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Dechlorane Plus in paired hair and serum samples from e-waste workers: Correlation and differences

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HIGHLIGHTS

- Elevated serum and hair DP levels were found in e-waste workers.
- Hair DP levels positively correlated with serum DP levels in paired samples.
- Hair showed different DP isomer composition with serum.
- The correlation between hair and serum DP levels in female was weaker than in male.

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ABSTRACT

Dechlorane Plus (DP) and a dechlorinated product of DP were measured in 34 matched human hair and serum samples (19 males and 15 females) collected from e-waste recycling workers in South China. The DP (sum of *syn*- and *anti*-DP) concentrations in hair and serum samples ranged from 6.3 to 1100 ng g^{-1} dry weight and from 22 to 1400 ng g^{-1} lipid weight (lw). The levels of *anti*-Cl_{11}-DP ranged from 0.02 to 1.8 ng g^{-1} in hair and from not detected to 7.9 ng g^{-1} lw in serum. Significant positive correlations for both DP and *anti*-Cl_{11}-DP concentrations between hair and serum samples were found ($p < 0.05$), indicating hair to be a suitable matrix for human DP exposure. However, a significant difference was found in the DP isomer composition between hair and serum, suggesting stereoselective bioaccumulation during the absorption of DP into hair. A sharp gender difference was found in the levels of DP in hair. Moreover, *syn*-DP, *anti*-DP and *anti*-Cl_{11}-DP in hair significantly correlated with those in serum for male samples, but not for female samples. The observed gender differences in the present study may be, in part, ascribed to the much longer hair exposure time for females than males due to the difference in sampling distance from the scalp.

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1. Introduction

Dechlorane Plus (DP, $C_{18}H_{12}Cl_{12}$), developed by Hooker Chemical in the 1960s as a substitute for Dechlorane, is widely used as an additive flame retardant in the coatings of electrical wires and cables, computer and television connectors, and plastic roofing materials (Bettes, 2006; Sverko et al., 2011). Commercial DP comes as three technical products, DP-25, DP-35, and DP-515, which differ in particle size but are similar in composition. Each of them consists of two isomers, *syn* and *anti*-DP, in a ratio of 1:3 (Hoh et al., 2006). DP has been categorized as a high production volume chemical by the U.S. Environmental Protection Agency (Xian et al., 2011). The usage of DP is projected to rise as it has been identified by European Commission as a possible replacement for the restricted decabromodiphenyl ether flame retardant (Sverko et al., 2011). Previous studies have suggested that DP and its analogs may be persistent, bioaccumulative, and subject to long-range transport, which can be characterized as persistent organic pollutants (Sverko et al., 2011). It was also found that DP exposure induced oxidative hepatic damage and led to an alteration of gene

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expression involved in carbohydrate, lipid, nucleotide, and energy metabolism in rats and mice (Wu et al., 2012; Li et al., 2013).

Several sources, including industrial use of DP technical mixtures and the use/disposal of consumable products containing DP, have been identified as contributing to the occurrence of DP in the environment (Wang et al., 2010). In China, the demand for flame retardants, including DP, has grown dramatically over the past decades due to the increasing use of textiles and plastics in houses and offices (Wang et al., 2010). There is a DP manufacturing plant located in Huai'an City (Jiangsu Province, China) with an annual DP production estimated in 2010 to be 2100–7000 tons (Wang et al., 2010). In addition to the emissions generated by productive processes, e-waste is another important source of DP in China due to intensive e-waste recycling activities conducted in various, unspecified locations throughout China (Ren et al., 2008). As a result of these e-waste recycling activities, elevated DP levels have been identified in abiota, biota and human samples taken from e-waste recycling regions (Ren et al., 2009; Zheng et al., 2010; Xian et al., 2011; Feo et al., 2012; Ben et al., 2013).

The identification of suitable biomonitoring indicators for the assessment of human exposure to chemicals is essential. Blood is an ideal matrix for human contaminant biomonitoring, but it is not always available in sufficient amount for reliable analysis, especially for children. Hair is a keratinous matrix and contains 3–4% lipids (Altshul et al., 2004). It can be conveniently collected, transported, and stored. Thus, human hair is a suitable matrix for the analysis of organic pollutants. In fact, several previous studies have used hair analysis to report human exposure to organic pollutants such as PCDD/PCDFs, OCPs, PCB, PBDEs and PAHs (Schramm et al., 1992; Schramm, 1997; Covaci et al., 2002; Toriba et al., 2003; Nakao et al., 2005; Chan et al., 2007; Zhang et al., 2007; Wen et al., 2008; Tadeo et al., 2009). In a previous study, Zheng et al. (2010) reported human DP exposure in populations located in an e-waste recycling area, a rural region and an urban region using hair as biomonitoring matrix. However, the correlation between hair and internal human matrices has not yet been clearly demonstrated.

