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Human health risks estimations from polycyclic aromatic hydrocarbons in serum and their hydroxylated metabolites in paired urine samples☆

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are compounds with two or more benzene rings whose hydroxylated metabolites (OH-PAHs) are excreted in urine. Human PAH exposure is therefore commonly estimated based on urinary OH-PAH concentrations. However, no study has compared PAH exposure estimates based on urinary OH-PAHs to measurements of PAH levels in blood samples. Estimates of PAH exposure based solely on urinary OH-PAHs may thus be subject to substantial error. To test this hypothesis, paired measurements of parent PAHs in serum and OH-PAHs in urine samples from 480 participants in Guangzhou, a typical developed city in southern China, were used to investigate differences in the estimates of human PAH exposure obtained by sampling different biological matrices. The median PAH concentration in serum was 4.05 ng mL^{-1}, which was lower than that of OH-PAHs in urine (8.33 ng mL^{-1}). However, serum pyrene levels were significantly higher than urinary levels of its metabolite 1-hydroxypyrene. Concentrations of parent PAHs in serum were not significantly correlated with those of their metabolites in urine with the exception of phenanthrene, which exhibited a significant negative correlation. Over 28% of the participants had carcinogenic risk values above the acceptable cancer risk level of 10^{-6}. Overall, estimated human exposure and health risks based on urinary 1-hydroxypyrene levels were only 13.6% of those based on serum pyrene measurements, indicating that estimates based solely on urine sampling may substantially understate health risks due to PAH exposure.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in environments where emissions due to human activity dominate. They are mainly produced by the incomplete combustion of organic substances such as coal, oil, gasoline, and wood (Abdel-Shafy et al., 2016; Xia et al., 2016). They are also highly lipophile and can enter and accumulate in the human body via inhalation, dietary intake, and skin exposure (Wang et al., 2012). Many PAH isomers present health hazards to humans because they are mutagenic and carcinogenic; accordingly, PAH exposure increases the risk of cancer (Belpomme et al., 2007), hormonal imbalance (Archibong et al., 2002), and adverse effects on neural development (Wormley et al., 2004). Sixteen PAH isomers have therefore been identified as priority control pollutants by the United States Environmental Protection Agency (USEPA) (Qiao et al., 2006).

China's energy consumption has increased markedly in recent decades due to its rapid urbanization and industrialization (Li et al., 2016), leading to an increase in emissions and atmospheric PAH concentrations (Zhang et al., 2007). Both external and internal exposure assessments have been used to assess the human exposure and health risks of PAHs (Kim et al., 2013). External exposure assessments are based on measurements of PAH concentrations in matrices such as air, food, water,

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and other media together with information on the intake rate of each matrix (Jiang et al., 2019; Lei et al., 2015; Wang et al., 2012; Yu et al., 2015). However, because oral and inhalation bioavailability/bioaccessibility data are rarely available, the accuracy of this approach is limited (Lu et al., 2021; Ren et al., 2020). Consequently, there may be inconsistencies in the findings of different studies concerning the main human exposure pathways (Li et al., 2015; Yu et al., 2012).

To avoid these problems, internal human exposure is generally estimated instead by sampling biometrics such as blood, breast milk, lipid, urine, or hair. Biomonitoring has become the preferred method for studying environmental exposure because it measures exposure via all possible pathways and allows unexpected exposure to be detected while also reflecting differences in absorption or genetic susceptibility between individuals (Lin, 2005). Since the 1980s, several studies have investigated human PAH exposure by analyzing PAHs and their metabolites in human tissues and body fluids including cord blood, breast milk, urine, and hair (Aquilina et al., 2010; Wilhelm et al., 2007).

In organisms, PAHs can be metabolized into hydroxylated derivatives (OH-PAHs) that are excreted in urine. OH-PAHs are predominantly eliminated through the urine and in a lesser extent in other human fluids (e.g. breast milk) (Mta et al., 2007). The compound 1-hydroxypyrene (1-OH-Pyr), a metabolite of pyrene (Pyr), has been widely used as a biomarker of human exposure to PAHs (Cavanagh et al., 2007; Kato et al., 2004). Although the half-lives of PAHs are only approximately 6–35 h (Jongeneelen et al., 1990; Huang et al., 2007), the parent PAHs can still be detected in blood samples (Song et al., 2013). For example, in one study using measurements of urinary 1-OH-Pyr to estimate Pyr exposure, it was found that only 6.7% of the uptaken parent compound was excreted as the hydroxylated metabolite in the urine (Han et al., 2006). Although several studies have investigated PAHs in blood, there are no published studies comparing exposure levels estimated by measuring PAH levels in blood serum samples to those obtained by measuring OH-PAH levels in paired urine samples.

We hypothesized that estimates of human PAH exposure based only on measurements of urinary OH-PAH levels may be subject to substantial error, especially for isomers such as Pyr whose urinary excretion ratios are low. To test this hypothesis, 480 matched blood and urine samples were collected in a typical developed city (Guangzhou in South China) and analyzed. We chose Pyr as a representative substance for evaluating PAH exposure, and calculated its total estimated daily intake (TEDI) based on its concentration in serum and the concentration of 1-OH-Pyr in urine. The results presented herein reveal challenges in the assessment of human exposure and health risks due to these low excretion ratio PAH isomers and provide important information for biomonitoring.

