



Urinary carboxylic acid metabolites as possible novel biomarkers of exposures to alkylated polycyclic aromatic hydrocarbons

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ABSTRACT

Previous studies have found that alkylated polycyclic aromatic hydrocarbons (alkyl-PAHs) were more abundant in petrogenic sources (e.g., crude oil and its refined products) than pyrogenic sources of incomplete combustion. While urinary hydroxylated metabolites of unsubstituted PAHs have been widely used as biomarkers of PAHs exposures, little information is available as to the occurrence of alkyl-PAH metabolites. In this study, we have detected carboxylic acid metabolites of alkyl-naphthalene (2-NAPCA) and alkyl-phenanthrene (2-PHECA) in 314 urine samples repeatedly collected from 45 Los Angeles residents before, during, and after they spent ten weeks in Beijing in summers of 2014–2017. We found that traveling from Los Angeles to Beijing led to 348% (95% CI: 243 to 485%) and 209% (95% CI: 149 to 282%) increases in 2-NAPCA and 2-PHECA concentrations, respectively, which returned to baseline levels after participants came back to Los Angeles. The concentration ratio between 2-PHECA and hydroxy-phenanthrenes was significantly ($p < 0.05$) lower in Beijing (median: 0.40, IQR: 0.27–0.53) than in Los Angeles (median: 0.51, IQR: 0.32–0.77), where more than 5,000 active gas and oil wells were located. From 2014 to 2017, the concentration ratio of 2-PHECA to hydroxy-phenanthrenes increased by 28.7 (95%CI: 12.3 to 47.6) %/yr in Los Angeles and 18.6 (95%CI: 7.9 to 30.3) %/yr in Beijing, likely resulted from both cities' efforts to reduce pyrogenic emissions (e.g. vehicle exhaust). These results provided indirect evidence supporting the use of 2-PHECA to hydroxy-phenanthrene ratio as an index to reflect the relative exposure contributions from petrogenic and pyrogenic sources. While our study suggested that urinary PAHCAs may be novel biomarkers of exposure to alkyl-PAHs, future studies with external exposure characterization are warranted to further validate these biomarkers.

1. Introduction

Both unsubstituted and substituted (e.g., alkylated) polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants, originating from both pyrogenic (i.e. incomplete combustion) and petrogenic (e.g., crude oil and its refined products) sources. With a chemical structure featuring two or more aromatic rings bonded in linear, cluster, or angular arrangements (Kim et al., 2013), PAHs can be mutagenic, carcinogenic, or neurotoxic and have been associated with

various diseases (e.g., lung cancer, reproductive disorder, cardiovascular diseases) (Alshaarawy et al., 2016; Ben et al., 2004). Although substantial actions have been taken to reduce PAH emissions by controlling pyrogenic sources (Kong et al., 2018; Shen et al., 2013), such as coal combustion and vehicle exhaust, less efforts have been documented to prevent the evaporative emission of petrogenic sources. Remarkably, the ambient PAHs levels in some places became even higher in recent years due to increased natural gas extraction and mining activities (Malaysian and Agency, 2007; Schuster et al., 2019).

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Substantial evidence indicates that pyrogenic and petrogenic sources release both unsubstituted and alkylated PAHs. However, larger proportions of alkylated PAHs have been observed from petrogenic sources of unburned fossil fuels as compared with pyrogenic sources of combustions (Boonyatumanond et al., 2007; Pereira et al., 1999; Saha et al., 2009). For example, the ratios of methylated phenanthrenes (MePHEs) to unsubstituted phenanthrene (PHE) were generally smaller than 0.5 for pyrogenic sources (e.g. coal burning), but greater than 2 for petrogenic sources (e.g. crude oil) (Saha et al., 2009). Thus, alkylated PAHs, particularly MePHEs, have been used as tracers of ambient PAHs from petrogenic sources (Saha et al., 2009). Notably, previous studies have found that alkylated PAHs could be more toxic than the unsubstituted PAHs (Ott et al., 1978), raising the possibility that PAHs and their substituted forms from different sources could have different health effects.

Our ability to study the health effects of PAHs from different sources in human studies has been limited by the lack of source-specific biomarkers. Having been widely used to assess exposures to PAHs (Strickland et al., 1996), urinary hydroxylated PAHs (OH-PAHs) are metabolites of unsubstituted PAHs that are more abundant in pyrogenic sources. Previous studies have suggested that the metabolism of alkylated PAHs may go through side chain hydroxylation and oxidation (Huang et al., 2017; Malmquist et al., 2015). This metabolic pathway, for example, resulted in the production of PAH carboxylic acids (PAH-CAs) and their glucuronidated conjugates as major metabolites of 2-methylnaphthalene (2-MeNAP) and 2-methylphenanthrene (2-MePHE) in vivo (Malmquist et al., 2015). However, no data are available from human urine samples to inform whether PAH-CAs could be used as biomarkers of alkylated PAHs exposure.

