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Variability in urinary biomarkers of human exposure to polycyclic aromatic hydrocarbons and its association with oxidative stress

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. Urinary concentrations of mono-hydroxylated metabolites of PAHs (OH-PAHs) have been used as biomarkers of these chemicals' exposure in humans. Little is known, however, with regard to intra- and inter-individual variability in OH-PAH concentrations and their association with oxidative stress. We conducted a longitudinal study of measurement of urinary concentrations of 15 OH-PAHs and 7 oxidative stress biomarkers (OSBs) of DNA damage [8-hydroxy-2′-deoxyguanosine (8-OHdG)], lipid [malondialdehyde (MDA) and F_2-isoprostanes ($PGF_{2\alpha}$)] and protein [*o,o′* -dityrosine (diY)] peroxidation in 19 individuals for 44 consecutive days. Metabolites of naphthalene (OHNap), fluorene (OHFlu), phenanthrene (OHPhe), and pyrene (OHPyr) were found in >70% of 515 urine samples analyzed, at sum concentrations (∑OH-PAH) measured in the range of 0.46–60 ng/mL. After adjusting for creatinine, OHNap and ∑OH-PAH concentrations exhibited moderate predictability, with intra-class correlation coefficients (ICCs) ranging from 0.359 to 0.760. However, ICC values were low (0.001–0.494) for OHFlu, OHPhe, and OHPyr, which suggested poor predictability for these PAH metabolites. Linear mixed-effects analysis revealed that an unit increase in ∑OH-PAH concentration corresponded to 4.5%, 5.3%, 20%, and 21% increase in respective urinary 8-OHdG, MDA, $PGF_{2\alpha}$, and diY concentrations, suggesting an association with oxidative damage to DNA, lipids, and proteins. The daily intakes of PAHs, calculated from urinary concentrations of OH-PAHs, were 10- to 100-fold below the current reference doses. This study provides valuable information to design sampling strategies in biomonitoring studies and in assigning exposure classifications of PAHs in epidemiologic studies.

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CRediT authorship contribution statement

Hongkai Zhu: Data curation, Formal analysis, Writing - original draft. **Maria-Pilar Martinez-Moral:** Formal analysis, Writing - review & editing. **Kurunthachalam Kannan:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106720.

Keywords

Hydroxy polycyclic aromatic hydrocarbons; Biomonitoring; Variability; Intra-class correlation coefficients; Oxidative stress biomarkers

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants, which are formed from incomplete combustion of fossil fuel and crop residues (Kim et al. 2013). Human exposure pathways to PAHs include diet, inhalation and dermal contact (Domingo and Nadal 2015; Fernando et al. 2016; Lao et al. 2018). Following exposure, PAHs are metabolically transformed into mono-, di-, and tetrahydroxylated metabolites (OH-PAHs) in human bodies (Carmella et al. 2004; Klotz et al. 2011). Among these metabolites, mono OH-PAHs are used as biomarkers of PAHs exposure through the analysis of specimens such as urine, plasma, breast milk, and hair (Guo et al. 2013; Lin et al. 2020; Oliveira et al. 2020; Sun et al. 2020; Zhu et al. 2019).

PAHs have short biological half-lives ($t_{1/2}$), on the order of a few hours, in human bodies. Li et al. (2012) reported $t_{1/2}$ of 10 mono OH-PAHs in the range of 2.5–6.1 h. It is presumed that the urinary concentrations of OH-PAHs vary depending on the time elapsed between exposure and sample collection. Due to the episodic nature of exposure and variable pharmacokinetics, measurements of OH-PAHs made in a single spot urine may reflect a recent exposure, and may not represent an integrated exposure over time. Several biomonitoring studies have measured OH-PAH concentrations in spot urine or first morning void samples (Guo et al. 2018; Urbancova et al. 2017; Wang et al. 2019b). For those chemicals that have temporal variations in exposure, analysis of a single spot urine sample to generalize long-term exposure will lead to exposure misclassification and, consequently, biased dose-response functions. Yet, there is little information available on intra-individual variability in urinary OH-PAH concentrations in non-occupationally exposed populations. Li et al. (2013) reported intra-individual variability in OH-PAH concentrations measured in spot, first-morning void, and 24-h composite urine samples collected from 8 non-occupationally exposed adults and showed that simulated 24-h composite samples provided least variable measure of exposure. However, that study was conducted over a duration of less than one week with a sample size of 8 individuals.

PAHs are endocrine disrupters, reproductive toxicants, neurotoxicants and carcinogens (Madeen and Williams 2017; Yin et al. 2017). Long-term exposure to PAHs has been linked to lung, skin and bladder cancers and diabetes (Uppstad et al. 2011; Ramesh et al., 2011). The biological mechanism of PAH toxicity is not clear, but these chemicals have been shown to elicit oxidative damage, which can alter the balance between reactive oxygen species (ROS) production and antioxidant defense. The excess ROS could target biological molecules such as DNA, proteins and lipids, resulting in their oxidation. Several oxidative stress biomarkers (OSBs) have been identified as the products of these oxidation processes. Studies have examined the association of oxidative stress with PAH exposures (Bortey-Sam

et al. 2017; Cao et al. 2020b). Nevertheless, the earlier studies have measured only a limited number of OSBs or select PAH metabolites.

To address the knowledge gaps identified above, 515 urine samples collected consecutively from 19 healthy individuals for over a 44-day study period, were analyzed for a panel of 15 mono OH-PAHs. Seven OSBs, namely, *o,o′*-dityrosine (diY), 8-hydroxy-2′-deoxyguanosine (8-OHdG), malondialdehyde (MDA), and four F_2-isoprostane isomers (i.e., 8-isoprostaglandin$F_{2\alpha}$ [8-$PGF_{2\alpha}$], 11β-prostaglandin$F_{2\alpha}$ [11-$PGF_{2\alpha}$], 15 (*R*)-prostaglandin$F_{2\alpha}$ [15-$PGF_{2\alpha}$], and 8-iso,15(*R*)-prostaglandin$F_{2\alpha}$ [8,15-$PGF_{2\alpha}$]) were also measured in the same set urine samples, as reported earlier (Martinez-Moral and Kannan 2019). The aims of this study were to describe intra-individual variability in OH-PAH concentrations in urine collected from 19 individuals consecutively for up to 44 days and to investigate the association between urinary OH-PAH concentrations and OSBs on a temporal basis. To the best of our knowledge, this is the first longitudinal study to elucidate the link between PAHs exposure and multiple OSBs measured in urine.