2. Materials and methods

2.1. Reagents and materials

Sixteen PAH standards, i.e., acenaphthylene (Acy), acenaphthene (Ace), anthracene (Ant), benzo [k]fluoranthene (BkF), benzo [b]fluoranthene (BbF), benzo [a]pyrene (BaP), benzo [ghi]perylene (BghiP), benz [a]anthracene (BaA), chrysene (Chr), fluoranthene (FluA), fluorene (Flu), phenanthrene (Phe), dibenz [a,h]anthracene (DahA), indeno [1,2,3-cd]pyrene (IcdP), pyrene (Pyr), and naphthalene (Nap) were bought from AccuStandard Inc. (New Haven, Connecticut, USA). Surrogate standards d_{10}-Phe, d_{12}-Chr, d_8-Nap, d_{10}-Ace, and d_{12}-Pyr were purchased from AccuStandard Inc. Thirteen OH-PAHs, i.e., 9-hydroxyfluorene (9-OH-Flu), 6-hydroxychrysene (6-OH-Chr), 2-hydroxyfluorene (2-OH-Flu), 3-hydroxybenzo(a)pyrene (3-OH-BaP), 1-hydroxynaphthalene (1-OH-Nap), 3-hydroxyfluorene (3-OH-Flu), 9-hydroxyphenanthrene (9-OH-Phe), 2-hydroxynynaphthalene (2-OH-Nap), 3-hydroxyphenanthrene (3-OH-Phe), 1-hydroxyphenanthrene (1-OH-Phe), 4-hydroxyphenanthrene (4-OH-Phe), 2-hydroxyphenanthrene (2-OH-Phe), and 1-hydroxypyrene (1-OH-Pyr) were purchased from Chiron AS (Trondheim, Norway), Toronto Research Chemicals (Toronto, Canada) and Dr. Ehrenstotfer (Augsburg, Germany). Four isotope-labeled internal standards, d_9-1-OH-Pyr, d_9-2-OH-Flu, d_7-2-OH-Nap, and d_{11}-3-OH-Bap were obtained from Toronto Research Chemicals (Toronto, Canada), and $^{13}C_{12}$-3-OH-Phe was from Cambridge Isotope Laboratories (Andover, USA). Acetonitrile and methanol (both HPLC grade) were bought from Merck (Darmstadt, Germany). β-Glucuronidase/sulfatase used for hydrolysis was from Roche Diagnostics GmbH (Mannheim, Germany). Solid phase extraction (SPE) columns (Captive EMR-Lipid, 6 mL, 600 mg) were bought from Agilent Technologies (California, USA).

2.2. Sample collection

In 2019, a total of 480 serum and 480 urine paired samples were collected from participants in four districts (TH: Tianhe, NS: Nansha, ZC: Zengcheng, and HD: Huadu) of a typical developed city, Guangzhou, in South China with a population of over 10 million. 120 paired samples were collected from each district. In each district, 120 participants (60 males, 60 females) were asked to fill in a detailed questionnaire and give their informed consent before sampling. Serum (8 mL) and urine samples (50 mL) were collected in Teflon centrifuge tubes and stored at −20 °C until analysis. The Ethics Committee of Guangdong University of Technology approved the experiments.

2.3. Extraction of PAHs in serum and OH-PAHs in urine

Serum samples were extracted using the QuEChERS approach, which is a lipid-removing solid phase extraction column purification process. This method is often used to extract drugs from blood and human tissue samples. The specific protocol used was a modification of that reported by Daljit (2010). Urine samples were treated using a protocol similar to that applied in a previous study (Li et al., 2020). Briefly, after enzymatic hydrolysis of urine samples and protein removal, target analytes were detected using an on-line solid phase extraction-liquid chromatography-mass spectrometry (SPE-LC-MS/MS) approach. Details of the extraction methods are given in the Supporting Information.

2.4. Instrumental analysis

PAHs were analyzed using an Agilent 7890 B gas chromatography (GC) instrument coupled with an Agilent 5977 B mass spectrometer operating in the electron impact ionization mode. A 30 m DB-5MS column (0.25 mm i. d., 0.25 μm film thickness, Agilent Technologies) was used for chromatographic separation (Yu et al., 2015). The ions monitored are listed in Table S1 in the Supporting Information. Urinary OH-PAHs were detected using an online solid phase extract-liquid chromatography-mass spectrometry (SPE-LC-MS/MS) Agilent 1260–6470 series instrument with an injection volume of 100 μL (Li et al., 2020). The ions monitored are listed in Table S2. Full details of the instrumental analyses are given in the Supporting Information.

2.5. Quality assurance and quality control

One matrix blank and one spiked sample were analyzed for each batch of 10 serum samples and 20 urine samples. Traces of PAHs were detected in the matrix blanks (Calf serum as matrix blank). Therefore, the concentrations of PAHs in the samples were blank-corrected. However, negligible quantities of OH-PAH compounds were detected in the matrix blanks (which consisted of diluted mixed urine). Therefore, the concentrations of OH-PAHs were not blank-corrected. The recoveries of PAHs and OH-PAHs in the matrix spiked samples ranged from 50% to 136% and from 74% to 121%, respectively. This result was not significantly different from the recovery rate of serum PAHs and urine OH-PAHs extracted by Wang et al. (2015) and Guo et al. (2013). Low

molecular weight PAH isomers (Nap, Acy, and Ace) had poor recoveries due to their high volatility. Limits of detection (LODs) were defined as the minimum detectable amounts of each analyte giving a signal-to-noise ratio of 3:1. If the concentration of a chemical was below its LOD, it was reported as not detected and assigned a concentration of zero. If an analyte was present at a concentration above its detection limit but below its quantitation limit, its concentration was recorded as being half of its limit of quantitation (LOQ). The limits of detection of PAHs in serum ranged from 0.003 to 0.041 ng mL^{-1} while those of OH-PAHs in urine were 0.0003–0.649 ng mL^{-1}. Values for individual analytes are given in the supporting information (Table S3-S4). The concentrations of the isomers 1-OH-Phe and 9-OH-Phe are reported jointly 1/9-OH-Phe because they could not be separated effectively during the LC-MS/MS analysis. Similarly, the concentrations of BbF and BkF are reported as their sum (BbF/BkF).