In our previous studies, we have followed a cohort of Los Angeles residents who traveled to Beijing and observed higher urinary levels of OH-PAHs when they were in Beijing than when in Los Angeles, likely due to more severe air pollution in Beijing mainly originated from pyrogenic (combustion) sources (Lin et al., 2016, 2019). Remarkably, there was a drastic difference in petrogenic sources between Los Angeles and Beijing, with more than 5000 active gas and oil wells in close proximity to residential areas in Los Angeles but not in Beijing (Shamasunder et al., 2018). This allows a natural experimental opportunity to contrast biomarkers related to pyrogenic and petrogenic sources. In the present study, we report, for the first time, the concentrations of 2-naphtholic acid (2-NAPCA) and 2-phenanthrene-carboxylic acid (2-PHECA) in 314 urine samples collected from 45 healthy adults who traveled from Los Angeles to Beijing between 2014 and 2017. Specifically we aim to (1) determine to what extent the travel affected urinary concentrations of the two PAH-CAs; (2) investigate the relationship between PAHCA and OH-PAH levels; and (3) explore the use of concentration ratio between PAH-CAs and OH-PAHs to reflect relative contribution of pyrogenic and petrogenic sources to human exposures.

2. Method

2.1. Participants and sample collection

This longitudinal study was built upon a joint research program between University of California Los Angeles (UCLA) and Peking University (PKU), which supported ~ 15 UCLA students to visit PKU for ten weeks in the summer of each year. A detailed description of participant recruitment was available in our previous studies (Lin et al., 2016, 2019, 2020). Briefly, we recruited 55 participants (10 in 2012 as a pilot study and 45 in 2014–2017 in this study) who traveled from Los Angeles to Beijing for a ten-week summer research program. Due to the absence of PAHCA measurement in 2012, the current study only included the 45 participants enrolled in 2014–2017. For each participant, early morning urine samples were repeatedly collected in Los Angeles (LA-before), Beijing, and in Los Angeles again (LA-after) with detailed information shown in Table 1. In 2014, we collected one, five, and three samples

Table 1

Demographic information of study participants from 2014 to 2017.

	Total	2014	2015	2016	2017	p-value ^a
Number of subjects	45	14	13	8	10	
Number of samples	68/	11/	24/	14/	19/	
(LA-before/ Beijing/LA-after)	152/	64/37	35/26	23/13	30/18	
Age (yr)	24.2 ± 8.3 ^b	23.3 ± 5.6	27.8 ± 13.6	22.6 ± 3.2	22.0 ± 2.7	0.32
BMI (kg/m ²)	21.4 ± 2.4	21.7 ± 2.8	21.3 ± 2.1	20.1 ± 2.3	22.1 ± 2.4	0.34
Race (Asian/ Others)	33/12	8/6	12/1	6/2	7/3	0.23
Sex (M/F)	23/22	9/5	3/10	3/5	8/2	0.03
Smoking (yes/no)	2/43	0/14	0/13	2/6	0/10	0.02

^a Between-year differences were tested by one-way ANOVA for age and BMI, and chi-square test for race, sex, and smoking status.

^b Mean ± standard deviation.

from each participant in LA-before, Beijing, and LA-after, respectively. While in 2015, 2016, and 2017, two, three, and two samples were collected from each participant in LA-before, Beijing, and LA-after, respectively. The urine samples were not collected until one week after the arrival at a new city and the time intervals for two consecutive collections were more than one week. This wash-off period is based on the half-lives of unsubstituted PAHs (i.e. <24 h) in the human body, aiming to diminish the exposure from the previous city or collection (Li et al., 2012; St Helen et al., 2012). Each urine collection was coupled with a questionnaire focusing on activities in the past three days that might influence PAHs exposures, including cooking behaviors (cooking frequency, cooking fuel, and barbecuing), diet (consumption of barbecue or baked meat), traffic-related activities (driving hours, public transportation usage, and time spent near heavy traffic), and passive smoking. We also obtained the average concentration of ambient fine particles (PM_{2.5}), nitrogen dioxide (NO₂), sulfur dioxide (SO₂), and carbon monoxide (CO) in the past three days from air monitoring stations within 30 km of UCLA (n = 4) and PKU (n = 18) as described previously (Lin et al., 2019). Participants were asked to fast for 8 h before sample collection to diminish dietary effects. The study was performed in accordance with guidelines and approval of the Institutional Review Boards of both University of California Los Angeles (UCLA) and Peking University.

2.2. Analytical method

Chemical standards of 2-NAPCA, 2-PHECA, 1- and 2- hydroxylated naphthalenes (OH-NAPs), and 1-, 2-, 3-, 4-, and 9-hydroxylated phenanthrenes (OH-PHEs) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Dr. Ehrenstrofer (Ausberg, Germany). All solvents used in this study were residue grade from Fisher Scientific (Hampton, NH, USA). Urinary concentrations of PAH-CAs and OH-PAHs were simultaneously determined with a previous established protocol (Lin et al., 2016, 2019). Briefly, 2 mL of urine was spiked with ¹³C-labeled 2-OH-NAP and 3-OH-PHE (Cambridge Isotope Laboratories, Andover, MA) and then incubated with β-glucuronidase-sulfatase (Helix pomatia, Sigma-Aldrich), followed by liquid-liquid extraction, diazomethane derivatization, and purification by column chromatography. Finally, samples were concentrated under a nitrogen stream and quantified by gas chromatography-mass spectrometry (GC-MS, Agilent 7890A-5975C). Blank samples were prepared for each of the eight urine samples, and negligible contamination was observed (Table S1). The recoveries of ¹³C-labeled 2-OH-NAP and 3-OH-PHE were 65.5 ± 22.9% (mean ± standard deviation) and 96.4 ± 16.2%, respectively. The peak areas of OH-PAHs were corrected by the peak areas of their corresponding ¹³C-labeled standards while those of PAH-CAs were not corrected. The recovery of all analytes in spiked samples (10 ng/sample)