To our best knowledge, only one study has investigated the correlation of DP levels between hair and blood of occupationally exposed workers in a DP manufacturing plant (Zhang et al., 2013). A significant positive correlation ($p < 0.05$) was obtained between the paired blood and hair samples. However, the difference between hair and blood in reflecting human DP exposure, and the factors which might influence the correlation between hair DP and serum DP, were not discussed. Though a correlation between hair and blood DP levels has been tentatively found, further insight into how different factors affect this relationship is still limited, and the differences between hair and blood in reflecting human DP levels and composition have rarely been taken into consideration.

In the present study, 34 matched hair and serum samples, including 19 paired male and 15 paired female samples, were collected from an e-waste recycling area in South China. The objective of this study was to investigate the correlation between the two important matrices (hair and serum) in indicating human DP levels. Moreover, we also investigated the effect of gender in the use of hair as a bioindicator of human DP exposure.

2. Materials and methods

2.1. Sample collection

A total of 34 matched human hair and serum samples (19 male and 15 female) were collected from e-waste recycling workers from Longtang, Qingyuan city in Guangdong province. This study was approved by the Ethics Committee of School of Life Sciences, Sun Yat-sen University. Consent was obtained from all participants after they were clearly informed of the study's objectives. A short questionnaire and general physical examination were completed, in which data on age, gender, weight, height, and occupational history of each participant were compiled. An approximately 8–10 mL venous blood sample was collected from each volunteer in an anticoagulant-free tube by medical professionals in a local hospital. The serum was isolated from the blood by centrifugation at 3000 rpm for 5 min and kept at −80 °C prior to chemical analysis. Hair samples were also collected from these volunteers using stainless steel scissors in a local barbershop. Hair samples were wrapped in aluminum foil, sealed in polyethylene zip bags, and kept at −20 °C prior to chemical analysis.

2.2. Chemicals

Anti-DP, *syn*-DP, and 1,6,7,8,9,14,15,16,17,17,18-octadeca-7,15-diene (*anti*-Cl_{11}-DP, lot NO. a-Cl_{11}DP 0708) standards were purchased from Wellington Laboratories (Ontario, Canada). BDE128 and BDE181 were obtained from AccuStandard Inc (New Haven, US). Organic solvents were redistilled using a glass system.

2.3. Sample cleanup and analysis

The procedures used for the extraction and cleanup of the hair and blood samples in the present study were the same as those previously used for our work on polybrominated diphenyl ethers (Zheng et al., 2014a). These methods are briefly described below. Hair samples were purified by rinsing with Milli-Q water, freeze-dried and cut into small pieces (2–3 mm) with scissors. Approximately 2 g of hair from each sample was weighed and spiked with an internal standard, BDE 128. The hair samples were then incubated for 12 h with hydrochloric acid (4 M) and a hexane/dichloromethane mixture (4:1, v/v), followed by liquid–liquid extraction. Serum samples were denatured using hydrochloric acid (6 M) and 2-propanol, and subsequently extracted with hexane/methyl-tert-butyl ether mixture (1:1, v/v). Concentrated sulfuric acid was used to remove the lipids from the serum extraction. Both hair and serum extracts were purified with a multilayer silica/alumina column. Finally, the cleaned extracts were condensed to a volume of 100 μL under a gentle stream of N_2. Before instrument analysis, known amount of BDE 181 was added to each sample as a recovery standard. The total lipid content was calculated from the total triglyceride and cholesterol values measured in the serum (Rylander et al., 2006).