2.6. Exposure estimation and health risk assessment

Human exposure to PAHs was estimated by calculating TEDI values based on the measured concentrations of OH-PAHs in the urine samples using the method of Peng et al. (2020). Health risks were evaluated by considering the non-carcinogenic and carcinogenic risks of PAHs, which were assessed based on the hazard quotient (HQ) and cancer risk (CR), respectively. The USEPA considers an HQ of less than 1 to indicate no significant potential health risk (Lei et al., 2015) and defines an acceptable level of total carcinogenic risk to be in the range of 1×10^{-6} to 1×10^{-4}, with risks below 1×10^{-6} being considered negligible (Lei et al., 2015; USEPA, 2000; Williams et al., 2013). The relevant equations and parameters are given in Table 2 and Table S5.

2.7. Statistical analysis

All statistical analyses were performed with SPSS (IBM SPSS). Because the concentration of PAHs and OH-PAHs were not normally distributed, median values were used in the statistical analyses. The Mann-Whitney *U* test for nonparametric independent samples was used to evaluate the significance of differences, and the distributions of PAH and OH-PAH concentrations were illustrated using histograms and curves. Spearman's correlation coefficient was used to estimate the correlation between concentrations measured in the urine and serum samples. A significance threshold of $p < 0.05$ was applied in all cases.

3. Results and discussion

3.1. Socio-demographic characteristics

A total of 480 volunteers participated in the study. Their socio-demographic characteristics were collected using a questionnaire. Information on the age, sex, body mass index (BMI), and other characteristics of the participants are presented in Table 1. There were equal numbers of males and females. Their ages ranged from 6 to 79 years, with an average of 34.6 ± 22.5 years. The mean BMI of the subjects was 22.3 ± 11.4, with approximately 55% being classed as overweight or underweight. To assess the possible influence of smoking and drinking on human exposure to PAHs, the participants' status as smokers and/or drinkers was also recorded; 15.6% of the participants were smokers and 4.6% drank alcohol.

3.2. Occurrence of PAHs in blood and OH-PAHs in urine

The detection frequencies of the PAH isomers in serum ranged from 13.5% to 89.6%, with 99% for total PAHs (Table 3), indicating the ubiquitous occurrence of PAHs in human serum. Among the 16 analyzed PAH isomers, Pyr had the highest detection frequency (up to 90%); this value is similar to that reported by Wang et al. (2015). The next most frequently detected isomers were BghiP (81%), BaA (67%), Acy (66%) and Chr (65%); the remaining isomers had detection frequencies below 60%, with Flu having the lowest (13.5%). OH-PAHs were detected in all urine samples; the detection frequencies of the isomers ranged from 18% to 99%, with 6-OH-Chr and 2-OH-Nap being the least and most frequently detected, respectively. The high molecular weight compounds 6-OH-Chr (18%) and 3-OH-Bap (29%) had lower detection frequencies than the other isomers. Similar results were reported previously (Guo et al., 2013; Lou et al., 2019; Lu et al., 2016; Wang et al.,

Table 1
Selected variable characteristics of participants.

Variable	Number (%)
Gender	
Male	240
Female	240
Age (year, mean ± S.D.)	34.6 ± 22.5
6–11	95 (19.8)
12–18	97 (20.2)
19–39	95 (19.8)
40–59	97 (20.2)
60–79	96 (20.0)
Smoking status	
Yes	75 (male: 90.7, female: 9.3)
No	405 (84.3)
Drinking status	
Yes	22 (4.6)
No	458 (95.4)
Body mass index (kg/m^2, mean ± S.D.)	22.3 ± 11.4
Under weight (<18.5)	138 (28.8)
Normal weight (18.5–25)	217 (45.2)
Over weight (>25)	125 (26.0)

S.D.: standard deviation.

Table 2
Formulae and parameters used for the calculation of health and cancer risks.

Formula	Calculated parameters	Reference
$v\frac{dC}{dt} = TEDIs \times BW \times A \times f - b \times V \times C$	C (ng mL^{-1}): concentration of individual PAHs in serum V (L): amount of blood (males: 8% of body weight with 80 mL blood per kilogram of body weight, and females: 7%) A (dimensionless): the absorption rate of the dose ingested (0.90) f (dimensionless): the proportion of absorbed dose distributed in the blood (0.052) b: the elimination rate constant (0.068)	Haddad et al. (1997) Haddad et al. (1998) Viau et al. (2002)
$TEDIu = \frac{C \times V \times M1}{f \times BW \times M2}$	C (ng mL^{-1}): concentration of individual OH-PAHs in urine V (L): amount of urine excreted $M1$ (g mol^{-1}): molecular weight of parent PAH $M2$ (g mol^{-1}): molecular weight of metabolite OH-PAH BW (kg): body weight f (dimensionless): ratio of OH-PAH excreted in the urine to the total exposure dose (Nap: 100%; Flu: 60%; Phe: 11%; and Pyr: 6.8%)	Anderson et al. (2001); Chen et al. (2018); Chen et al. (2019); Li et al. (2012); Lu et al. (2018); Peng et al. (2020); Yu et al. (2020)
$HQ = \frac{TEDI}{RfD}$	RfD (μg/kg-bw/day): USEPA reference dose (Nap: 20; Flu: 40; Phe: 40; and Pyr: 30)	Lei et al. (2015); USEPA, 2020
$BaPeq = TEF \times TEDI$	TEF (dimensionless): toxic equivalency factor (Nap: 0.001; Flu: 0.001; Phe: 0.001; and Pyr: 0.001)	Ian and Peter (1992)
$CR = BaPeq \times CSF$	CSF: carcinogenic slope factor of BaP with 7.3 (mg/kg-bw/day)$^{-1}$	Lei et al. (2015)

Table 3
Concentrations (ng mL^{-1}) of PAHs in serum and OH-PAHs in urine.