ranged from 83.2 to 102.7%, and the relative standard deviation of replicate analyses ranged from 9.2 to 18.6% (Table S1). Specifically, the recoveries of 2-NAPCA and 2-PHECA were $91.5 \pm 9.7\%$ and $96.4 \pm 18.6\%$, respectively. The method limit of quantification (LOQ) was 0.015 and 0.031 ng/mL for 2-NAPCA and 2-PHECA, respectively (Table S1). Urinary creatinine levels were measured based on the Jaffe reaction and were used to normalize the concentrations of PAHs metabolites (Toora and Rajagopal, 2002).

2.3. Statistical Analysis.

All reported biomarkers were detected in more than 86.2% of the samples, with undetected biomarkers assigned with a concentration of 50% of the LOQ (Table S1). We tested whether demographic variables are significantly different between different years with one-way ANOVA or chi-square test for continuous or categorical variables, respectively. We tabulated the means ($\pm$ standard deviations) or median with the interquartile ranges of biomarkers by phase (i.e., LA-before, Beijing, and LA-after), as appropriate. Then, we built different mixed effects models with random intercept at the participant level to study the relationship of PAHCA concentrations with other variables as follows. First, we studied the effects of phase and participants' characteristics on PAHCA levels in models with the fixed effect of phases, sex, age, BMI, race, and smoking status. Second, we studied the temporal trend of biomarker concentrations in each city in models with the fixed effect of year, sex, age, BMI, race, and smoking status. Third, in each city (or phase), we explored potential behavioral determinants of PAHCA concentrations in models with the fixed effect of activities or the length of stay in each phase. Last, we tested the city-specific associations of PAHCA concentrations with other exposures (i.e. unsubstituted PAHs or air pollution) in models with the fixed effect of urinary OH-PAHs or ambient air pollutant levels adjusting for activities. Statistical significance was reached with a p -value < 0.05 . All analyses were performed in the R Software.

3. Results and discussion

The current study included 45 participants (23 males and 22 females) recruited from 2014 to 2017, most of whom were Asian young non-smokers. The average ($\pm$ standard deviation) age and BMI were 24.2 (± 8.3) years and 21.4 (± 2.4) kg/m², respectively. We observed significant differences in sex and smoking status ($p < 0.05$), but not in age, BMI, and race across different years (Table 1).

3.1. Identification of PAHCAs in Urines

Authentic standards of 2-NAPCA and 2-PHECA were methylated by diazomethane and then injected into GC-MS. Mass spectra obtained under the electron impact mode showed intensive signals for molecular ions $[\text{ArCOOCH}_3]^+$ for both chemicals (Fig. 1). Additionally, fragments of $[\text{ArCO}]^+$ and $[\text{Ar}]^+$ were also observed for both analytes. Similar fragmentation pathways in mass spectra, together with clear peaks of molecular ions in chromatograms, were observed for both analytes in urine samples (Fig. 1), indicating the presence of methyl esters of 2-NAPCA and 2-PHECA.

To explore the precursor of PAH methyl esters detected in the urine, we selected a subgroup of 35 urine samples ($n = 13$ in Beijing and $n = 22$ in Los Angeles) with high 2-PHECA methyl ester concentrations, and performed the measurement with the same protocol but without the incubation with β -glucuronidase-sulfatase. We found that the levels of 2-PHECA methyl ester in urine samples without β -glucuronidase-sulfatase incubations were 11.0% (median; IQR: 6.8 to 19.7%) of those with incubations, indicating that the majority of 2-PHECA methyl esters detected in the urine originated from conjugated 2-PHECA, rather than free 2-PHECA or its methyl esters. Although we did not measure 2-NAPCA methyl esters in these urines, similar results were expected as conjugated 2-NAPCA is more water-soluble than free NAPCA and NAP methyl esters (Luch and Baird, 2005) and therefore might be excreted through urine faster.

Although the precursors of urinary PAHCAs and their conjugates were not investigated in the present study, recent animal studies have