2. Materials and methods

2.1. Standards and reagents

Fifteen mono OH-PAH standards, namely two hydroxynaphthalenes (1- and 2-Nap), five hydroxyphenanthrenes (1-, 2-, 3-, 4-, and 9-Phe), three hydroxyfluorenes (2-, 3-, and 9-Flu), hydroxypyrene (1-Pyr), two hydroxychrysenes (1- and 6-Chr), 3-hydroxybenzo-[*c*]-phenanthrene (3-BcP), and 1-hydroxybenz[*a*]-anthracene (1-BaA) were purchased from Sigma-Aldrich (purity ≥ 97% for all standards; St. Louis, MO, USA) or AccuStandard (purity ≥ 97%; New Haven, CT, USA). Ten isotopically labeled internal standards, $^{13}C_{12}$-1-Nap, $^{13}C_{12}$-2-Nap, $^{13}C_{12}$-1-Phe, $^{13}C_{12}$-2-Phe, $^{13}C_{12}$-3-Phe, $^{13}C_{12}$-6-Chr, $^{13}C_{12}$-2-Flu, $^{13}C_{12}$-1-Pyr, $^{13}C_{12}$-3-BcP, and $^{13}C_{12}$-1-BaA, were purchased from Cambridge Isotope Laboratories (purity ≥ 99% for all; Andover, MA, USA). High performance liquid chromatography (HPLC)-grade water, methanol, toluene, and pentane were purchased from J.T. Baker (Center Valley, PA, USA), Fisher Scientific (Waltham, MA, USA), Avantor Performance Materials (Phillipsburg, NJ, USA), and Sigma-Aldrich (St. Louis, MO, USA), respectively. BGALA-RO β-glucuronidase from *E. coli* K12 liquid enzyme (activity: 140 units/mg protein) was purchased from Roche Diagnostics GmbH (Mannheim, Germany).

2.2. Sample collection

Urine samples were collected from 19 healthy volunteers from Albany area of New York State, USA, in 2018. Demographic information [e.g., gender, age, body mass index (BMI), and ethnicity] and lifestyle variables (e.g., alcohol use, smoking status, exercise frequency, and dietary supplement use) were collected (Table 1). Of the 19 participants, 11 were males and 8 were females; 13 were Asians and 6 were Caucasians. The average age was 34 years (range: 11–56 years); mean BMI, height, and body weight were 24 kg/m^2 (19–36 kg/m^2), 168 cm (145–181 cm) and 68 kg (40–108 kg), respectively. None of the participants were active smokers and were rarely or occasionally consumed alcohol (14 rare vs 5 occasional); 11 participants reported regular exercise daily and 10 used dietary supplements.

Urine samples were collected for 44 consecutive days during the period of February to April 2018, in 50 mL polypropylene (PP) tubes, stored immediately in a refrigerator, and then transferred to a freezer at −20 °C within few hours of collection. Samples were collected on a daily basis, in most cases, but, for some individuals (due to travel and other logistical issues), the collection interval ranged from 2 to 18 days. A total of 515 urine samples, comprising 272 spot urine (SU) and 243 first morning voids (FMV) were collected (see Table S1 in the Supporting Information for details; SI). From each volunteer an average of 27 urine samples (13–40 samples per individual) were analyzed. Samples were anonymized prior to analysis, and the study was approved by the Institutional Review Board of the New York State Department of Health. These urine samples were analyzed for a wide range of environmental chemicals including pesticides, melamine, and organophosphate esters previously (Li et al. 2019b; 2020; Martinez-Moral and Kannan 2019; Wang et al. 2019a; Zhu and Kannan 2019).

2.3. Chemical analysis

The concentrations of OH-PAHs in urine samples were determined using an isotope dilution method described previously (Zhu et al. 2019), with minor modifications. Briefly, following the addition of labeled internal standards (2.5 ng for each compound), an aliquot of 0.5 mL of urine was buffered with 0.5 mL of 1 M ammonium acetate buffer (pH = 5.5) containing 10 μL of β-glucuronidase. Glucuronide conjugates of OH-PAHs were enzymatically deconjugated through incubation overnight at 37 °C, and extracted with 3 mL of pentane/toluene (4:1; v/v) twice. The mixture was shaken in an orbital shaker for 60 min and centrifuged at 3500 × g for 20 min. The supernatants were combined in a clean glass tube, purified with 1 mL of HPLC-grade water, concentrated to near-dryness, and re-dissolved in 0.25 mL of methanol:water mixture (1:1, v/v) for instrumental analysis. An ABSciex 5500 tandem mass spectrometer (MS/MS; Applied Biosystems, Foster City, CA, USA) connected to a Shimadzu LC-30AD Series HPLC (Shimadzu Corporation, Kyoto, Japan) was used for identification and quantification of the target analytes. An analytical column, Zorbax Eclipse Plus C18 (100 mm × 4.6 mm, 3.5 μm; Agilent Technologies, Santa Clara, CA, USA) was used for chromatographic separation. The mobile phase consisted of methanol and HPLC-grade water pumped at a flow rate of 0.2 mL/min. Due to co-elution, concentrations reported for 1/9-Phe and 2/3/9-Flu were the sum of 1- and 9-Phe and 2-, 3-, and 9-Flu, respectively. The sum concentrations of 15 OH-PAHs, 1- and 2-Nap, and 1/9-, 2-, 3-, and 4-Phe are denoted as ΣOH-PAH, ΣNap, and ΣPhe, respectively. The concentrations of creatinine and seven OSBs in the same set of urine samples were determined by HPLC-MS/MS as reported in our previous study (Martinez-Moral and Kannan 2019) and the details are presented in Texts S1–S2 and Table S2 in the SI.

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

Each analytical batch included analyses of a procedural blank (HPLC-grade water used in place of urine), matrix-spiked sample (10 ng/mL of each analyte spiked into urine), and two standard reference materials (SRMs 3672 and 3673; purchased from the National Institute of Standards and Technology, Gaithersburg, MD, USA). Trace levels of 1/9-Phe (mean: 0.026 ng/mL) were found in procedural blanks, and a background subtraction was performed for this analyte to report sample concentrations. Acceptable recoveries of target

compounds, ranging from 81 to 101% and from 76 to 95%, were found in matrix-spiked sample and SRMs, respectively. A total of 30 urine samples were analyzed in duplicate for the evaluation of method precision. The relative standard deviation (RSD) of replicate analyses of samples was < 15% for all OH-PAHs. The limit of detection (LOD) ranged from 0.003 ng/mL (3-Phe) to 0.48 ng/mL (2/3/9-Flu) (Table 2).