PAHs	P25	Median	Mean	P75	Range	DF	OH-PAHs	P25	Median	Mean	P75	Range	DF
Nap	N.d.	N.d.	0.08	0.03	N.d.–5.52	44.2%	2-OH-Nap	1.55	3.16	9.40	8.24	N.d.–307	99.4%
Acy	N.d.	0.08	0.47	0.27	N.d.–17.7	66.3%	1-OH-Nap	0.49	1.53	5.51	4.63	N.d.–89.9	76.2%
Ace	N.d.	N.d.	0.06	0.06	N.d.–7.14	30.4%	∑OH-Nap	2.27	4.66	14.90	12.70	0.08–308	–
Flu	N.d.	N.d.	0.10	N.d.	N.d.–3.76	13.5%	3-OH-Flu	N.d.	N.d.	6.24	5.31	N.d.–137	40.1%
Phe	N.d.	N.d.	0.49	N.d.	N.d.–13.0	24.0%	2-OH-Flu	0.16	0.33	0.91	0.71	N.d.–15.6	99.4%
Ant	N.d.	N.d.	0.06	0.02	N.d.–1.55	31.3%	∑OH-Flu	0.20	0.72	7.15	5.97	N.d.–137	–
FluA	N.d.	0.01	0.25	0.29	N.d.–3.67	50.2%	2-OH-Phe	0.03	0.06	0.15	0.11	N.d.–1.39	95.6%
Pyr	0.75	1.64	2.02	2.77	N.d.–25.2	89.6%	3-OH-Phe	0.11	0.17	0.27	0.27	N.d.–6.67	98.1%
BaA	N.d.	0.01	0.03	0.04	N.d.–1.44	67.1%	4-OH-Phe	0.05	0.10	0.18	0.20	N.d.–2.90	88.7%
Chr	N.d.	0.01	0.01	0.02	N.d.–0.51	64.6%	1/9-OH-Phe	N.d.	0.03	0.06	0.06	N.d.–0.83	67.0%
BbF/BkF	N.d.	0.07	0.96	0.18	N.d.–28.7	53.5%	∑OH-Phe	0.21	0.36	0.61	0.65	N.d.–11.67	–
BaP	N.d.	0.07	1.36	1.08	N.d.–29.7	53.1%	1-OH-Pyr	0.05	0.14	0.35	0.33	N.d.–10.9	96.7%
IcdP	N.d.	N.d.	0.55	0.05	N.d.–18.8	40.2%	6-OH-Chr	N.d.	N.d.	0.02	N.d.	N.d.–1.07	17.7%
DBahA	N.d.	0.03	0.47	0.09	N.d.–29.6	50.2%	3-OH-Bap	N.d.	N.d.	0.04	0.01	N.d.–2.01	29.0%
BghiP	0.01	0.04	0.98	0.16	N.d.–7.51	80.6%	$\sum_{12}$OH-PAH	3.42	8.33	23.1	24.4	0.26–310	–
$\sum_{16}$PAH	2.00	4.05	7.89	12.9	N.d.–49.7	–							

DF: detection frequency; N.d.: not detected; Nap: naphthalene, Acy: acenaphthylene; Ace: acenaphthene; Flu: fluorene; Phe: phenanthrene; Ant: anthracene; FluA: fluoranthene; Pyr: pyrene; BaA: benz[a]anthracene; Chr: chrysene; BbF: benzo[b]fluoranthene; BkF: benzo[k]fluoranthene; BaP: benzo[a]pyrene; IcdP: indeno[1,2,3-cd]pyrene; DBahA: dibenz[a,h]anthracene and BghiP: benzo[ghi]perylene. 2-OH-Nap: 2-hydroxynaphthalene; 1-OH-Nap: 1-hydroxynaphthalene; 3-OH-Flu: 3-hydroxyfluorene; 2-OH-Flu: 2-hydroxyfluorene; 2-OH-Phe: 2-hydroxyphenanthrene; 3-OH-Phe: 3-hydroxyphenanthrene; 1/9-OH-Phe: 1/9-hydroxyphenanthrene; 1-OH-Pyr: 1-hydroxypyrene; 6-OH-Chr: 6-hydroxychrysene; 3-OH-BaP:3-hydroxybenzo(a)pyrene. $\sum_{16}$PAH: sum of 16 PAHs; $\sum_{12}$OH-PAH: sum of 12 OH-PAHs.

2015; Yu et al., 2020).

The (non-lipid adjusted) Σ_{16}PAH (sum of the concentrations of 16 PAH isomers) in serum ranged from 0 to 49.7 ng mL^{-1}, with a median of 4.05 ng mL^{-1} (Table 3). Among the detected isomers, Pyr had the highest median concentration (1.64 ng mL^{-1}) and was also the isomer with the highest detection frequency. Its metabolite is often used as a biomarker of human exposure to PAHs (Morgan et al., 2015; Mucha et al., 2006). Generally, lower molecular weight isomers are found in higher concentrations in serum. However, it is likely that the serum concentrations of PAHs and their metabolite do not depend solely on the concentrations of the PAHs in environmental matrices such as food, air, and house dust; their different rates of metabolism are also probably important. PAHs are relatively easily metabolized substances with short half-lives (Morgan et al., 2015). Isomers with low molecular weights are first metabolized into water-soluble metabolites of glucuronic acid conjugates, which are then excreted in urine, whereas those with higher molecular weights have longer half-lives and are excreted mainly in feces (Li et al., 2008; Marie et al., 2010; Ramesh et al., 2004).