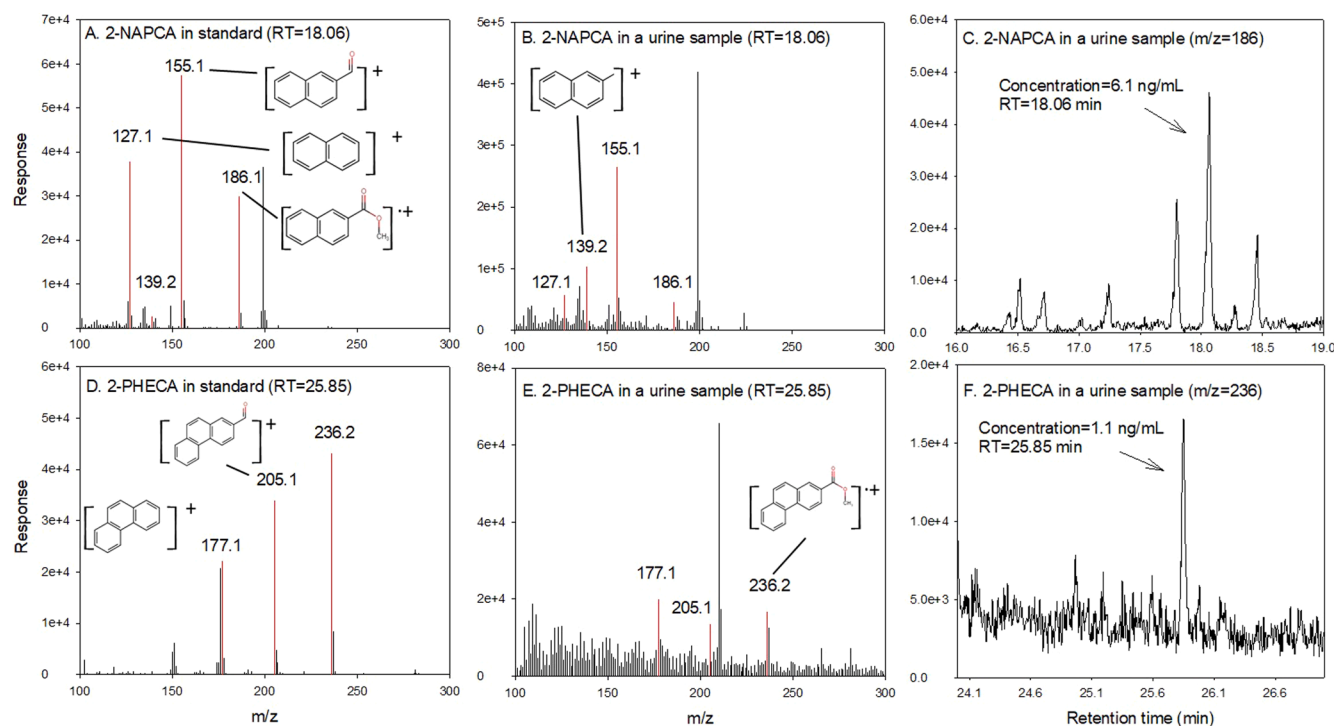


Fig. 1. Full scan mass spectra for methylated 2-NAPCA (nominal molecular weight = 186.1) and 2-PHECA (nominal molecular weight = 236.1) in authentic standards (panels A and D) and urine samples (panels B and E) obtained under electron impact (EI) mode, and chromatograms of urine samples (panels C and F).

identified PAHCAs as the primary metabolites of alkylated PAHs (Malmquist et al., 2015), which have been found in various environmental matrixes (e.g., air, soil, and sediments) (Boonyatumanond et al., 2007; Lin et al., 2015; Ma et al., 2016; Notar et al., 2001; Schuster et al., 2019). Hence, the urinary 2-NAPCA and 2-PHECA detected in our study are likely resulting from, at least partially, participants' exposure to alkylated PAHs. Notably, NAPCAs have been detected at high levels in petroleum (Clemente and Fedorak, 2005), which might be another source of 2-NAPCA in the urine. However, the occurrence of NAPCAs in receptor sites was less documented and it is unclear whether general population is exposed to NAPCAs. While we cannot exclude the contribution of other potential precursors in the environment (e.g. PAH esters) to urinary PAHCAs, little information is available as to their environmental levels and bioavailability.

3.2. Levels of urinary PAHCAs and their determinants

2-NAPCA and 2-PHECA were detected in more than 96% of urine samples (Table S1), with median concentrations of 0.15 (IQR: 0.07 to 0.31) and 0.28 (IQR: 0.14 to 0.42) $\mu\text{g/g}$ creatinine, respectively. We found that the levels of 2-NAPCA and 2-PHECA were increased by 348% (95% CI: 243 to 485%) and 209% (95% CI: 149 to 282%), respectively, after participants traveling from Los Angeles to Beijing, a city with more severe air pollution due to substantial pyrogenic emissions (Lin et al., 2015). Notably, the PAHCA levels had reached the steady state within <10 days after the arrival of participants in Beijing (Fig. S1). On the other hand, we observed a significant decline in the 2-PHECA level ($p < 0.05$) 4–11 weeks after participants returning to Los Angeles (Fig. S1), and there were no significant differences in the levels of 2-NAPCA ($p = 0.15$) or 2-PHECA ($p = 0.07$) before (LA-before) and after the travel (LA-after; Fig. 2). These results suggested predominant effects of changes in environments (i.e. city) on urinary PAHCA levels.

We further explored whether urinary PAHCA concentrations were influenced by participants' characteristics. Sex was found to be a significant predictor for both PAHCAs, with higher levels among the females ($p < 0.01$; Table S2). In addition, age was negatively associated with 2-NAPCA ($p < 0.05$, Table S2). On the other hand, we found that PAHCA levels were also associated with environmental or behavioral variables. Urinary PHECA levels were positively associated with ambient levels of $\text{PM}_{2.5}$ and CO (Fig. S2) in Beijing but not in Los Angeles, and there is a positive association between urinary NAPCA levels and participants' time spent near heavy traffic ($p < 0.05$, Table S3) in Los Angeles but not in Beijing. These results suggest possible

differences in sources or exposure pathways between the two PAHCAs between Beijing and Los Angeles.