2.5. Data analysis

All statistical analyses were performed using SPSS ver. 25 (SPSS Inc., Chicago, IL, USA). Concentrations below the LOD were substituted with a value of LOD divided by square root of 2. Due to the skewed distributions of measured values (as determined by Shapiro-Wilk test), urinary OH-PAH and OSB concentrations were log-transformed prior to statistical analysis. Differences in concentrations across sample/chemical categories were tested using linear models, with potential covariates as independent variables and OH-PAH concentrations as dependent variables. Linear mixed-effects analysis, after adjusting for confounding factors (e.g., gender, age, BMI, ethnicity, and lifestyle characteristics), was used to determine associations between OH-PAH and OSB concentrations in urine. The statistical significance was set at $p < 0.05$.

Intra-class correlation coefficients (ICCs, a ratio of between-individual variance to the sum of between- and within-individual variances) were calculated as a measure of predictability of OH-PAH concentrations over time (Wu et al. 2010). Between- and within-individual variances were calculated using one-way random effect model. To evaluate the effect of demographic and life style variables on OH-PAH concentrations, ICC calculations were stratified by gender, age, BMI, ethnicity, sample type (SU or FMV), and lifestyle factors. The ICC values were categorized as excellent (ICC ≥ 0.75), moderate (0.75 > ICC ≥ 0.40), and poor (ICC < 0.40) for the predictability of OH-PAH concentrations between repeated samples from the same individual (Morgan et al. 2018).

On the basis of the measured urinary OH-PAH concentrations, daily intakes (DIs) of naphthalene (Nap), phenanthrene (Phe), fluorene (Flu), and pyrene (Pyr) were estimated using the following equation:

$$\mathrm{DI} = C \times V \times \frac{\mathrm{M}_1}{\mathrm{M}_2} \times \frac{1}{f}$$

where DI is the daily intake of PAH (μg/day), C is the urinary OH-PAH concentration (μg/L), V is the daily urine excretion volume (L/day) (presumed at 2.0 L), M_1 and M_2 are the respective molecular weights of parent PAH and its metabolite (g/mol), and f is the ratio of OH-PAH excreted in urine relative to exposure of its parent compound. A pharmacokinetic study reported that 100%, 60%, 11%, and 6.8% of ingested Nap, Flu, Phe, and Pyr, respectively, were excreted in human urine (Li et al. 2012) and these values were used for 'f'.

3. Results and discussion

3.1. Urinary concentrations of PAH metabolites

Of the 15 target PAH metabolites determined, 2-Nap and 2-Phe were found in 100% of the urine samples, which were followed by 1/9-Phe (94%), 3-Phe (85%), 1-Pyr (85%), 2/3/9-Flu (82%), 1-Nap (79%) and 4-Phe (72%). Metabolites of chrysene, benz[*a*]anthracene, and benzo[*c*] phenanthrene (1-Chr, 6-Chr, 1-BaA, and 3-BcP) were rarely detected in urine samples (<3% of the samples). This may be due to the fact that these high molecular-weight (HMW) PAHs are excreted mainly through feces rather than in urine (Li et al. 2008). Thus, urine may not be the suitable matrix for the assessment of exposure to HMW PAHs.

The mean, median, and range of urinary concentrations of OH-PAHs as uncorrected (expressed in ng/mL) and creatinine-corrected (μg/g creatinine) values are displayed in Table 2. Urinary ∑OH-PAH concentrations ranged from 0.46 to 60 ng/mL (0.59–110 μg/g creatinine), with an overall median value of 7.8 ng/mL (5.7 μg/g creatinine). 2-Nap was the most abundant compound found at a median concentration of 2.6 ng/mL, followed by 2/3/9-Flu (1.3 ng/mL), and 1-Nap (0.66 ng/mL). 2-Nap exposure arises primarily from the use of known naphthalene sources such as mothballs, burning of wood and fossil fuel and tobacco smoke (Bortey-Sam et al. 2017). 1-Nap exposure can also arise from carbamate insecticide, carbaryl, herbicide, napropamide, and the beta-blocker propranolol (Li et al. 2016). Meeker et al. (2007) suggested that the ratio of 1-Nap to 2-Nap > 2 was indicative of exposure to sources such as carbaryl. In our study, 68 of 515 urine samples showed 1-Nap/2-Nap ratio > 2; especially most of the urine samples provided by two Asian males had 1-Nap/2-Nap ratios > 2.

Among the four OH-Phe isomers analyzed, 3-Phe was the abundant one, with a median concentration of 0.44 ng/mL, followed by 1/9-Phe (0.21 ng/mL), 2-Phe (0.15 ng/mL), and 4-Phe (0.04 ng/mL). 1-Pyr is a commonly used urinary biomarker of PAHs exposure. The urinary concentrations of 1-Pyr measured in this study (median: 0.38 ng/mL; 0.28 μg/g creatinine) were comparable to those reported from Poland (median: 0.36 μg/g creatinine) (Sochacka-Tatara et al. 2018), but higher than those reported from the United States (mean/median: 0.11–0.13 ng/mL) (CDC 2019), Canada (mean/median: 0.09–0.11 ng/mL) (Health Canada 2017), Czech Republic (median: 0.18 μg/g creatinine) (Urbancova et al. 2017), and Australia (0.06–0.14 ng/mL) (Thai et al. 2020; Thai et al. 2015), and an order of magnitude lower than those reported from China (mean/median: 3.1–6.4 μg/g creatinine) (Cao et al. 2020a; Li et al. 2015a).

The reported urinary concentrations of OH-PAHs from various countries, either creatinine-adjusted or unadjusted, were comparable to those found in our study (Table S3). ΣNap was the dominant compound, accounting for 60–90% of the total OH-PAHs in urine collected from Asian countries, followed by ΣPhe (3–16%), ΣFlu (3–20%), and 1-Pyr (2–8%) (Guo et al. 2013). ∑OH-PAH concentrations measured in our study were similar to those of the mean/median values reported previously, but significantly lower than those reported for occupationally exposed populations including firefighters and smokers (Table S3) (Li et al. 2015a; Oliveira et al. 2017a).

