

Sensitive biomarker of polycyclic aromatic hydrocarbons (PAHs): urinary 1-hydroxypyrene glucuronide in relation to smoking and low ambient levels of exposure

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Abstract

The study was conducted in a Chinese population with occupational or environmental exposures to polycyclic aromatic hydrocarbons (PAHs). A total of 106 subjects were recruited from coke-oven workers (workers), residents in a metropolitan area (residents) and suburban gardeners (gardeners). All subjects were monitored twice for their personal exposures to PAHs. The biological samples were collected for measurements of 1-hydroxypyrene (1-OHP) and cotinine in urine. The geometric means of personal exposure levels of pyrene, benz(a)anthracene (BaA) and benzo(a)pyrene (BaP) in workers were 1.470, 0.978 and 0.805 $\mu\text{g m}^{-3}$, respectively. The corresponding levels in residents were 0.050, 0.034 and 0.025 $\mu\text{g m}^{-3}$; and those in gardeners were 0.011, 0.020 and 0.008 $\mu\text{g m}^{-3}$, respectively. The conjugate of 1-OHP with glucuronide (1-OHP-G) is the predominant form of pyrene metabolite in urine and it showed strong associations with exposures not only to pyrene, but also to BaA, BaP and total PAHs. Most importantly, a significant difference in 1-OHP-G was even detected between the subgroups with exposures to BaP at <0.010 and >0.010 but <0.020 $\mu\text{g m}^{-3}$, suggesting that 1-OHP-G is a good marker that can be used for the risk assessment of BaP exposure at levels currently encountered in ambient air. Furthermore, multiple regression analyses of 1-OHP-G on PAHs exposure indicated that cigarette smoke was a major confounding factor and should be considered and adjusted for while using 1-OHP to estimate PAHs exposure.

Keywords: *Polycyclic aromatic hydrocarbons (PAHs), environmental exposure, 1-hydroxypyrene, urinary metabolites, biomarkers*

(Received 23 August 2005; accepted 1 February 2006)

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ISSN 1354-750X print/ISSN 1366-5804 online © 2006 Informa UK Ltd.
DOI: 10.1080/13547500600626883

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread pollutants in the environment due to significant contributions from incomplete combustion of fossil fuels in transportation, residential heating and power generation. These compounds can be absorbed into the body through the skin, lungs and gastrointestinal tract from various sources, such as occupational and environmental exposures, mainstream and sidestream tobacco smoke, as well as dietary intakes (IARC 1983, Juliane et al. 2000, Jürgen & Jürgen 2000, Matthias et al. 2001, Hecht 2002, Brandt & Watson 2003). Many of them, including benzo(a)pyrene (BaP), are animal and human carcinogens (IARC 1983). The potential carcinogenic effects of PAHs on humans at low exposure levels have attracted increasing public concerns. However, the relative contributions to the increased cancer risk in humans from low-level ambient exposures to PAHs are difficult to estimate due to the lack of sensitive and reliable methods to examine the relationship between low-level exposure and biological effects. The ambient levels of PAHs are extremely low, e.g. the strongly carcinogenic BaP was typically found in the range $1-20 \text{ ng m}^{-3}$ in Europe and around 1 ng m^{-3} in the USA (Menichini 1992). To assess the potential effects of such low levels of PAH exposures, it is desirable and important to develop specific and sensitive biomarkers. Valid and sensitive biomarkers can be very useful in estimating the risk in humans exposed to currently encountered low levels of PAHs and to establish what level of exposure to PAHs in the general environment constitutes an acceptable risk.

Urinary 1-hydroxypyrene (1-OHP), a metabolite of pyrene, was first proposed as a biological marker of exposure to PAHs in humans by Jongeneelen et al. (1985). Since then a large number of studies for urinary 1-OHP as a marker of exposure to PAHs in humans have been reported and were discussed in most recent reviews (Jongeneelen 2001, Brandt & Watson. 2003, Castano-Vinyals et al. 2004). However, the findings obtained in these studies, including occupational and non-occupational exposures, were not always consistent. It was reported that urinary 1-OHP correlated well with exposure levels of PAHs in some studies (Zhao et al. 1990, Ny et al. 1993, Angerer et al. 1997), but in other studies, the associations with PAHs exposures were weak or uncertain probably due to potential effects of confounding factors such as smoking (Van Rooij et al. 1993, Hara et al. 1997, Mielzynska et al. 1997).