3.3. Relationship between PAHCA and OH-PAH Concentrations.

The urinary level of 2-PHECA (median: 0.28; IQR: 0.14 to 0.42 $\mu\text{g/g}$ creatinine) was at the same magnitude of that of $\Sigma\text{OH-PHEs}$ (median: 0.64; IQR: 0.32 to 1.11 $\mu\text{g/g}$ creatinine), which coincided with previous studies showing comparable levels of alkylated and unsubstituted phenanthrenes in petrogenic and pyrogenic sources, as well as various environmental matrixes of receptor sites worldwide (Boonyatumanond et al., 2007; Lin et al., 2015; Ma et al., 2016; Notar et al., 2001; Schuster et al., 2019). Notably, the levels of alkylated and unsubstituted phenanthrenes have been found to be highly correlated in environmental samples (Lin et al., 2015; Liu et al., 2017), because both of them are emitted by petrogenic or pyrogenic sources albeit to different extents. Likewise, we observed positive associations between concentrations of 2-PHECA and the sum of OH-PHEs ($\Sigma\text{OH-PHEs}$) in both Los Angeles and Beijing ($p < 0.001$; Fig. 3).

In comparison, levels of 2-NAPCA (median: 0.15; IQR: 0.07 to 0.31 $\mu\text{g/g}$ creatinine) were much lower than those of $\Sigma\text{OH-NAPs}$ (median: 2.95; IQR: 2.01 to 4.82 $\mu\text{g/g}$ creatinine), and the 2-NAPCA concentrations were significantly associated with $\Sigma\text{OH-NAPs}$ concentrations only in Beijing ($p < 0.001$), but not in Los Angeles ($p = 0.13$; Fig. 3). This is likely because $\Sigma\text{OH-NAPs}$ in the urine may not necessarily originate from pyrogenic or petrogenic sources. For example, naphthalene could be released by mothballs, fumigants, and deodorizers (Jia and Batterman, 2010), and the metabolism of carbaryl pesticides may also produce 1-OH-NAP (Meeker et al., 2007). Notably, previous studies have indicated comparable concentrations of ambient naphthalene and urinary $\Sigma\text{OH-NAPs}$ in Los Angeles and Beijing (Eiguren-Fernandez et al., 2007; Liu et al., 2007; Lu et al., 2005; Ma et al., 2011), but the levels of non-naphthalene PAHs in the air and their metabolites in the urine were markedly higher in Beijing as compared with Los Angeles (Eiguren-Fernandez et al., 2007; Ma et al., 2011). These results suggest that the source of naphthalene in Los Angeles might be different from other PAHs, which might contribute to the lack of associations between 2-NAPCA and $\Sigma\text{OH-NAPs}$ concentrations in Los Angeles.

3.4. Ratios of 2-PHECA to $\Sigma\text{OH-PHEs}$ Levels.

With consistent associations between 2-PHECA and $\Sigma\text{OH-PHEs}$ concentrations in both cities, the ratio of 2-PHECA to $\Sigma\text{OH-PHEs}$

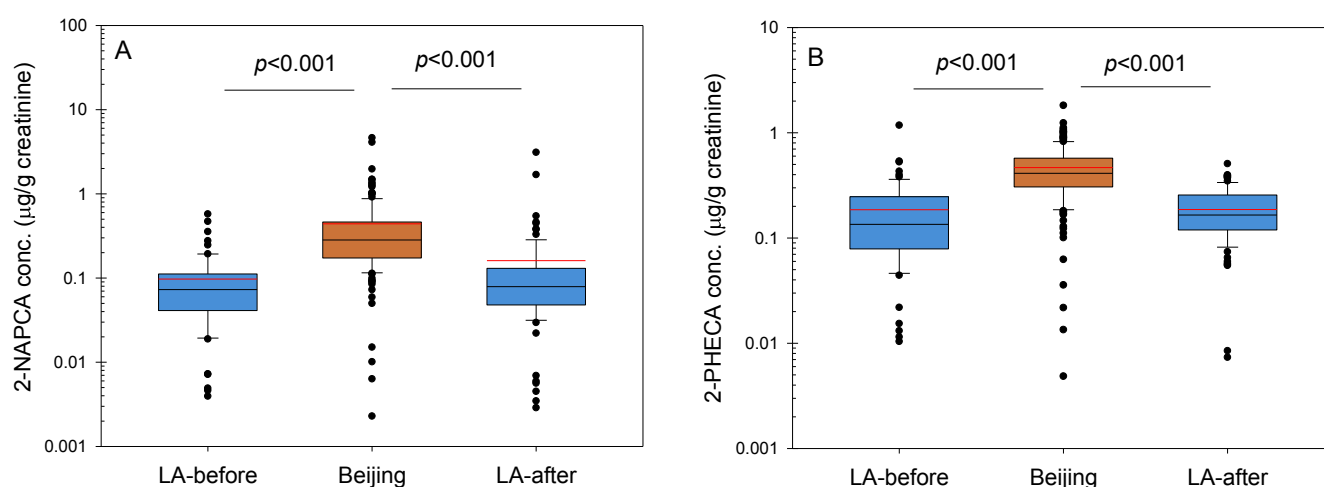


Fig. 2. Concentrations of urinary 2-NAPCA (panel A) and 2-PHECA (panel B) in LA-before, Beijing, and LA-after. Blue and orange boxes indicate data in Los Angeles and Beijing, respectively. The solid horizontal line represents the median, and the red horizontal line represents the 25th–75th percentiles, and the whiskers represent the 10th and 90th percentiles. Differences were tested by mixed effects models with random intercept of participants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