In the present study, 12 OH-PAH isomers were determined in paired urine samples. The median value of Σ_{12}OH-PAH (the summed concentrations of 12 OH-PAH isomers) in the urine samples was 8.33 ng mL^{-1}, with a range of 0.26–310 ng mL^{-1}, which was much higher than the corresponding serum concentrations. Of the individual OH-PAHs, 2-OH-Nap showed the highest concentration with a median of 3.16 ng mL^{-1}, followed by 1-OH-Nap (1.53 ng mL^{-1}). 1-OH-Pyr, which has been used as a biomarker of human exposure to PAHs, exhibited a median concentration of 0.14 ng mL^{-1}, which is more than an order of magnitude lower than the concentrations of OH-Nap. 1-OH-Pyr also had a lower concentration than ∑OH-Flu (0.72 ng mL^{-1}) and ∑OH-Phe (0.36 ng mL^{-1}). Similar results have been reported in analyses of urine samples from seven Asian countries (Qin et al., 2011), in samples from children in Shenzhen (Yu et al., 2020), and in a study of 222 children in Manhattan (New York, USA) (Oliveira et al., 2019). Whatever the rate of Pyr metabolism, human exposure to this compound can be very significant. For example, the concentrations of 1-OH-Pyr in urine from coke oven workers and residents living in an E-waste dismantling area were reportedly as high as 13.5 and 20 ng mL^{-1}, respectively (Kuang et al., 2013; Lu et al., 2016).

The relationships between parent PAHs in serum and metabolized OH-PAHs in urine were also analyzed. However, no correlation was found between the total amount of OH-PAHs in urine and that of the corresponding parent PAHs in serum. When considering individual compounds, the detection frequencies of 3-OH-BaP and 6-OH-Chr were too low to analyze. There were also no consistent trends among the other compounds. For example, there were significant correlations between Phe and five OH-Phe isomers ($r_p = 0.263$, $p < 0.01$) (Fig. S1), but no significant correlations for the other chemicals. These results may be due to the different metabolic rates of PAHs in the human body, the different velocities of PAHs entering the body via different routes, and the different bioaccumulation characteristics of PAHs (Zhang et al., 2014). For example, PAHs absorbed via inhalation may reach systemic circulation faster than those absorbed via oral ingestion because PAHs ingested orally must pass through the gastrointestinal system and liver (where they may be metabolized) before entering the systemic circulation. Moreover, PAH metabolites are not only excreted in the urine; they can also be excreted via routes such as the feces, in which case they will not necessarily be converted into hydroxyl derivatives. Some may even form DNA adducts or be excreted as prototypes (Li et al., 2008; Tang et al., 2006; Motorykin et al., 2015).

3.3. Composition profiles of PAHs and OH-PAHs

The compositions of PAHs in serum and OH-PAHs in urine samples are shown in Fig. 1. The proportion of low molecular weight PAHs (88.4%) in serum was much higher than that of high molecular weight PAHs (11.6%). Pyr (83.6%) was the dominant compound. Similar results were obtained in analyses of umbilical cord serum by Yin et al. (2017), who found that the proportion of low molecular weight PAHs was higher than that of high molecular weight isomers, Pyr (43.3%) was also the main component in that case. Ye et al. (2020) similarly found that Pyr accounted for a relatively high proportion of total serum PAHs. Interestingly, the PAH profiles of the serum samples varied between districts (Fig. 1A): NS and ZC had similar composition profiles, whereas TH and HD had different profiles. However, although TH and NS represent urban areas and ZC and HD are classified as suburbs in administrative terms, TH is in the center of the city whereas NS and ZC are just outside the city center and HD is in the outer suburbs. Moreover, the main industry in HD's sampling sites is leather working, and there are many tanneries around it. The differences in serum PAH profiles may thus reflect differences in the living environments of the sampled individuals.

OH-Nap (i.e., the summed concentrations of 1-OH-Nap and 2-OH-Nap) was the most abundant OH-PAH in the urine samples, accounting for 85.1% of the totals, although the relative abundance of 2-OH-Nap (57.4%) was almost twice that of 1-OH-Nap (27.8%) (Fig. 1B). 2-OH-Flu accounted for approximately 5.9% of total OH-PAHs, while the proportion of 3-OH-Flu was negligible given that its detection rate was

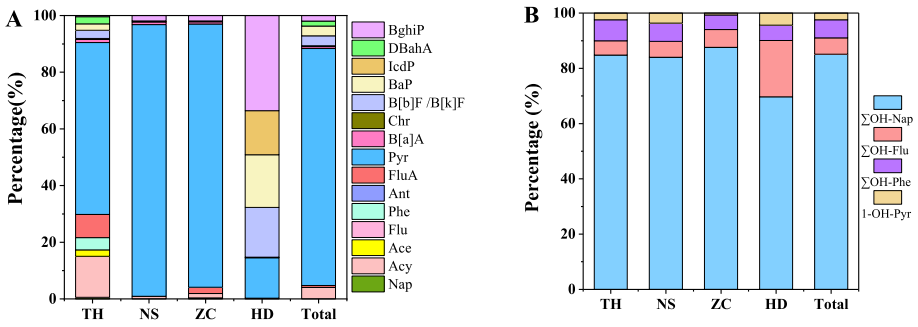

Fig. 1. Composition profiles of PAHs in serum and OH-PAHs in urine from four districts in Guangzhou, South China.

below 50%. The five OH-Phe isomers accounted for 6.5% of the total OH-PAHs. Similar results have been reported previously (Guo et al., 2013; Yu et al., 2020). In contrast to the composition of PAHs in serum, 1-OH-Pyr accounted for only 2.4% of total OH-PAHs. The differences between the composition profiles of PAHs in serum and OH-PAHs in urine are probably largely due to the differing rates of metabolism of the PAH isomers. It is harder to explain why there were no obvious regional differences in the urinary OH-PAH profiles, but this might reflect differences in the metabolism of different PAH isomers and the excretion routes of their metabolites. Moreover, the available data on the excretion rates of PAHs suggest that there can be delays of up to 6–35 h between environmental/occupational exposure to PAHs and urinary excretion of major metabolites (Oliveira et al., 2016). As mentioned above, metabolites of high molecular weight PAHs are mainly detected in feces (Marie et al., 2010; Ramesh et al., 2004). In keeping with this result, low molecular weight PAHs accounted for 99.7% of the total OH-PAHs detected in the urine samples examined in this work. This implies that estimates of human PAH exposure and the associated health risks based solely on measurements of urinary OH-PAHs may be inaccurate. Further work is needed to clarify the impact of this issue.