The present study was conducted in Chinese populations with broad range of PAHs exposures to determine whether or not 1-OHP can be used as a sensitive and reliable biomarker to differentiate PAHs exposure levels within the range currently encountered in ambient air.

Materials and methods

The human subject protocol for this study was approved by the IRBs of both the New York University School of Medicine and the Peking University Health Science Center. Written informed consent was obtained from all participating subjects.

Chemical reagents

EPA 619 Polynuclear Aromatic Hydrocarbon Mix ($100-2000 \mu\text{g ml}^{-1}$), the reference standard mixture, was purchased from Supelco (Bellefonte, PA, USA). The standard

of free 1-OHP (1-OHP-F) was ordered from Sigma Chemical Co. (St Louis, MO, USA). The standards of both conjugates of glucuronide (1-OHP-G) and sulphate (1-OHP-S) were purchased from Midwest Research Institute, NCI Chemical Carcinogen Reference Standard Repository (Kansas City, MO, USA).

Subject recruitment and sample collection

A total of 106 subjects (77 male and 29 female) were recruited to the study through questionnaire interview, physical examination and personal exposure monitoring for PAHs. The participating subjects include 28 coke-oven workers, 49 residents living about 15 miles away from the coke oven factory and 29 gardeners in north-west suburban area in Beijing. The coke-oven workers were provided with protective gloves and clothes but no respirators during their operation. Therefore, inhalation is the major concern about the exposure to PAHs. All recruited subjects were monitored for their personal exposure twice within 4 weeks. On the day of the last personal exposure monitoring, each participating subject provided about 50 ml urine in the morning before starting their work and was then monitored for PAHs exposures for the entire workshift. They were asked to provide urine samples again at the end of the workshift. Each sample was collected in a sterile specimen container and then an aliquot was transferred into a 15-ml cell culture centrifuge tube which was labelled with a pre-assigned four-digit code. These aliquots of urine samples were kept at 4°C after collection in the field and during transportation to a local laboratory. All urine samples were then stored at -20°C until packed in dry ice and shipped to the USA. In addition, to investigate any error inherent in the technical procedures, ten quality-control samples were made from a pooled sample (mixture of leftover urine samples from control subjects). These samples were coded to appear like regular study samples and analysed randomly so that those performing the analyses were unaware that a given sample was an aliquot from the pooled sample.

To evaluate the clearance of 1-OHP in urine after exposure to PAH, nine subjects were selected from the 28 exposed workers to conduct a time-course study. They were first asked to provide urine samples in the morning before work on Friday, and then kept being monitored for PAHs exposure. At the end of the workshift they were asked to donate urine sample again (the '0' time point). Additional urine samples were collected in the following mornings of Saturday (16 h), Sunday (40 h) and Monday before work (64 h).

Personal exposure sampling and PAHs analyses

The personal exposure monitors consist of a 2-µm pore size, 37-mm diameter polytetrafluoroethylene (PTFE) filter followed by a two-section sorbent (100 mg/200 mg) tube containing washed XAD. All study subjects were monitored twice for PAHs exposures with a flow rate of about 2 l min⁻¹ for their entire workshifts. After sampling, the monitors were wrapped in aluminium foil and refrigerated to avoid potential sublimation and/or degradation due to ultraviolet light exposure. In addition, both blank and spiked monitors (10% of the total samples) were also prepared in the field. Three major PAHs components, including pyrene, benz(a)anthracene (BaA) and BaP, were analysed in the exposure monitors by high-pressure

liquid chromatography (HPLC) with fluorescence and ultraviolet light detectors according to the NIOSH Standard Procedure No. 5506 (NIOSH 1994).

