indicator for retrospective human exposure to PFRs. All detected PFRs exhibited higher concentrations in the distal segment than the proximal segment of hair from females. Segmental analysis showed that PFRs with higher log K_{OA} values tended to accumulate more rapidly in hair. The concentrations of PFRs in male hair were lower than those in the combined segments of female hair. The opposite was true when using the proximal segment (0-5 cm from the root) for both genders. Due to the small sample size in the study, more extensive studies are needed to conform these conclusions. However, our results provide vital evidences that sample collection of different segments or the position of the hair would mislead the estimation of human exposure to PFRs and likely other organic pollutants, demonstrating the importance of segmental analysis when using hair as a

biomarker of PFR exposure. The gender differences in PFR concentrations in hair should be investigated using the same segment in females and males. This study was the first to conduct segmental analysis of PFRs in hair; further study is still needed to verify our conclusions, to explore the accumulation mechanism of PFRs in hair segments and to standardize the sample collection method when using hair as a human biomonitoring matrix.

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Table 1. PFR concentrations (ng/g dry weight) in hair of male and female (combined segments)

	LOQ s	total (n=49)				male (n=27)				female (n=22)			
		range (min-max)	mean	median	detection frequency	range (min-max)	mean	median	detection frequency	range (min-max)	mean	median	detection frequency
TEP	0.48	nd - 3.84	0.44	0.30	51.0%	nd - 3.84	0.46	nd	37.0%	nd - 1.42	0.42	0.39	68.2%
TiPrP	0.81	nd - 12.4	2.82	2.43	93.9%	nd - 5.71	2.23	1.80	88.9%	0.89 - 12.4	3.54	2.58	100%
TPP	2.46	nd - 4.91	0.84	nd	32.7%	nd - 4.91	0.77	nd	25.9%	nd - 4.56	0.93	nd	40.9%
TNBP	0.61	nd-25.4	5.58	3.30	98.0%	nd - 21.7	4.71	2.22	96.3%	0.92 - 25.4	6.65	4.51	100%
TCEP	3.50	nd - 64.9	8.25	3.61	57.1%	nd - 64.9	11.0	5.66	59.3%	nd - 19.9	4.93	3.24	54.5%
TCIPP	6.53	nd - 141	52.6	43.9	98.0%	nd - 122	46.9	38.3	96.3%	13.7 - 141	59.5	53.3	100%
TDCIPP	1.04	nd - 73.8	8.84	4.14	85.7%	nd - 35.4	6.36	3.13	77.8%	nd - 73.8	11.9	6.57	95.5%
TPHP	1.43	nd - 352	36.8	20.5	83.7%	nd - 142	19.1	10.3	70.4%	3.62 - 352	58.5	28.3	100%
EHDPP	5.78	nd - 78.0	16.6	11.8	71.4%	nd - 51.5	7.78	4.57	51.9%	nd - 78.0	27.4	22.1	95.5%
TEHP	0.05	nd - 151	37.0	24.1	98.0%	nd - 151	27.5	14.7	96.3%	4.60 - 109	48.7	42.9	100%
Total PFRs	-	10.1 - 604	170	148	100%	10.1 - 331	127	111	100%	62.7 - 604	222	202	100%

nd, not detected (<LOQ)

Table 2. PFR concentrations (ng/g dry weight) in distal and proximal segments of female hair

	proximal segments (n=15)				distal segments (n=15)			
	range (min-max)	mean	median	detection frequency	range (min-max)	mean	median	detection frequency
TEP	nd - 0.99	0.20	nd	26.7%	nd - 1.06	0.29	nd	46.7%
TiPrP	1.65 - 11.6	3.48	2.34	100.%	1.76 - 13.9	4.66	3.41	100%
TPP	nd - 7.03	0.83	nd	20.0%	nd - 3.65	0.83	nd	26.7%
TNBP	nd - 23.0	3.87	2.24	86.7%	1.54 - 26.9	7.57	4.30	100%
TCEP	nd - 7.30	0.49	nd	6.67%	nd - 20.5	5.84	5.00	60.0%
TCIPP	nd - 61.5	24.8	24.1	86.7%	19.9 - 196	91.2	86.3	100%
TDCIPP	nd - 17.1	2.59	nd	46.7%	4.20 - 111	18.9	10.2	100%
TPHP	nd - 17.3	2.93	nd	26.7%	20.8 - 536	88.2	44.2	100%
EHDPP	nd - 22.0	1.47	nd	6.67%	13.0 - 114	47.8	37.4	100%
TEHP	2.81 - 36.7	12.0	7.83	100%	nd -136	67.8	64.8	93.3%
Total PFRs	8.42 - 110	52.7	41.0	100%	127 - 904	333	306	100%

nd, not detected (<LOQ)

Figure 1. The composition profiles of PFRs in hair of male and female (combined segments)

Figure 2. Levels in distal and proximal segments in paired hair samples

Figure 3. Correlation analysis between log (PFR concentrations-distal/PFR concentrations-proximal) and log K_{OA}