3.4. Factors associated with human exposure to PAHs and OH-PAHs

Regional differences ($p < 0.05$) were observed in the concentrations of Σ_{16}PAH (Fig. 2A). However, levels of the most abundant PAH isomer, Pyr, exhibited no significant regional differences ($p > 0.05$). Σ_{12}OH-PAH was significantly higher in ZC and HD than at the other sites ($p < 0.05$). The most abundant metabolite in urine, OH-Nap, also exhibited significant regional differences in concentration ($p < 0.05$), as did the biomarker OH-Pyr. For PAHs, the Σ_{16}PAH of HD differed markedly from that for the three other areas. High molecular weight PAHs accounted for 85.5% of the total PAHs in this area but for less than 20% of the total in other areas. This may indicate that residents in HD suffered higher exposure to high molecular weight PAHs than residents in the other

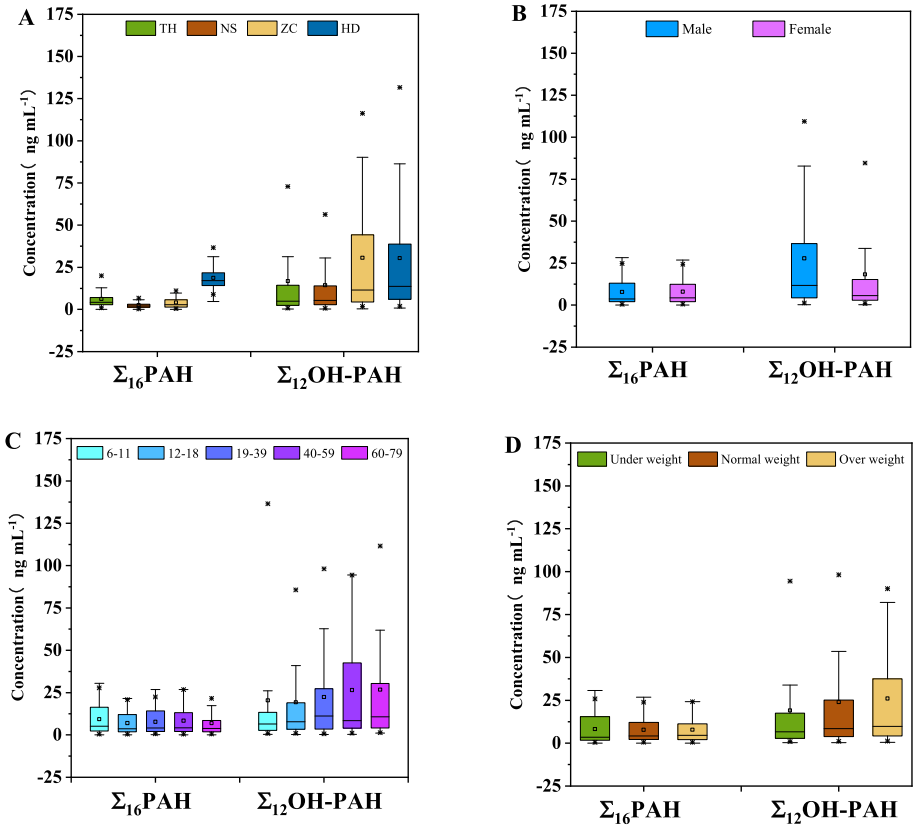

Fig. 2. Concentrations of 16 PAHs in serum and 11 OH-PAHs in urine for different district (A), gender (B), age (C) and BMI (D) groups in Guangzhou. The upper and lower limits of each box denote the 25% and 75% quartiles, whereas the open square symbols represent the median value. The horizontal lines within the boxes show the mean value. The whiskers extend to 1.5 times the quartile range of the last observation.

areas. This trend was also reflected in the urinary OH-PAH concentrations. High OH-PAH concentrations were also observed in ZC; this may be because HD and ZC are located in the suburbs of Guangzhou, whose economy is relatively underdeveloped. Consequently, the living habits of people in these areas may differ from those of people living in the other two areas, and the environmental protection infrastructure may be worse.

There was no significant difference in Σ_{16}PAH between males and females (Fig. 2B). However, males had significantly higher concentrations of urinary OH-PAHs than females, mainly due to high levels of OH-Nap (median: 5.99 vs. 4.14 ng mL^{-1}). Similar trends were observed for OH-Flu (males: 1.35 ng mL^{-1}; females: 0.48 ng mL^{-1}), OH-Phe (males: 0.40 ng mL^{-1}; females: 0.33 ng mL^{-1}), and 1-OH-Pyr (males: 0.15 ng mL^{-1}; females: 0.10 ng mL^{-1}). Although significant sex differences were not observed in previous studies (Guo et al., 2013; Thai et al., 2016; Yu et al., 2020), there is some evidence of sex-related differences. For example, a study on the general population of Korea concluded that gender was a major factor for predicting urinary 1-OH-Pyr concentrations (Sul et al., 2012). The higher levels of urinary OH-PAHs in males may be due to smoking or secondary smoking exposure; the smoking rate for males among the study participants was 28.3%, compared to only 2.9% for females. There were also statistically significant differences in the levels of Σ_{12}OH-PAH, OH-Nap, OH-Flu, OH-Phe, and OH-Pyr between smoking and non-smoking males. Studies have shown that low molecular weight PAHs are major components of tobacco smoke (Ding et al., 2005). However, it should be noted that the concentrations of OH-PAHs were not adjusted on the basis of creatinine levels in this work, which may partly explain the observed gender differences.