Analyses for 1-OHP and cotinine in urine

Three forms of urinary 1-OHP, including 1-OHP-F, 1-OHP-G and 1-OHP-S, were determined simultaneously using HPLC with a fluorescence detector according to the method developed by Melikian et al. (2003). Briefly, to 180 μ l urine sample, 20 μ l DMSO were added and well mixed by stirring vigorously for 1 min. After being centrifuged at 10 000 rpm for 1 min, the supernatant was transferred into a tube and loaded onto the autosampler for injection and analysis. Analysis was carried out using an HPLC system (Water Model-1525, Milford, MA, USA) consisting of an autosampler (Water Model-717). Separation was achieved on a stainless steel ZORBAX SB-phenyl column (5 μ m, 4.6 mm i.d. $\times$ 250 mm, Aligent, Newport, DE, USA) with a 10 $\times$ 2 mm i.d. guard column. The mobile phase consisted of a mixture of acetonitrile and 10 mM sodium phosphate supplied at a flow rate of 1 ml min⁻¹. The peaks of 1-OHP-G, 1-OHP-S and 1-OHP-F were monitored at excitation and emission wavelengths of 242 and 388 nm with fluorescence detector (Water Model-2475 Multi λ Fluorescence Detector), respectively.

During the analyses, additional quality control procedures were carefully performed. These include: (1) HPLC was calibrated every day before starting analysis; (2) three working standards of 1-OHP-G were injected after every tenth sample, beginning after sample 10 on each day; (3) every tenth sample, beginning at sample 5 on each day, was repeated, i.e. two injections were made for precision of analysis. The analyses were halted, the HPLC was restarted and calibrated again if the variation was equal or greater than 5%. The LOD was 0.02 μ g l⁻¹ with a linear calibration curve obtained ranging from 0.05 to 5 μ g l⁻¹.

Cotinine, one of the major metabolites of nicotine, was selected as an indicator of smoking status for confounding analysis. It was quantified by radioimmunoassay at the American Health Foundation's Clinical Biochemistry Facility according to previously described methods (Melikian et al. 1993). Urinary creatinine was used to adjust the concentration of cotinine in urine samples for variations in liquid uptake between subjects. Creatinine was determined with a Kodak Ektachem 500 Computer-Directed Analyzer.

Statistical analyses

Log transformation was performed on both urinary 1-OHP and PAHs levels in air to improve the normality. Pearson correlation was used to evaluate the associations between PAHs exposure and urinary 1-OHP. Differences in the levels of urinary 1-OHP among groups were compared by a two-sample Student's *t*-test. Multiple linear regression analysis was used to evaluate the impact of PAHs exposure on 1-OHP while controlling potential confounders such as age, gender and cotinine levels. A mixed-effects model was used to calculate the half-life of 1-OHP-G; and the Delta method (Oehlert 1992), a method to estimate the standard error of a transformed parameter, was used to calculate its confidence interval. The data were analysed using SAS statistical packages (SAS Institute 2004). All *p*-values were two-sided.

Results

Reproducibility and reliability of urinary 1-OHP analyses

The reliabilities of measurements for urinary 1-OHP were evaluated by determining the variability among measurements of the QC samples prepared in the field from a homogeneous pool of urine. The coefficients of variation (CV) for 1-OHP-G and total 1-OHP in the 10 QC samples were 10.3 and 11.6%, respectively. Note that these values reflected the combined variations of two independent measurements, including assays of 1-OHP and creatinine in urine. The CVs for 1-OHP-G and total 1-OHP without modification of creatinine were all less than 10%, suggesting that the methods employed in this study were valid and reliable and, therefore, the results of 1-OHP were reproducible.