3.2. OH-PAH concentrations stratified by demographic and lifestyle characteristics

Although the number of individuals participated in the study is small, both creatinine-adjusted and unadjusted urinary concentrations of OH-PAHs were compared between different subgroups (n = 8), stratified by demographic (i.e., sex, age, BMI, and ethnicity) and lifestyle characteristics (i.e., alcohol consumption, exercise frequency, and dietary supplement use), as well as sample types (SU and FMV) (Table 1). Between gender, the median concentration of ∑OH-PAH in females was (7.7 μg/g creatinine) 1.8-fold higher than that in males (4.4 μg/g creatinine; $p < 0.001$). Individuals ≤ 30 years of age with normal BMI (≤25) had significantly higher urinary concentrations of ∑OH-PAH than did the older (>30 years old; median: 7.4 vs 4.7 μg/g creatinine) and overweight/obese individuals (BMI > 25; 6.6 vs 2.7 μg/g creatinine) ($p < 0.001$). However, no significant difference was found in unadjusted urinary concentrations of ∑OH-PAHs between the subgroups of age (8.3 vs 7.4 ng/mL) and BMI (7.6 vs 8.2 ng/mL) ($p > 0.05$). Unadjusted ∑OH-PAH concentrations were significantly higher in Caucasians (9.4 ng/mL) than those in Asians (6.6 ng/mL) ($p < 0.001$). However, this difference disappeared after the concentrations were corrected for creatinine (5.6 vs 6.3 μg/g creatinine; $p > 0.05$). ∑OH-PAH concentrations were significantly higher in FMV (9.8 ng/mL) than in SU type samples (5.3 ng/mL; $p < 0.001$), even after adjusted for creatinine levels (6.8 vs 4.7 μg/g creatinine; $p < 0.01$). Significantly higher ∑OH-PAH concentrations were found in individuals who consumed alcohol occasionally (8.6 vs 4.6 μg/g creatinine), exercised regularly (8.6 vs 4.3 μg/g creatinine), and used dietary supplements (6.3 vs 4.9 μg/g creatinine) than those who did not ($p < 0.01$).

Earlier studies that examined determinants of urinary OH-PAH concentrations showed inconsistent results. Li et al. (2008) reported that mean urinary 2-Nap concentration in U.S. females (2.64 ng/mL) was slightly higher than that in males (2.19 ng/mL). In contrast, mean concentrations of 2-Nap reported for Iranian (4.26 vs 2.69 ng/mL) and Korean (5.26 vs 2.77 ng/mL) males were 1.8-fold higher than those in females (Hoseini et al. 2018; Sul et al. 2012). In a Chinese national survey, higher urinary OH-PAH concentrations were found in females (mean: 6.36 μg/mmol creatinine) than in males (5.22 μg/mmol creatinine) ($p < 0.0001$) (Cao et al. 2020a). All of our 19 participates were non-smokers and lived in a suburban area near Albany, New York State, USA. Age was not a determinant of urinary concentrations of 2-Nap in a Korean population (Sul et al. 2012). However, urine from Iranian adolescents (12–20 years) contained higher 2-Nap concentrations than those who were < 12 or > 20 years of age (Hoseini et al. 2018). A BMI of > 30 kg/m^2 was significantly associated with higher OH-PAH concentrations than those with a BMI of < 25 kg/m^2, in a population in Boston, although this relationship was not found for a population in Puerto Rico (Cathey et al. 2018). Urine from non-Hispanic whites contained higher concentrations of 1-Nap, 2/3/9-Flu, and 1-Phe than those from Mexican Americans (Li et al. 2008). Individual differences in dietary habits including cooking practices and exposure to traffic related air pollution can contribute to discrepancies in OH-PAH concentrations in the above studies (Cao et al. 2020a).

3.3. Longitudinal and intra-individual variability in urinary OH-PAH concentrations

The measured concentrations of urinary OH-PAHs spanned over 2–3 orders of magnitude among the 19 study participants over the 44-day study period. The intra-individual

variabilities in urinary OH-PAH concentrations were calculated (Table 3). Creatinine normalization of urinary ∑OH-PAH concentrations increased ICC values from 0.517 to 0.630. Further analysis of data, therefore, was based on creatinine-normalized concentrations of OH-PAHs. In the same set of samples, ICC values were reported for universal pesticides, neonicotinoid insecticides, organophosphate esters, OSBs, and melamine derivatives, which were in the ranges of 0.05–0.24, 0.06–0.42, 0.31–0.68, 0.58–0.96, and 0.541–0.763, respectively (Li et al. 2019b; 2020; Martinez-Moral and Kannan 2019; Wang et al. 2019a; Zhu and Kannan 2019). In comparison to SU, FMV samples showed higher ICC values (0.648 vs 0.626). Similarly, an earlier study on urinary OH-PAHs showed the highest ICC values for 24-h composites (0.44–0.77), followed by FMV (0.33–0.65), and SU samples (0.30–0.55) (Li et al. 2013). Relatively higher within-individual variability in OH-PAH concentrations in SU may be attributed to the short half-lives of these metabolites in humans (Li et al. 2012).

Among various sub categories, ICC values were higher for males (0.689), and those with normal BMI (0.528) than did for females (0.432) and overweight/obese (0.361) individuals. Ethnicity and age were not significant determinants of ICC values (0.616 vs 0.649 for Asians and Caucasians; 0.602 vs 0.523 for ages ≤ 30 and > 30 years). Urine samples collected from participants who were not regular alcohol consumers and those who did not exercise nor used dietary supplements showed higher ICC values for OH-PAHs than those who did (0.604–0.730 vs 0.359–0.406). Among OH-PAH species analyzed, fair to good predictability/reliability was found for ∑Nap (0.373–0.760), which suggested that the concentrations of ∑Nap varied only slightly during the study period. However, ∑Phe (0.014–0.494), 2/3/9-Flu (0.016–0.179), and 1-Pyr (0.001–0.049) levels in urine showed poor predictability (ICCs, 0.020–0.370), which suggested variable exposures.

Daily variation in urinary OH-PAH concentrations measured in individuals for over a week (Sunday through Saturday) is shown in Fig. 1. Urinary ∑OH-PAH concentrations peaked during weekends (measurement made for samples collected on Monday morning represents exposure from Sunday) for half of the study participants (e.g., P1, 3, 5, 7, 9, 12, 15, and 16). ∑OH-PAH concentrations in weekend samples (mean: 10 μg/g creatinine) were significantly higher than those of weekday samples (7.0 μg/g creatinine) ($p < 0.001$). Reason for this pattern is unclear, but can be related to dietary exposures. Cooking activities and consumption of grilled food during weekends can increase PAH exposure (Alomirah et al. 2011). Nevertheless, it should be noted that for some individuals (P6, 13, 17, and 19), the urinary ∑OH-PAH concentrations peaked on Fridays.