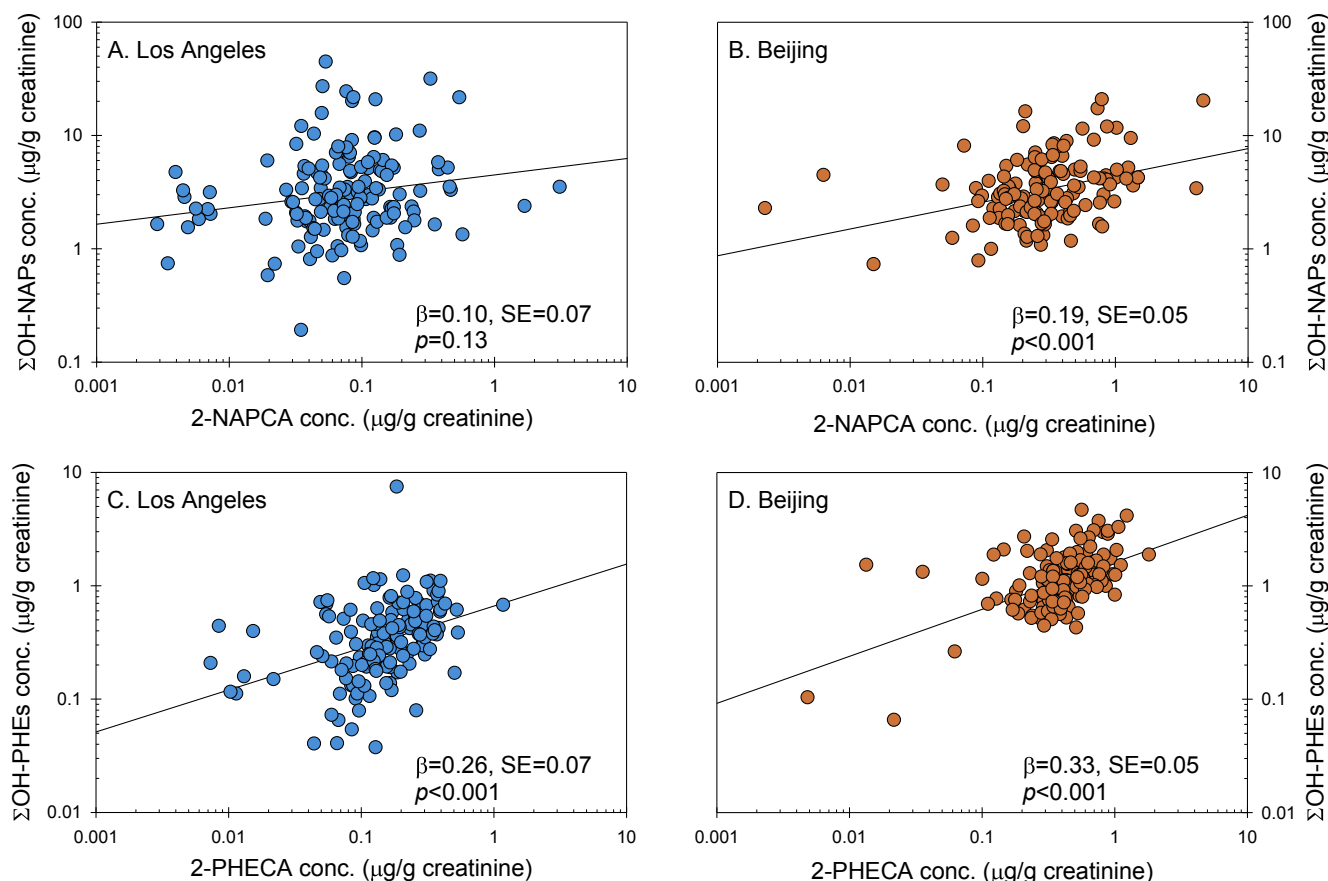


Fig. 3. Associations between urinary concentrations of PAHCAs and OH-PAHs in Los Angeles (panels A and C) and Beijing (panels B and D). Associations were tested by mixed-effect models with random intercept of participants adjusting for activities.