Personal exposure levels

The demographic characteristics of participating subjects and the levels of their personal exposures to pyrene, BaA and BaP monitored on the day of urine sample collections are shown in Table I. The personal exposures to BaP in coke-oven workers were extremely high with the mean and geometric means of 4.3 and $0.81 \mu\text{g m}^{-3}$, respectively. The BaP exposures in gardeners were within the ambient levels currently encountered and were the lowest among the three groups. The exposure ranges were broad and well-suited for examining the biomarker exposure–response relationship. Further analysis indicated that the individuals' exposure levels of pyrene were correlated very well with corresponding exposures to BaA ($r=0.925$, $p<0.0001$) and BaP ($r=0.9723$, $p<0.0001$), suggesting that the relative contribution of each monitored component of PAHs is constant and the measurement of individual's pyrene exposure or its metabolite can be used to well predict his/her exposures to the other two components of PAHs.

Urinary 1-OHP: response to PAHs exposure and confounding factors

Figure 1 shows the relative contributions of each form of 1-OHP to the total pyrene metabolite in urine. As expected, 1-OHP-G is the predominant form excreted in urine. It represents about 94% of total 1-OHP in highly exposed workers and 75% in gardeners with ambient levels of exposures. These results further support the finding from other investigators that 1-OHP-G is the major metabolite of pyrene in human urine (Strickland et al. 1994, Singh et al. 1995). Compared with 1-OHP-G, the

Table I. Characteristics of the study subjects and the levels of their personal exposures to pyrene, BaA and BaP monitored on the day of urine sample collections.

	Gender (M/F)	Age, mean $\pm$ SD (years)	Cotinine in urine ($\mu\text{g g}^{-1}$ creatinine)	Pyrene exposure (ng m^{-3})	BaA exposure (ng m^{-3})	BaP exposure (ng m^{-3})
Coke oven workers	28/0	34.4 ± 5.5	1370.5 ± 1381.4	1470.1 ± 6.6	977.9 ± 5.1	805.2 ± 5.2
Residents nearby	20/29	34.8 ± 4.2	995.4 ± 1331.9	49.5 ± 1.7	34.1 ± 2.4	25.1 ± 1.6
Gardeners in suburbs	29/0	30.4 ± 11.5	406.4 ± 673.2	11.4 ± 2.1	20.3 ± 1.4	8.3 ± 1.9

Levels of pyrene, BaA and BaP are expressed as geometric mean $\pm$ geometric standard deviation.

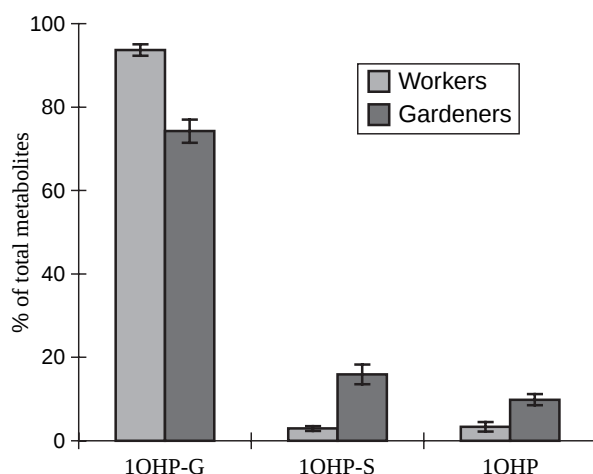


Figure 1. Distribution of three forms of pyrene metabolites in urine samples.

contributions from the free form of 1-OHP and its conjugate of sulfate are minor. Therefore, the levels of 1-OHP-G and total of 1-OHP were used for data analysis.

The correlation analyses showed strong associations of 1-OHP-G with not only exposure levels of pyrene, but also the exposures to BaA, BaP, and the sum of the three components analysed, suggesting that 1-OHP-G is a good exposure marker for pyrene and also other carcinogenic components of PAHs exposures (Figure 2). Similar to 1-OHP-G, the levels of total 1-OHP were also significantly correlated with each component of PAHs measured (data not shown). To determine the sensitivity of 1-OHP-G as an exposure marker, the subjects were divided into five subgroups according to their BaP exposure levels. As shown in Figure 3, the lowest two exposure groups represent the subjects exposed to BaP at different ambient levels. And the levels of BaP for the other three subgroups are believed to cover the entire range of BaP exposure in most occupational settings. In addition to an exposure response trend observed, a significant difference was also detected in 1-OHP-G between the lowest two exposure subgroups ($p < 0.05$). This suggests that 1-OHP-G is a specific and sensitive biomarker that can be used for risk assessment of BaP exposures at ambient levels.