DP was analyzed by a Shimadzu 2010 gas chromatograph coupled with a mass spectrometer with electron-capture negative ionization in selected ion monitoring mode. The target chemicals were separated by a DB-XLB (30 m × 0.25 mm × 0.25 μm) capillary column. Each sample (1 μL) was manually injected in splitless mode. The column temperature program was as follows: held at 110 °C for one min, then increased to 180 °C at 8 °C min^{-1} and held for one min, then to 240 °C at 2 °C min^{-1} and held for 5 min, before being increased to 280 °C at 2 °C min^{-1} and held for 15 min, and finally, to 310 °C at 10 °C min^{-1} and held for 5 min. The ions monitored were *m*/*z* 653.8 and 651.8 for DP isomers, *m*/*z* 618.0 and 620.0 for *anti*-Cl_{11}-DP, and *m*/*z* 79 and 81 for BDE 128 and BDE 181.

2.4. Quality assurance and quality control (QA/QC)

The QA/QC measures in this study were the same as those in our recent study on PBDEs, including the use of recovery standard, procedural blanks, spiked blanks, and spiked matrices. The recovery of the internal standard, BDE 128 was in the range of 72–113% for the hair samples and 71–110% for the serum samples. Recoveries of *syn*-DP, *anti*-DP, and *anti*-Cl_{11}-DP in the spiked blanks were in

the range of 78–96%, 92–103%, and 80–95%, respectively, for hair samples and 80–92%, 89–98%, and 83–91%, respectively, for serum samples. No corrections for difference in recovery between the internal standard and target analytes were applied. Milli-Q water was used in the serum procedural blanks; hydrochloric acid (4 M) and hexane/dichloromethane mixture incubated without any hair sample was used for the hair procedural blanks. No targeted compound was detected in these procedural blanks. The limit of quantification (LOQ) was defined as a signal-to-noise ratio of 10. The LOQs for *syn*-DP, *anti*-DP, and *anti*-Cl_{11}-DP were 2.9, 2.6, and 1.8 pg g^{-1}, respectively, for hair samples based on the average sample masses, and 3.1, 1.3 and 0.6 ng g^{-1} lipid weight (lw) for serum samples using an average sample lipid weight of 0.0191 g.

2.5. Statistical analysis

Data analysis was performed using SPSS for Windows Release 17.0 (SPSS Inc.). The statistical significance of DP concentrations between genders was calculated by Mann–Whitney test. Linear regression analysis was conducted to investigate the correlation between DP concentrations in hair and serum, as well as between working years and human DP levels. A logarithmic transformation was applied to achieve a normal distribution of all data before linear regression analysis. Statistical significance was set at $p < 0.05$ throughout the manuscript.

3. Results and discussion

3.1. Levels of DP and its dechlorination product

Both *syn*- and *anti*-DP were consistently detected in the examined samples. The ΣDP concentrations (sum of *syn*- and *anti*-DP) in hair and serum samples ranged from 6.3 to 1100 ng g^{-1} dry weight and from 22 to 1400 ng g^{-1} lw, respectively (Table 1). *Anti*-Cl_{11}-DP, a dechlorination product of *anti*-DP, was detected in all hair samples and 26 of 34 serum samples. The levels of *anti*-Cl_{11}-DP ranged from 0.02 to 1.8 ng g^{-1} for hair and from n.d. to 7.9 ng g^{-1} lw for serum samples (Table 1). A significant correlation ($p < 0.001$) was found between *anti*-Cl_{11}-DP and *anti*-DP in both the serum and hair samples, in agreement with previous studies (Zheng et al., 2010; Yan et al., 2012).

The serum DP levels in this study (median 190 ng g^{-1} lw) were lower than those found in occupational workers in a DP manufacturing plant (median 860 ng g^{-1} lw) and comparable to those found in non-occupationally exposed residents near the DP manufacturing plant (median 240 ng g^{-1} lw) (Zhang et al., 2013). Ren et al. (2009) has reported serum DP levels ranging from 7.8 to 465 ng g^{-1} lw, with a median value of 43 ng g^{-1} lw, in residents living in a different e-waste recycling area (Guiyu). These values are lower than those in the present study.