concentrations might be used to estimate the relative contribution of petrogenic and pyrogenic sources to human exposures, in analogue to the use of MePHEs/PHE ratios for environmental samples (Boonyatumanond et al., 2007; Saha et al., 2009). We found that the ratios between 2-PHECA and $\Sigma\text{OH-PHEs}$ levels in Los Angeles (median: 0.51, IQR: 0.32–0.77) and Beijing (median: 0.40, IQR: 0.27–0.53) were at the same magnitude with those between 2-MePHE and PHE in various environmental matrixes (range from 0.06 to 0.95, Table S4 and Fig. S3) (Boonyatumanond et al., 2007; Hedberg et al., 2002; Lin et al., 2015; Liu et al., 2017; Nalin et al., 2016; Pereira et al., 1999). In particular, the median ratio of 2-MePHE/PHE in 33 fine particle samples, collected in Beijing during the non-heating season in our previous study in 2012–2013 (Lin et al., 2015), was 0.24 (IQR: 0.22 to 0.25), which was very close to the 2-PHECA/ $\Sigma\text{OH-PHEs}$ ratio in Beijing in 2014 (median: 0.29; IQR: 0.24 to 0.41) as compared with in 2015–2017 (median: 0.47; IQR: 0.33 to 0.62) or in Los Angeles (median: 0.51; IQR: 0.32 to 0.77). Nevertheless, it is important to note that besides the exposure to PAHs, the 2-PHECA/ $\Sigma\text{OH-PHEs}$ ratio might be influenced by the metabolism of PAHs. Therefore, the cut-off value of 2-MePHE/PHE that was used for source identification cannot be directly extrapolated to 2-PHECA/ $\Sigma\text{OH-PHEs}$ ratios.

There were more than 5000 active oil wells in Los Angeles (Shamasunder et al., 2018) and the natural gas extraction in the United States has been shown to increase population's exposure to PAHs (Paulik et al., 2018), hence the oil and gas industry in Los Angeles may lead to higher contribution of petrogenic sources to population's exposure to PAHs (Shamasunder et al., 2018). Moreover, the positive association between 2-PHECA and ambient air pollutant levels in Beijing but not in Los Angeles (Fig. S2) also suggests a greater contribution of pyrogenic sources to PAHs exposures in Beijing, since pyrogenic sources (e.g.

vehicle emission) have been shown to be the major contributor to air pollution in both city (Gao et al., 2017; Parrish et al., 2016). Unlike the higher 2-MePHE/PHE ratios observed in petrogenic sources (Fig. S3), the 2-PHECA/ $\Sigma\text{OH-PHEs}$ ratio was significantly higher in Los Angeles than in Beijing (Fig. 4A). These results suggest that the 2-PHECA/ $\Sigma\text{OH-PHEs}$ ratio might be a useful index to evaluate the relative contribution of petrogenic and pyrogenic sources to human exposures to PAHs. In addition, we have found that the 2-PHECA/ $\Sigma\text{OH-PHEs}$ ratio increased by 28.7% (95%CI: 12.3 to 47.6%) per year in Los Angeles and 18.6% (95%CI: 7.9 to 30.3%) per year in Beijing from 2014 to 2017 (Fig. 4B and 4C), albeit the 2-PHECA levels were not increased from 2014 to 2017 in both cities (Fig. S4). These trends are consistent with the fact that current emission control efforts in both cities are mostly targeted at pyrogenic sources, such as vehicle emission and coal combustion (Gao et al., 2017; Parrish et al., 2016). As a result, the level of population's exposure to PAHs may decrease due to reduced pyrogenic emissions, making the contribution of petrogenic sources relatively greater.

3.5. Limitations and Implications

One of the limitations of this study is that we did not measure study participants' external exposures to alkylated PAHs, thus a direct linkage between alkylated PAHs and urinary PAHCA levels cannot be established. Nevertheless, we observed comparable levels of 2-PHECA and $\Sigma\text{OH-PHEs}$ in the urine and consistent positive associations between 2-PHECA and $\Sigma\text{OH-PHEs}$ levels in both cities, suggesting that the precursors of 2-PHECA should have similar sources and comparable environmental levels with unsubstituted phenanthrene. In this regard, alkylated phenanthrenes are the most likely precursors of 2-PHECA, since alkylated and unsubstituted phenanthrenes were found to be