3.4. Associations between urinary OH-PAHs and OSBs

Among seven OSBs, MDA was the most abundant biomarker measured, with urinary concentrations ranging from 0.95 to 198 ng/mL (median: 11.5 ng/mL), followed by 8-OHdG (0.05–38.6 ng/mL; 3.65 ng/mL) and diY (<LOD–19.8 ng/mL; 2.13 ng/mL), and $\sum PGF_{2\alpha}$ (sum of four F_2-isoprostane isomers; 0.14–23.9 ng/mL; 1.56 ng/mL) (Table S2).

8-OHdG is a biomarker of oxidative DNA damage. Previous studies have reported significant correlation between urinary OH-PAHs and 8-OHdG in coke oven workers (Kuang et al. 2013), general populations (Chen and Xu 2019; Hou et al. 2019; Sun et

al. 2017; Zhou et al. 2016), school students (Fan et al. 2012; Li et al. 2015a; Li et al. 2015b; Lin et al. 2016), pregnant women (Lou et al. 2019; Peng et al. 2020), and people living near e-waste recycling facilities (Lu et al. 2016; Yang et al. 2015). In our study, a significant correlation was found between ∑Phe or 2/3/9-Flu and 8-OHdG ($p < 0.05$); whereas, no such relationship was found between ∑Nap, 1-PYR, or ∑OH-PAH and 8-OHdG ($p > 0.05$). With every unit increase in log-transformed urinary ∑Phe or 2/3/9-Flu concentrations, 8-OHdG correspondingly increased by 9.3% or 9.8%, after adjustment for potential confounders (Fig. 2).

Urinary MDA concentrations are used as indicators of lipid peroxidation. Lu et al (2016) reported that urinary 1-Pyr concentration was significantly correlated with MDA in e-waste workers, whereas no such correlation was found between OH-PAHs and MDA in urban reference populations. Bortey-Sam et al. (2017) reported that urinary concentrations of 2-Nap, 2/3/9-Flu, 1/9-Phe, 2-Phe, and 4-Phe increased concomitantly with MDA, in people living in urban areas. MDA concentrations were significantly correlated with ΣOH-PAH in 10 individuals who traveled from Los Angeles to Beijing (Lin et al. 2016). In our study, a significant correlation was found between urinary concentrations of ∑Phe or 2/3/9-Flu and MDA ($p < 0.01$). Linear mixed-effects analysis showed that with every unit increase in ∑Phe or 2/3/9-Flu concentrations, MDA levels in urine increased by 13% and 12%, respectively, which fell within the range of 9.8–31% reported by Zhang et al (2020).

A significant positive correlation between the concentrations of several OH-PAHs (∑Phe, 2/3/9-Flu, 1-Pyr, or ΣOH-PAH) and $\Sigma PGF_{2\alpha}$ was found ($p < 0.01$ for all). However, urinary ΣNap was not correlated with $\Sigma PGF_{2\alpha}$ levels ($p > 0.05$). Each log-unit increase in ΣPhe, 2/3/9-Flu, 1-Pyr or ΣOH-PAH concentrations corresponded to an increment in 30%, 29%, 24%, or 21% of $\Sigma PGF_{2\alpha}$ levels, respectively, in urine. Among four F_2-isoprostane isomers analyzed, 8- and 8,15-$PGF_{2\alpha}$ were highly correlated with 6 of 9 OH-PAHs in urine ($p < 0.05$).

diY is a biomarker of protein oxidation (Li et al. 2019a; Li et al. 2019b; 2020; Li et al. 2019c). In our study, log-transformed creatinine-adjusted concentrations of diY were significantly correlated with all individual ∑OH-PAHs or ∑OH-PAH ($p < 0.01$). Moreover, each log-unit increase in urinary ∑Nap, ∑Phe, 2/3/9-Flu, 1-Pyr, or ΣOH-PAH concentrations corresponded to 11%, 24%, 14%, 19%, or 21% increase in urinary diY levels, respectively ($p < 0.05$). To the best of our knowledge, this is the first study to show the relationship between PAHs exposure and the marker of protein oxidation.

Analysis of multiple OSBs to examine associations with environmental chemical exposure has several advantages. For example, two different biomarkers of lipid peroxidation, namely MDA and $PGF_{2\alpha}$, yielded varying relationship with OH-PAH concentrations. Urinary 1-Pyr and ∑OH-PAH concentrations were significantly associated with $PGF_{2\alpha}$ ($p < 0.01$), but not with MDA. This highlights the importance of measuring multiple biomarkers of oxidative stress when associations with chemical exposures are made. These results suggest mixture or interactive effects from multiple chemical exposures. A few earlier studies reported non-linear dose–response relationships of OH-PAHs with OSBs (Huang et al. 2020; Zhou et al. 2016). In our study, the correlation between OH-PAH and OSBs was weak ($r < 0.400$).

This may due to the fact that other factors including exposure to other contaminants can confound the relationship between OSBs and OH-PAHs (Sezer et al. 2012).

3.5. Cumulative daily intake

The DI values estimated for Nap, Phe, Flu, and Pyr consecutively for 44 days are shown in Fig. S1. Among the exposure doses calculated for four parent PAHs, the median DI of Phe was the highest (15.7 μg/day), followed by Pyr (10.4), Nap (6.91), and Flu (3.92). The total DIs of Nap, Phe, Flu, and Pyr spanned over 2–3 orders of magnitude, ranging from 1.75 to 709 μg/day, in 515 urine samples. The DIs of four PAHs estimated in our study (median: 43.6 μg/day) were similar to those reported for populations in China (38.9 μg/day), India (39.5), Korea (26.4), Kuwait (28.1), and Vietnam (35.9), but 3–5-fold higher than those reported for Japan (13.8) and Malaysia (8.7) (Guo et al. 2013). A median DI of 21.2 μg PAHs/day was reported among Chinese mothers (Peng et al. 2020). Dietary exposure to PAHs by adults in Eastern China was reported to range from 42 to 160 μg/day (Li et al. 2019d; Wang et al. 2020). Falco et al. (2003) reported that dietary exposure to PAHs in Western Europe ranged from 1.6 to 8.42 μg/day. Thus, our PAH DI estimates fall within the range of dietary exposure doses reported in other countries.