Age affects PAH levels in the human body primarily via its effects on metabolic capacity and lifestyle. No statistically significant differences ($p > 0.05$) in serum Σ_{16}PAH levels were observed between age groups in this work (Fig. 2C), and no significant age differences were observed in the serum concentrations of individual PAHs either. There were also no significant differences ($p > 0.05$) between age groups with respect to Σ_{12}OH-PAH in urine, but there was a distinct parabolic trend with increasing age. However, significant differences were observed between OH-Nap and OH-Phe among the age groups. In particular, Σ_{12}OH-PAH levels in the 19–59 age group were higher than in children, teenagers, and the elderly (>60 years). This is similar to the findings of a previous study in Australia (Thai et al., 2016, 2020), which showed that the concentration of OH-PAHs was higher in adolescents and adults (15–59 years) than in the elderly (>60 years), children (5–14 years), and infants (0–4 years). The same age-dependence was also reported by CHMS II (Canada, 2013). However, this does not mean that OH-PAHs concentrations increase with age; it is known that PAHs are easily metabolized compounds that are excreted via the urine as OH-PAHs after consumption, with a half-life of about 6–35 h (Jongeneelen et al., 1990; Huang et al., 2007), indicating that rapid metabolism is more important than storage in vivo. People in different age groups may have different living habits, diets, contact environments, exposure sources, and metabolic rates. For example, a study in the United States showed that the smoking rate of young and middle-aged people is higher than that of other age groups (Bortey-Sam et al., 2017).

As shown in Fig. 2D, there were no statistically significant differences ($p > 0.05$) in Σ_{16}PAH in serum among the BMI groups, but there were significant differences ($p = 0.04 < 0.05$) in Σ_{12}OH-PAH in urine. In particular, Σ_{12}OH-PAH in the overweight group were much higher than in the other two groups. Significant differences in individual PAHs, e.g., 2-OH-Flu, 1-OH-Nap, 2-OH-Nap, 2-OH-Phe, and 1/9-OH-Phe, were observed among the BMI groups. Poursafa et al. (2018) suggested that exposure to 2-OH-Nap, 9-OH-Phe, and Σ_{12}OH-PAH is associated with an increased risk of obesity. Although this conclusion lacks verification based on creatinine correction, it is well known that dietary exposure is a major route of PAH exposure and that because PAHs are lipophilic, obesity may influence urinary Σ_{12}OH-PAH levels (Peng et al., 2020).

3.5. Human exposure and health risk assessment

To assess differences in human PAH exposure based on concentrations of PAHs in serum and OH-PAHs in urine, TEDI values were calculated for Pyr in serum and 1-OH-Pyr in urine (Table 4). The TEDI values for Pyr ranged from 0 to 3.11 and from 0 to 5.37 μg/kg-bw/day based on the concentrations of Pyr in serum and 1-OH-Pyr in urine, respectively. Although the highest TEDI of Pyr in urine was higher than that in serum, the median and mean TEDI values calculated from serum were all higher than those from urine (0.319 vs. 0.067 and 0.357 vs. 0.175, respectively). It thus seems that the TEDI of Pyr may be underestimated if determined on the basis of urinary 1-OH-Pyr levels. Specifically, when comparing the median and mean TEDI values from urinary 1-OH-Pyr to those obtained based on Pyr in serum, it appears that daily human exposure to Pyr may be underestimated by at least 79.0% and 51.0%, respectively, when using urinary data. Additionally, some amount of the ingested Pyr is metabolized in the serum. Therefore, TEDI values calculated based on serum concentrations will also underestimate true exposure levels. It thus appears that there is an urgent need for a more accurate way of estimating Pyr exposure.

To understand the health risks of PAH exposure, HQ and CR values were calculated to assess the carcinogenic and non-carcinogenic risks based on levels of Pyr in serum and 1-OH-Pyr in urine (Fig. 3). The median HQ value based on urine samples was 0.0015, which was approximately 13.6% of that calculated from serum Pyr (HQ: 0.011). All of the HQ values were below unity, suggesting that human exposure to PAHs does not pose a non-carcinogenic risk. However, the carcinogenic risk estimates indicated that PAH exposure does present an important health risk: the CR values of Pyr estimated based on serum concentrations ranged from 0 to 2.27×10^{-5} while those based on urinary OH-PAHs ranged from 0 to 3.92×10^{-5}, with means of 2.61×10^{-6} and 1.28×10^{-6}, respectively. The median CR of Pyr was 2.23×10^{-6} and 4.88×10^{-7} based on serum and urinary data, respectively. Additionally, 85.4% and 28.3% of the participants exceeded the acceptable cancer risk level of 10^{-6} based on CR values derived from serum and urine sampling, respectively. The HQ and CR values obtained from urine were far lower than those from serum. Therefore, analyses of urine samples may severely understate the health risk due to PAH exposure. Given the high carcinogenic risks of PAHs, further studies on this issue are needed.

3.6. Uncertainty in risk assessment

It is important to note that there are some important sources of uncertainty in the assessment of human PAH exposure and the resulting health risks. Firstly, PAHs can be metabolized in the human body, and PAH levels in serum do not completely reflect daily exposure to PAHs. For OH-PAHs in urine, there is also considerable uncertainty regarding the ratio of OH-PAHs excreted in the urine to the total exposure dose, as shown previously. For example, Chien and Yeh (2010) reported that the excretion rate of Pyr after dietary exposure was 2%–3%, whereas Viau et al. (2002) reported a range of 2.9%–4.5%. In the present study, the excretion rate of Pyr was estimated to be 6.8% (Li et al., 2012; Peng et al., 2020). This implies that variation in the excretion ratio could affect the calculated PAH exposure by as much as a factor of two. Additionally, some isomers were not considered in the estimates because

Table 4
Total estimated daily intake (TEDI, μg/kg-bw/day) of Pyr estimated based on the concentrations of Pyr in serum and 1-OH-Pyr in urine.