In the present study, the median ΣDP concentration in hair (46 ng g^{-1}) was 3 times higher than that previously reported in workers from the same e-waste recycling area (15 ng g^{-1}) (Zheng et al., 2010). Similarly, the median *anti*-Cl_{11}-DP concentration in the present study was also approximately 3 times higher than those in the previous study. This observation is likely due to differences in the accumulation in hair by gender, which will be discussed in detail later. Our study was composed of 15 female and 19 male workers, while all the hair samples in the Zheng et al. (2010) study were from male workers. When limiting our study to only male subjects, we obtain a median DP concentration of 19 ng g^{-1} dry weight, which is comparable to the 15 ng g^{-1} dry weight reported by Zheng et al. (2010). The median DP concentration in females in the current study was 200 ng g^{-1} dry weight, more than 10 times higher than in males. The DP levels in hair samples from DP manufacturing workers (170–2200 ng g^{-1}, median of 260 ng g^{-1}) and from residents living approximately 3 km away from the manufacturing plant (median: 82 ng g^{-1}) (Zhang et al., 2013) were remarkably higher than those in the present study. However, the ΣDP concentrations in this study were significantly higher than those found in general residents of rural (Yuantan, median: 1.0) and urban (Guangzhou, median: 0.87) areas (Zheng et al., 2010), confirming that e-waste recycling was an important non-manufacturing source of DP exposure.

3.2. The associations between hair and serum

To demonstrate the relationship between DP levels of hair and serum, linear regression analysis was performed on concentrations of DP and *anti*-Cl_{11}-DP in the hair and serum samples (Fig. 1). We found a moderate positive correlation for DP concentrations between human hair and serum samples ($r = 0.42$, $p = 0.01$). The correlation was stronger for *syn*-DP ($r = 0.48$, $p < 0.01$) than for *anti*-DP ($r = 0.36$, $p = 0.03$). The levels of *anti*-Cl_{11}-DP in hair were also correlated with those in the paired serum samples. A significant positive correlation ($p < 0.05$) between the DP concentrations in blood and hair was also obtained from samples collected from occupational workers from a DP manufacturing plant (Zhang et al., 2013). This consistent correlation between blood and hair indicated that DP in human hair could effectively reflect DP contamination state in human body, and thereby could be used as a reliable biomonitoring indicator for human DP exposure.

However, differences in DP composition between hair and serum samples were also observed in the present study. The f_{anti} values were significantly lower in human hair (median was 0.47) than in serum (median was 0.65) ($p < 0.05$) (Table 1). It is well known that organic pollutants are deposited on and in human hair via two major routes: endogenous (internal) and exogenous (external) sources (Schramm, 1997; Król et al., 2013). To figure out whether the sources determined the proportion of these chemicals, we compared the f_{anti} value in hair with serum samples (internal source) and dust samples (external source). The dust samples were collected from the same e-waste recycling area as in this study (Zheng et al., 2010). Hair samples showed significantly lower f_{anti} values (0.45 ± 0.11) than either the internal (0.64 ± 0.06) or

Table 1
Median and range of DP concentrations and f_{anti} values in hair (ng g^{-1}) and serum (ng g^{-1} lipid) of e-waste recycling workers.

	Serum			Hair		
	Male ($n = 19$)	Female ($n = 15$)	Total ($n = 34$)	Male ($n = 19$)	Female ($n = 15$)	Total ($n = 34$)
syn-DP	52 (7.5–190)	88 (16–420)	77(7.4–420)	8.3 (3.5–100)	95 (7.5–860)	23 (3.5–860)
anti-DP	120 (14–370)	140 (21–990)	120 (14–990)	11 (2.8–46)	91 (6.6–240)	24 (2.8–240)
ΣDP[a]	180 (22–510)	230 (37–1400)	190 (22–1400)	19 (6.3–150)	200 (17–1100)	46 (6.3–1100)
anti-Cl_{11}-DP	1.6 (nd[b]–4.3)	1.9 (nd–7.9)	1.8 (nd–7.9)	0.10 (0.02–0.32)	0.42 (0.06–1.8)	0.19 (0.02–1.8)
f_{anti}	0.64 (0.54–0.73)	0.65 (0.55–0.73)	0.65 (0.54–0.73)	0.51 (0.24–0.64)	0.45 (0.20–0.55)	0.47 (0.20–0.64)

[a] Sum of *anti*-DP and *syn*-DP.
[b] Not detected.