The Reference Doses (RfDs) set by the U.S. EPA for Nap, Flu and Pyr were 20, 40 and 30 μg/kg bw/day, respectively (Bulder et al. 2006). The DI of the sum of four PAHs listed above ranged from 0.032 to 8.27 μg/kg bw/day, with a median value of 0.640 μg/kg bw/day (Fig. 3). The estimated exposure doses were several orders of magnitude below the respective RfDs (Fig. 3). It should be noted that several uncertainties exist in our exposure calculations. The urinary excretion rates of target PAHs varied among studies and were dependent on exposure route-, matrix analyzed-, and even among individuals (Motorykin et al. 2015). For example, urinary excretion rate of Pyr varied widely from 2.9% to 22% (Chien and Yeh 2010; Li et al. 2012; Motorykin et al. 2015). The excretion rates adopted in our study were derived from a pharmacokinetic study based on oral exposure dose (Li et al. 2012), which was considered to be an important source of PAH exposure in the general population (Yebra-Pimentel et al. 2015). Nevertheless, inhalation can be an important source of exposure to volatile PAHs like Nap (Cao et al. 2020a; Oliveira et al. 2017b).

4. Conclusions

The longitudinal measurement of OH-PAHs in urine displayed moderate within-individual variabilities for ∑Nap during the 44-day study period. However, ∑Phe, 2/3/9-Flu, and 1-Pyr presented notable within-individual variabilities. Compared to spot urine samples, first morning voids are more suitable for biomonitoring as they appear to integrate exposure for a longer period of time. Urinary concentrations of ∑OH-PAH exhibited a significant positive correlation with all seven OSBs analyzed. To our knowledge, this is the first study to investigate within-individual variability in urinary OH-PAHs and to evaluate their relationships with oxidative damage to DNA, lipids, and proteins simultaneously. The findings are valuable to design sampling strategies in biomonitoring studies and in assigning exposure classifications of PAHs in epidemiologic studies. Although the study was conducted longitudinally for up to 44 days with 515 urine samples analyzed, this study was

based on a convenience sample of 19 individuals from a single geographic location; and the participants were generally similar in social characteristics. Therefore, caution should be exercised in generalizing our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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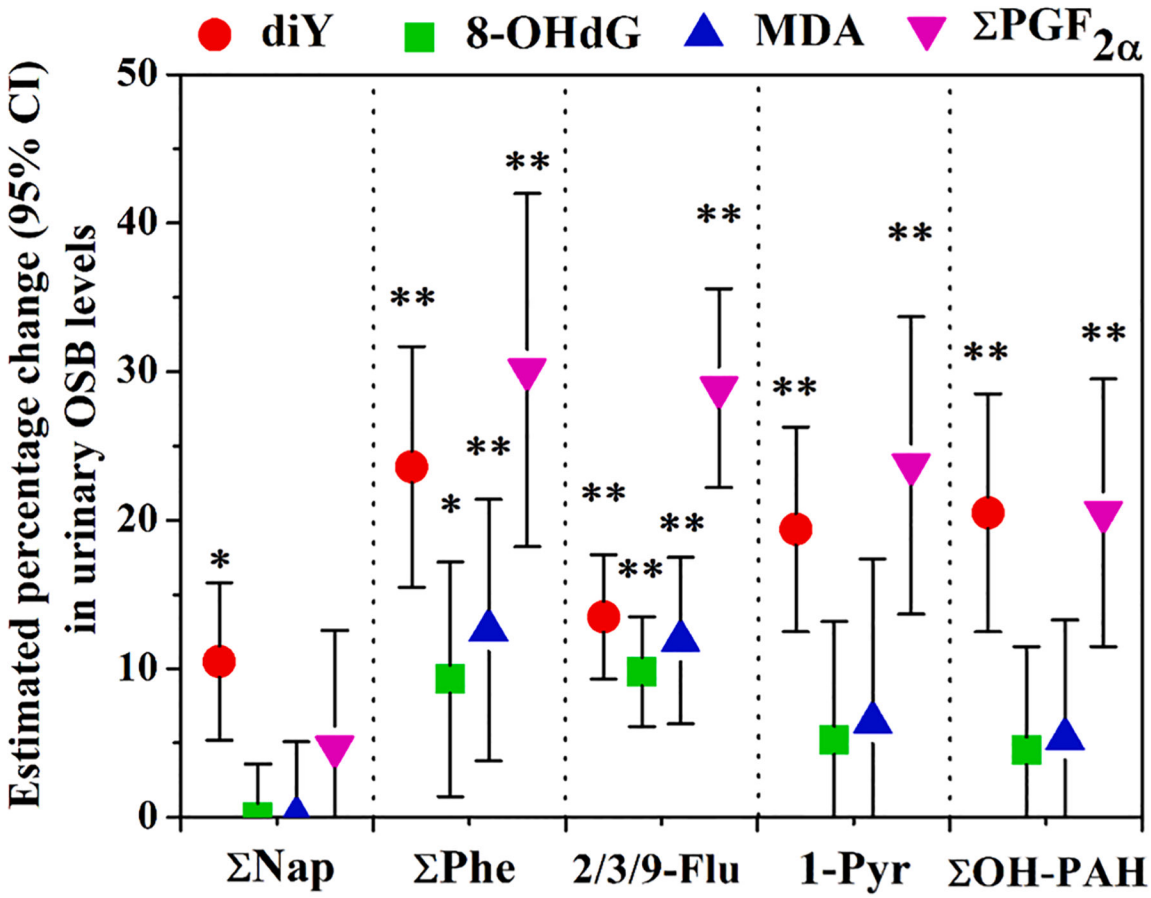

Fig. 1. Urinary OH-PAH concentrations in relation to change (%) in urinary OSB levels (error bar: 95% confidence intervals).

Linear mixed-effects model was obtained after adjustment for demographic [e.g., gender, age, body mass index (BMI), and ethnicity] and lifestyle variables (e.g., alcohol use, smoking status, exercise frequency, and dietary supplement use). *Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