To examine the effect of smoking on the levels of 1-OHP in urine, an initial analysis was first conducted to compare the levels of urinary 1-OHP-G between smokers and non-smokers among the residents and gardeners. As shown in Table II, all 78 study subjects without occupational exposures to PAHs were divided into three subgroups according to their cotinine levels in urine. The excretion of 1-OHP-G increased significantly in smokers and also showed an exposure-response trend with cotinine levels (Table II). Furthermore, multiple linear regression analyses of 1-OHP-G on PAHs exposures were conducted in both exposed and unexposed subjects with adjustment for potential confounders, including sex, age and cotinine levels. Again, smoking was identified as a major confounding factor to the formation of 1-OHP-G associated with PAHs exposure. However, after controlling for potential confounding by smoking, there were still very strong associations between PAHs exposures and 1-OHP-G (Table III).

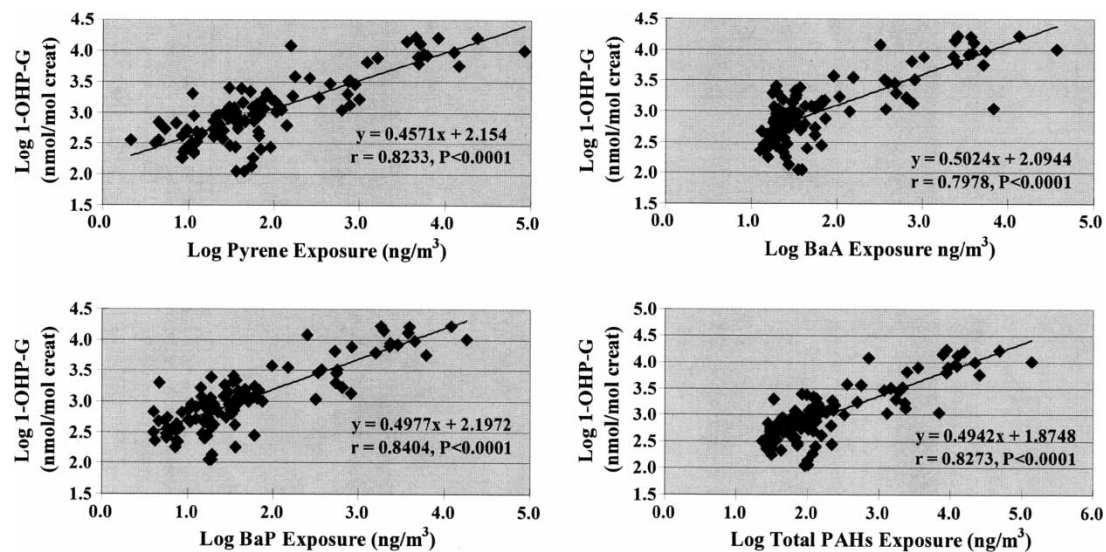


Figure 2. Correlations between 1-OHP-G in urine and personal exposures to pyrene, BaA, BaP and total PAHs.