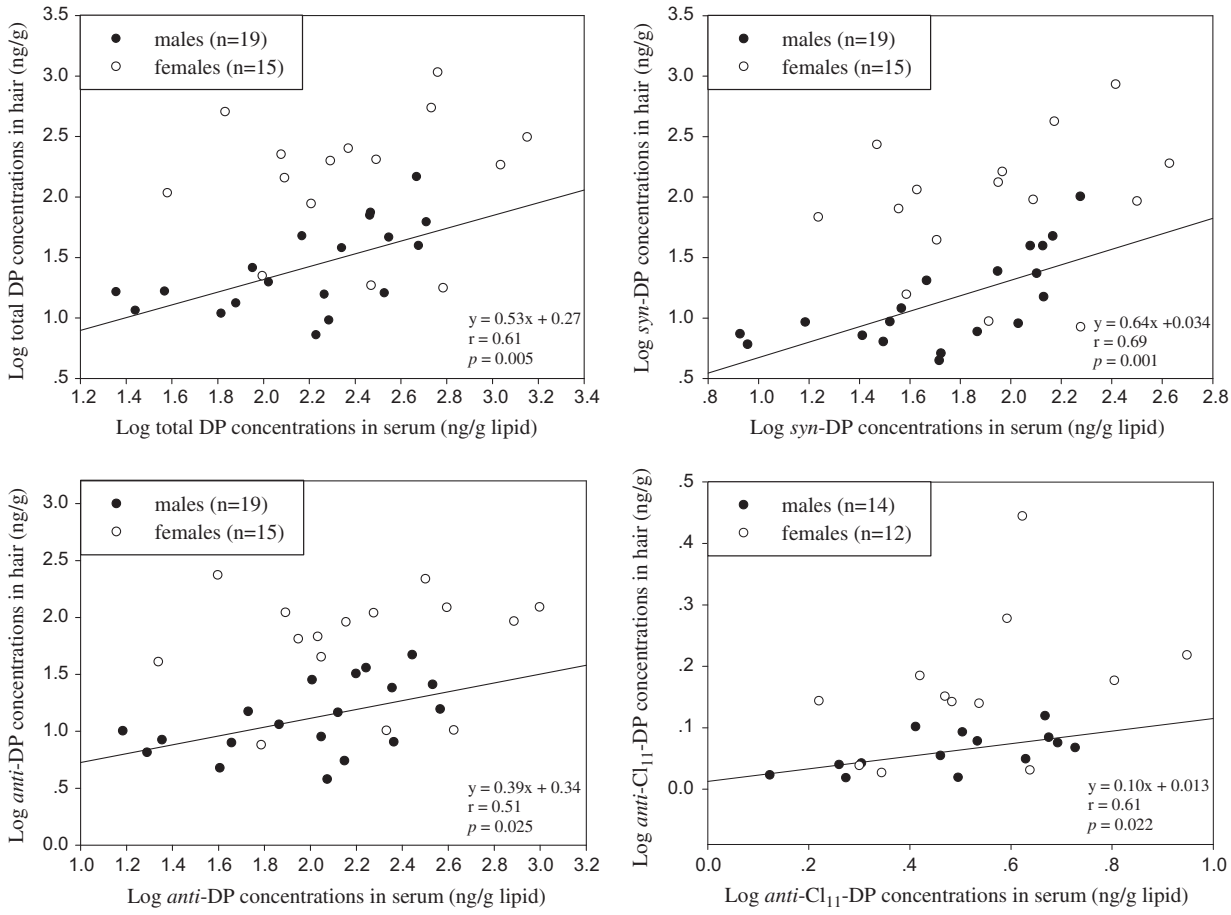

Fig. 1. Gender difference in the relationship between serum and hair DP levels for e-waste workers. The fitted line was only provided for males because a significant correlation between serum and hair samples was found for males (r = 0. 51–0.69, $p < 0.05$), but not for females (p = 0.2–0.7).

external source (0.54 ± 0.15) (Fig. 2). This result implied that different DP isomers might have different transport rates into hair, either from internal or external sources, leading to a stereoselective enrichment of DP in human hair. Studies on DP levels in fish, chicken and birds also found a selective enrichment of certain DP isomers (Wu et al., 2010; Chen et al., 2013; Zheng et al., 2014b). The possible stereoselective bioaccumulation of DP isomers during the absorbance of DP into hair suggested that data taken from hair could not be simply extrapolated to human internal exposure. The tissue-specific distribution of organic pollutants, as well as the effect of external exposure on contaminants in hair, must be better understood to enable hair to be an effective indicator to monitor human body burden of DP.