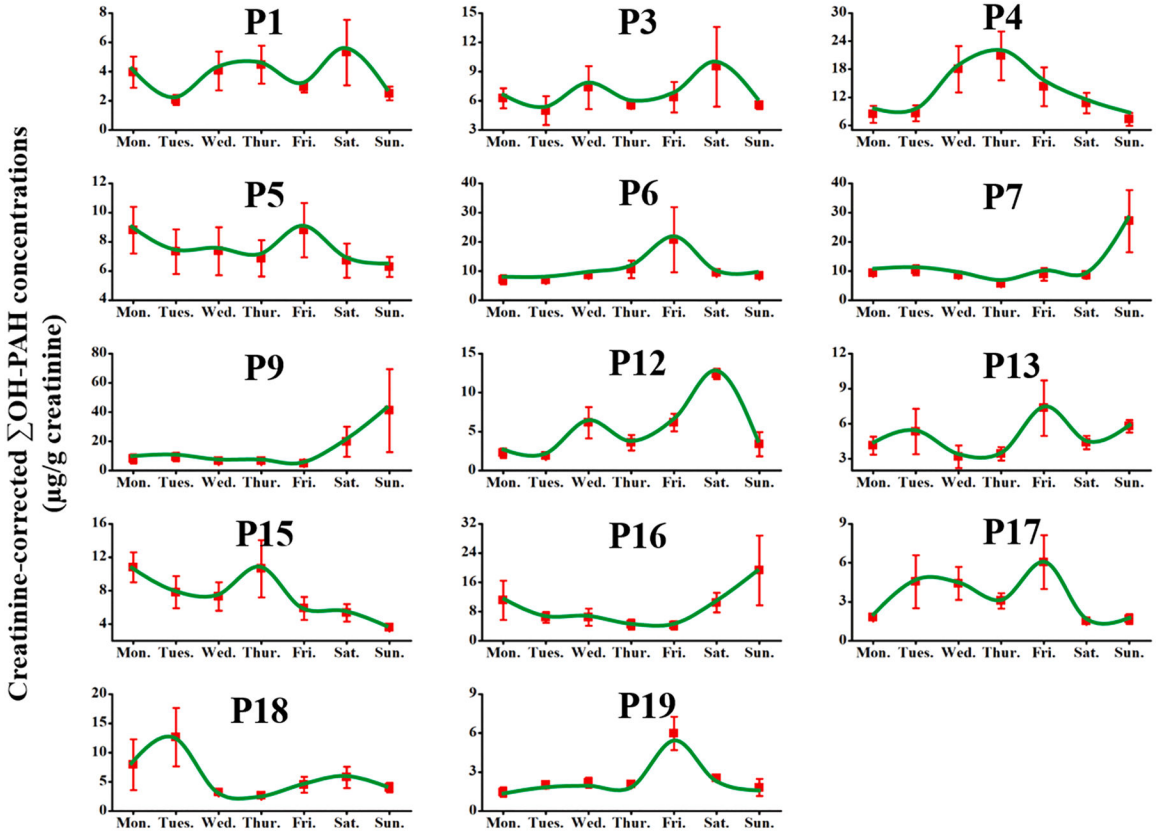

Fig. 2.

Daily variation of creatinine-adjusted concentrations of ∑OH-PAH (mean ± standard error) in urine samples collected from 19 study participants (P) for a week. Only those participants who provided at least triplicate samples for each day in a week are displayed here.

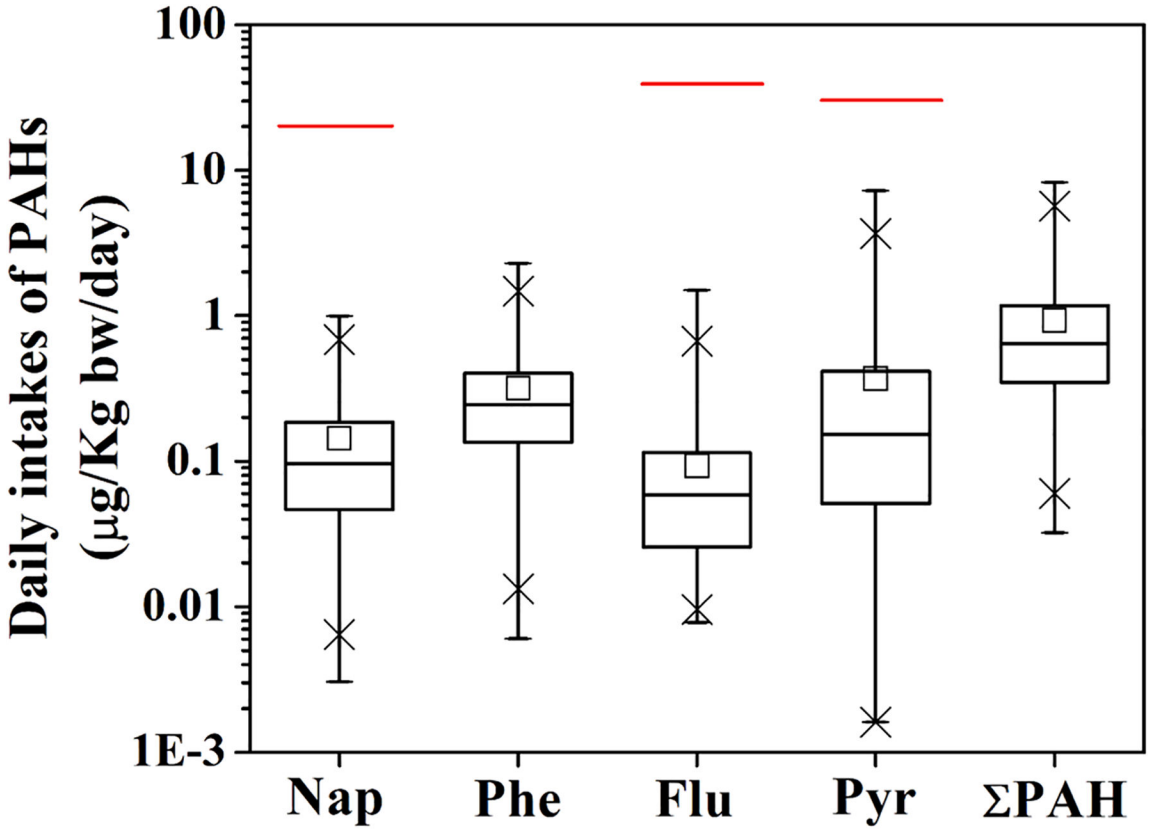

Fig. 3. Daily intakes of Nap, Phe, Flu, Pyr, and ∑PAH, estimated from urinary metabolite concentrations in 19 study participants, during a 44-day study period.

The red lines represent the Reference Doses (RfDs) set by the U.S. EPA for Nap, Flu and Pyr, which were 20, 40 and 30 μg/kg bw/day, respectively. The upper and lower limits of the box represent the 75th and 25th percentiles, the mean values are presented as "□", whereas the median values are presented as a line in the box. The top and bottom horizontal lines outside the box represent the maximum and minimum values. The top "×" and bottom "×" represent the 99th and 1st percentiles, respectively.

Table 1

Median concentrations (IQR) of urinary OH-PAHs stratified by demographic and lifestyle characteristics of the 19 study participants.