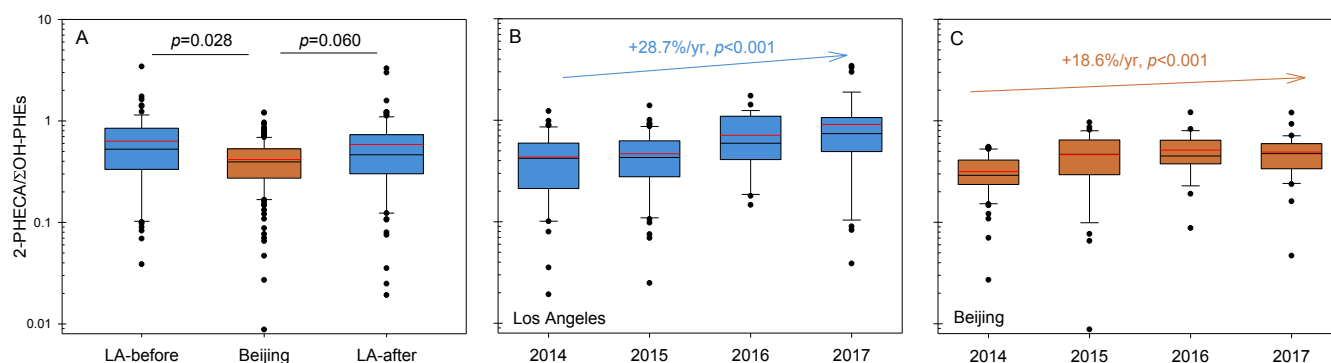


Fig. 4. Concentration ratios of urinary 2-PHECA to ΣOH-PHEs concentrations in LA-before, Beijing, and LA-after (panel A) and their temporal trends from 2014 to 2017 in Los Angeles (panel B) and Beijing (panel C). Blue and orange boxes indicate data in Los Angeles and Beijing, respectively. The solid horizontal line represents the median, and the red horizontal line represents the mean. The box represents the 25th–75th percentiles, and the whiskers represent the 10th and 90th percentiles. Between-phase differences and temporal trends were tested with linear mixed effects models with the random intercept of participants controlled for sex, age, BMI, race, and smoking status. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

highly correlated and at comparable levels in the air of Beijing (Lin et al., 2015). Different from 2-PHECA, there is insufficient evidence to infer the precursors of 2-NAPCA in this study, and therefore more research is needed before it can be used as an exposure biomarker of alkylated naphthalenes.

Our study is also limited by the small population size. Although we have found significant effects of demographic variables on PAHCA levels (Table S2), future larger population studies are needed to confirm these results. Nevertheless, we have performed repeated measurements among the travelers allowing each participant to serve as his/her own control. Thus, significant changes in PAHCA levels and their ratios to OH-PAH levels were observed during the travel, supporting the use of these biomarkers to reflect exposure changes. Finally, the biological half-times of PAHCAs are crucial to the use of these biomarkers to estimate exposures. While previous animal studies suggested rapid urinary excretion of 2-methyl-NAP within 48 h (USEPA. IRIS Toxicological Review of 2-Methylnaphthalene, 2003), little information is available as to the half-times of PAHCAs in humans. In our study, we have found that the levels of both PAHCAs reached the steady-state 10 days after the arrival at Beijing (Fig. S1), suggesting the possibility of short PAHCAs' half-lives. However, the 2-PHECA concentration significantly declined 4–11 weeks after participants returned to Los Angeles (Fig. S1), which otherwise suggested a longer half-life. Since it is unclear whether there are changes in external exposures in each phase (e.g. Beijing and LA-after), future studies are needed to better understand the biological half-times of PAHCAs.

The findings in the present study may have several public health implications. On one hand, increasing epidemiological evidence has associated residential proxy to oil and gas wells with various adverse effects (McKenzie et al., 2014; Rabinowitz et al., 2015; Tran et al., 2020). Meanwhile, with increased global fossil fuel consumption and technology advance to reduce combustion emissions (Shen et al., 2013), sources with evaporative emissions may become a more important contributor to ambient pollutant levels. While many countries have initiated the regulation of evaporative emissions (Yang et al., 2015), there is a need to evaluate the effectiveness of regulations in reducing population's exposures, and the 2-PHECA/ΣOH-PHEs ratios in the urine may be a promising index for this purpose. On the other hand, despite comparable levels of alkylated and unsubstituted PAHs in the environment, little evidence is available as to the health effects of alkylated PAHs. Recent studies have identified methylated-PAHs as key constituents responsible for the adverse health effects of outdoor and household air pollution mixtures (Jiang et al., 2019; Vermeulen et al., 2019), which is supported by earlier in vitro studies showing higher toxicity of alkylated PAHs as compared with unsubstituted ones (Ott et al., 1978). While the aforementioned evidence is mainly based on the direct

characterization of environmental samples, the simultaneous measurement of endogenous OH-PAHs and PAHCAs in the urine may provide another tool to better understand the differential health effects of alkylated and unsubstituted PAHs in future population studies.

In summary, we have discovered the occurrence of 2-NAPCA and 2-PHECA in human urine, which is sensitive to the changes of environments. The concentrations of PAHCAs were significantly associated with OH-PAHs and the 2-PHECA/ΣOH-PHEs ratios decreased after participants traveling from Los Angeles, a city with substantial petrogenic sources, to Beijing. These results provided indirect evidence suggesting that urinary PAHCAs might be used as biomarkers of exposure to alkyl-PAHs and supporting the use of 2-PHECA/ΣOH-PHEs ratio as a possible novel index to reflect the relative exposure contributions from petrogenic and pyrogenic sources. Future studies are warranted to confirm the parental compounds of PAHCAs in the environment, as well as to elucidate the biological half-lives of PAHCAs.

CRediT authorship contribution statement

Yan Lin: Investigation, Conceptualization, Data curation, Formal analysis, Writing - original draft, Visualization. **Xueyao Gao:** Formal analysis, Writing - original draft, Visualization. **Xinghua Qiu:** Conceptualization, Writing - review & editing, Visualization, Funding acquisition. **Jinming Liu:** Investigation, Data curation. **Chi-Hong Tseng:** Formal analysis. **Junfeng Jim Zhang:** Writing - review & editing. **Jesus A Araujo:** Writing - review & editing, Funding acquisition. **Yifang Zhu:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

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 material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.106325>.

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