Parent	Metabolite	P25	Median	P75	Mean	Range
Pyr		**Serum**				
		0.200	0.319	0.461	0.357	0–3.113
Pyr	1-OH-Pyr	**Urine**				
		0.026	0.067	0.154	0.175	0–5.37

Pyr: pyrene.

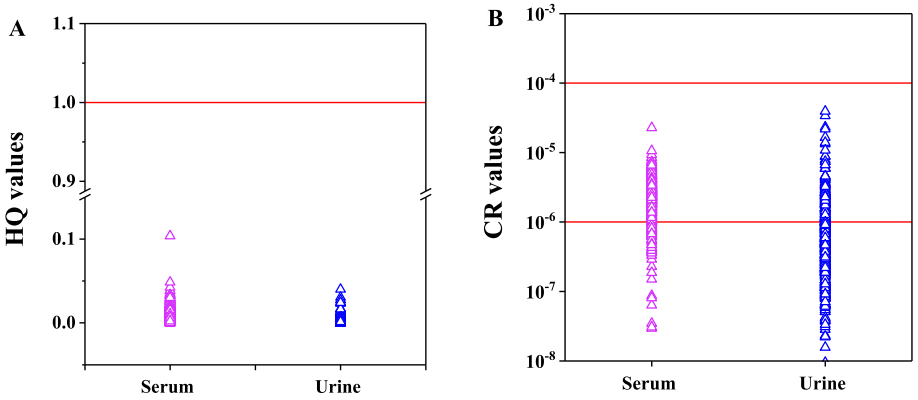

Fig. 3. Individual hazard quotient (HQ) and cancer risk (CR) of Pyr in serum and 1-OH-Pyr in urine samples from Guangzhou, China (HQ of less than 1 to be an indication of no significant potential health risk, an acceptable risk level of total carcinogenic risks in the range of 1×10^{-6} to 1×10^{-4}, and the risk lower than 1×10^{-6} considered negligible).

their detection rates were very low (e.g., BaP has an excretion rate of 0.07%) (Payan et al., 2009), which would lead to underestimation. Furthermore, the measured OH-PAH concentrations were not adjusted based on creatinine levels, which also affects the concentrations used for exposure assessment and associated health risks. Second, the RfD (US EPA reference dose) values were derived from long-term oral exposure (Bulder et al., 2006), whereas the results in the present study reflect all exposure pathways. Similarly, the CSF (carcinogenic slope factor) values of different exposure pathways are different. For example, the CSF values of BaP for oral intake, inhalation, and dermal contact exposure were estimated to be 7.3, 3.85, and 25 $(mg/kg\text{-}bw/day)^{-1}$, respectively (Vicente et al., 2019).

Despite these issues, the health risk results presented herein suggest that exposure to Pyr among the sampled population is high using the data of 7.3 $(mg/kg\text{-}bw/day)^{-1}$ considering BaP equivalent. In addition, due to the various possible mixtures of PAHs, there is always some uncertainty in assessments of their toxicity. For example, when using the TEF method of the European Food Safety Agency (EFSA) to evaluate the risk of PAHs, the relevant compounds are required to exert their toxicological effects via the same mechanism of action. However, PAHs can act via multiple carcinogenic pathways in the body, including binding to aryl hydrocarbon receptors and DNA. Moreover, there is no evidence that different PAHs are activated by the same metabolic pathway. Larsen et al. (1998) concluded that the carcinogenicity (expressed in BaP equivalents) of coal tar mixtures calculated using TEF values was underestimated 3.3-fold when compared to their actual observed carcinogenicity. Similarly, the carcinogenicity of PAHs might be underestimated by TEF method. In particular, variation in the relative abundance of different PAHs or types of PAHs in a given mixture may profoundly impact carcinogenicity due to differences in the compounds' bioavailability, binding site interactions, and carcinogenic properties. In general, the usefulness of TEF for estimating the carcinogenicity of PAHs may be limited. Underestimation of carcinogenicity and exposure may also occur because some isomers, especially low molecular weight PAHs, are mainly taken up by inhalation. Further studies are therefore needed to find better ways of assessing human PAH exposure and the associated health risks.

4. Conclusion

The concentrations of parent PAHs in serum and of their hydroxylated metabolites in urine were determined in matched pair samples collected from the general population of four districts in Guangzhou, South China. This is the first time that such a paired biological sample study has been conducted on PAH exposure. The target chemicals had high detection frequencies in serum and urine samples, demonstrating the ubiquitous exposure of humans to these pollutants. Although there were no significant correlations between the concentrations of total PAHs in serum and total OH-PAHs, significant correlations were found for Phe. There were also regional differences for both the serum and urine samples. PAHs levels in serum did not vary appreciably with gender, age, or BMI, but significant differences in urinary OH-PAH levels were observed based on gender and BMI. Health risk assessments revealed an appreciable carcinogenic risk due to PAH exposure in the studied population, but HQ values below unity were obtained for non-carcinogenic effects. Overall, the results obtained indicated that the risks of human exposure to PAHs (and Pyr in particular) may be underestimated if exposure is estimated based only on levels of OH-PAHs in urine.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.117975.

Author statement

Ziying Yang: Methodology, Formal analysis, and draft preparation. Chongshan Guo: Design and methodology. Qin Li: Sampling, Formal analysis. Yi Zhong: Design and sampling. Shengtao Ma: Methodology. Jinhua Zhou: Sampling. Xiaotong Li: Methodology. Rende Huang: Methodology. Yingxin Yu: Design, reviewing & editing.

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