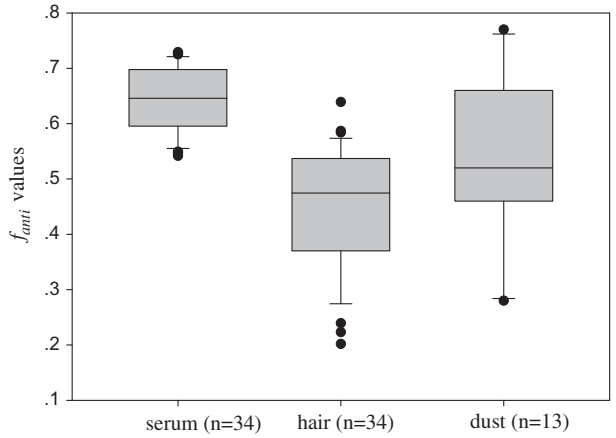

Fig. 2. f_{anti} values in hair, serum, and dust samples collected from the same e-waste site. Data for the f_{anti} values in dust came from our previous study (Zheng et al., 2010). The p values from multiple comparisons of ANOVA analysis for serum-hair, serum-dust, and hair-dust were <0.001, 0.043, and 0.003, respectively.

3.3. Gender differences in DP levels and in the relationships between hair and serum

Previous studies have investigated the influence of gender on DP levels in human serum samples, but no consistent conclusion was reached (Yan et al., 2012; Zhang et al., 2013). No difference in DP levels was observed between male and female workers from a DP manufacturing plant (Zhang et al., 2013). However, our previous study found significantly higher DP levels, as well as f_{anti} values, in females compared to males (Yan et al., 2012). As expected, in the present study, serum DP levels in females (median 230 ng g^{-1} lw) were slightly higher than in males (median 180 ng g^{-1} lw), though the difference was not significant, perhaps due to the smaller sample size of this study. The influence of age and occupational exposure time were ruled out as there was no significant correlation between serum DP levels and these two parameters.

A pronounced gender difference was found in the DP levels found in hair, with the median ΣDP level in females (200 ng g^{-1}) more than 10 times higher than in males (19 ng g^{-1}). The *anti*-Cl_{11}-DP concentrations (median, 0.42 ng g^{-1}) in female hair were also significantly higher than in hair samples from males (median,

0.10 ng g^{-1}) ($p < 0.01$). Longer external exposure time for female hair could be a main reason for the higher DP levels in female hair. In theory, hair should be cut as close to the root as possible to minimize the contribution of external exposure (Schramm, 2008). However, not all of the participants were willing to have their hair cut in such a fashion, especially females. In the present study, hair samples were collected during participants' routine haircut sessions. Thus, the female hair samples we collected were customarily far from scalp as compared to the male hair samples, indicating a longer external exposure time for female hair. Higher DP levels were found in the environmental matrix, such as air and dust samples collected from e-waste recycling areas (Zheng et al., 2010). Thus, longer external exposure time would elevate the hair DP levels in female hair. Additionally, higher internal exposure, reflected by the relative higher serum DP levels in females as compared to males, could be another important cause for the higher DP levels in female hair.

To illustrate whether a gender difference existed in the relationship between paired hair and serum samples, we grouped all 34 paired hair and serum samples by gender and analyzed the correlation. Interestingly, the *syn*-DP, *anti*-DP and *anti*-Cl_{11}-DP showed a significantly positive correlation between hair and serum from male workers ($n = 19$) ($r = 0.51–0.69$, $p < 0.05$) (Fig. 1). However, no significant correlation was found in female workers ($n = 15$) ($p = 0.2–0.7$). It suggested that the longer hair of females showed substantially different DP levels from blood. Longer hair reflects an accumulated exposure over a longer time period, while blood shows the real-time body burden (Altshul et al., 2004). Moreover, external exposure pathway might contribute a greater portion of DP in female hair than male hair, inducing a weaker positive correlation of DP concentrations between hair and serum.

4. Conclusions

In this study, the significant positive correlation between blood and hair DP levels indicated that human hair could be a reliable indicator for DP biomonitoring in males but not for females due to their typically longer hair. We found a gender difference between male and female hair DP levels and in the correlation between DP levels in hair and serum. The DP levels in female hair, which were several times higher than those in male hair, but not correlated with DP levels in matched serum samples, indicated that longer hair failed to reflect the current body burden and that external pathway might play an important role in the distribution of DP in hair.

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