Strata	n_i/n_s	UC-∑OH-PAH (ng/mL)		CR-∑OH-PAH μg/g creatinine)	
Total	19/515	7.8 (4.3, 12)		5.7 (3.0, 9.8)	
Gender					
Male	11/296	6.4 (3.7, 11)	***p* < 0.01**	4.4 (2.3, 7.8)	***p* < 0.001**
Female	8/219	9.0 (5.2, 13)		7.7 (4.4, 11)	
Age					
≤ 30 years	7/181	8.3 (4.4, 13)	$p = 0.523$	7.4 (4.2, 11)	***p* < 0.001**
> 30 years	12/334	7.4 (4.3, 11)		4.7 (2.7, 8.7)	
BMI					
BMI ≤ 25	16/425	7.6 (4.3, 12)	$p = 0.326$	6.6 (3.7, 10)	***p* < 0.001**
BMI > 25	3/90	8.2 (5.0, 12)		2.7 (1.9, 4.0)	
Ethnicity					
Asians	13/347	6.6 (3.8, 11)	***p* < 0.01**	5.6 (3.0, 9.3)	$p = 0.215$
Caucasians	6/168	9.4 (6.2, 14)		6.3 (3.0, 11)	
Alcohol consumption					
Seldom	14/400	7.4 (4.0, 12)	$p = 0.164$	4.6 (2.6, 9.1)	***p* < 0.001**
Occasionally	5/115	8.0 (5.6, 14)		8.6 (5.7, 12)	
Exercise frequency					
Seldom	11/333	6.4 (3.7, 11)	***p* < 0.01**	4.3 (2.4, 8.1)	***p* < 0.001**
Frequently	8/182	9.0 (5.9, 14)		8.6 (5.1, 12)	
Dietary supplement					
No	10/268	6.6 (3.7, 12)	***p* < 0.01**	4.9 (2.7, 9.1)	***p* < 0.01**
Yes	9/247	8.5 (5.1, 12)		6.3 (3.4, 10)	
Urine sample type					
Spot urine (SU)	272	5.3 (3.2, 8.8)	***p* < 0.001**	4.7 (2.8, 9.3)	***p* < 0.01**
First morning voids (FMV)	243	9.8 (7.2, 14)		6.8 (3.4, 9.9)	

IQR: interquartile range; BMI: body mass index; UC-∑OH-PAH: uncorrected concentration (ng/mL); CR-∑OH-PAH: creatinine-corrected concentration (μg/g creatinine); n_i: number of individuals; n_S: number of urine samples; linear models were used to compare differences across different subgroups.

Table 2

Concentrations of OH-PAHs in urine samples (n = 515) collected from 19 participants consecutively for 44 days.

	1-Nap	2-Nap	1/9-Phe	2-Phe	3-Phe	4-Phe	2/3/9-Flu	1-Pyr	∑OH-PAH
df/%	79	100	94	100	85	72	82	85	
LOD (ng/mL)	0.040	0.017	0.007	0.003	0.003	0.012	0.480	0.009	
Uncorrected concentration (ng/mL)									
min	<LOD	0.07	<LOD	0.01	<LOD	<LOD	<LOD	<LOD	0.46
max	37	33	4.1	3.7	4.8	1.5	25	23	60
mean	1.5	3.7	0.34	0.23	0.60	0.07	1.9	0.89	9.3
SD	3.1	3.7	0.47	0.28	0.70	0.11	2.3	1.9	7.4
median	0.66	2.6	0.21	0.15	0.44	0.04	1.3	0.38	7.8
IQR	(0.15, 1.6)	(0.91, 5.4)	(0.09, 0.41)	(0.08, 0.29)	(0.14, 0.79)	(0.01, 0.09)	(0.59, 2.4)	(0.13, 0.96)	(4.3, 12)
Creatinine-corrected concentration (μg/g creatinine)									
min	<LOD	0.10	<LOD	0.01	<LOD	<LOD	<LOD	<LOD	0.59
max	86	15	13	5.5	7.7	2.1	32	31	110
mean	1.6	2.7	0.34	0.23	0.44	0.07	1.9	0.87	8.2
SD	5.6	2.6	0.74	0.43	0.73	0.15	3.1	2.2	10
median	0.47	1.6	0.15	0.11	0.27	0.03	0.93	0.28	5.7
IQR	(0.12, 1.2)	(0.68, 4.2)	(0.06, 0.36)	(0.05, 0.25)	(0.10, 0.47)	(0.10, 0.08)	(0.43, 2.2)	(0.08, 0.88)	(3.0, 9.8)

∑OH-PAH: sum of 15 OH-PAHs; df: detection frequency; LOD: limit of detection; SD: standard deviation; IQR: interquartile range; 1-Chr, 6-Chr, 1-BaA, and 3-BcP were not included in the statistical analysis as they were seldom detected in urine samples (df < 3%). Due to co-elution of the peaks, 1/9-Phe and 2/3/9-Flu represent sum of 1- and 9-Phe and sum of 2-, 3-, and 9-Flu, respectively.

Table 3

Intra-class correlation coefficients (ICC) of repeated measurements of urinary OH-PAH concentrations for various demographic subgroups of 19 participants.

Strata	∑Nap	∑Phe	2/3/9-Flu	1-Pyr	∑OH-PAH
Urine sample type					
SU	0.621	0.144	0.179	0.027	0.626
FMV	0.622	0.135	0.124	0.040	0.648
Gender					
Female	0.489	0.038	0.037	0.013	0.432
Male	0.634	0.062	0.130	0.049	0.689
Ethnicity					
Asians	0.639	0.100	0.039	0.009	0.616
Caucasians	0.715	0.014	0.020	0.026	0.649
Age					
≤30 years	0.627	0.044	0.030	0.015	0.602
>30 years	0.556	0.494	0.114	0.017	0.523
Body mass index (BMI)					
BMI ≤ 25	0.567	0.372	0.052	0.001	0.528
BMI > 25	0.439	0.028	0.016	0.005	0.361
Alcohol consumption					
Seldom	0.648	0.154	0.090	0.021	0.622
Occasionally	0.483	0.425	0.052	0.015	0.406
Exercise frequency					
Seldom	0.662	0.086	0.104	0.010	0.604
Frequently	0.402	0.388	0.024	0.018	0.364
Dietary supplement					
No	0.760	0.107	0.099	0.012	0.730
Yes	0.373	0.427	0.041	0.026	0.359

Creatinine-corrected concentrations were used in the calculation; FMV: first morning void; SU: spot urine.