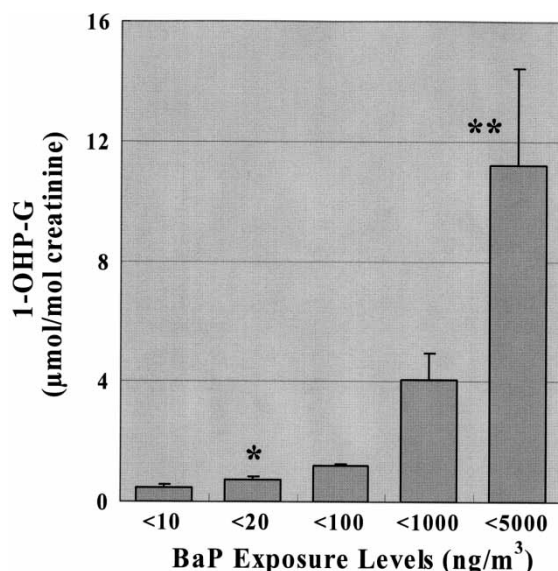


Figure 3. Levels of 1-OHP-G in urine samples collected from subjects grouped according to BaP exposures. *1-OHP-G in subjects with BaP exposures between 10 and 20 ng m^{-3} was significantly higher than in those exposed to BaP below 10 ng m^{-3} ($p < 0.05$). **Exposure-response trend ($p < 0.0001$).

Clearance of 1-OHP in urine after cessation of exposure to PAHs

Among the exposed workers recruited, nine subjects were selected for a time-course study to examine the clearance of 1-OHP after cessation of exposure to PAHs. Compared with the levels measured at the end of Friday workshift, about 50% of 1-OHP-G was detected in urine collected 16 h later and only around 30 and 20% could be found at 40 and 60 h after cessation of PAHs exposure, respectively (data not shown). It was estimated that the half-life of 1-OHP-G in the body was 15.4 h assuming a one-compartment model is involved.

Table II. Effects of cigarette smoking on the levels of 1-OHP-G and total 1-OHP in urine collected from both participating residents and gardeners.

Groups ^a	Number of subjects	Pyrene exposure (ng m^{-3}) ^b	1-OHP-G ($\text{nmol mol}^{-1} \text{Cr}$) ^c	Total 1-OHP ($\text{nmol mol}^{-1} \text{Cr}$) ^d
Non-smokers	28	21.2 ± 2.7	531.7 ± 1.8	712.5 ± 1.6
Moderate smokers	28	31.4 ± 2.4	584.9 ± 2.2	838.1 ± 1.9
Heavy smokers	22	38.2 ± 2.5	$820.2 \pm 2.1^{**,*}$	$989.1 \pm 2.0^{**}$

^aGrouped according to urinary cotinine levels (non-smokers: $<100 \mu\text{g g}^{-1}$ creatinine; moderate smokers: $<1000 \mu\text{g g}^{-1}$ creatinine; and heavy smokers: $\geq 1000 \mu\text{g g}^{-1}$ creatinine).

^bNo significant differences were detected among groups in the levels of personal exposures to pyrene (geometric mean $\pm$ geometric SD).

^cLevels of 1-OHP-G (geometric mean $\pm$ geometric SD) were significantly higher in heavy smokers than that in non-smokers ($^{**}p = 0.0012$) and moderate smokers ($^{***}p = 0.0325$).

^dLevels of total 1-OHP-G (geometric mean $\pm$ geometric SD) were significantly higher in heavy smokers than that in non-smokers ($^{**}p = 0.0061$).

Table III. Summary of multiple regression analyses of 1-OHP-G on PAHs exposures with adjustment of potential confounders.

Parameter	Estimate	SE	<i>p</i>
Intercept	6.1328	0.4842	<0.0001
Age	0.0172	0.0140	0.2232
Sex, F	-0.2485	0.2488	0.3203
Cotinine*	0.2622	0.0884	0.0037
Pyrene exposure*	0.0468	0.0121	0.0002
Intercept	6.3982	0.4249	<0.0001
Age	0.0153	0.0123	0.2167
Sex, F	-0.1027	0.2188	0.6399
Cotinine*	0.2625	0.0868	0.0049
BaA exposure*	0.1137	0.0262	<0.0001
Intercept	6.4355	0.3998	<0.0001
Age	0.1275	0.0162	0.2753
Sex, F	-0.0489	0.2064	0.8131
Cotinine*	0.2481	0.0823	0.0050
BaP exposure*	0.2456	0.0430	<0.0001

*For presentation purposes, in this regression model cotinine was expressed in mg g^{-1} creatinine and pyrene, BaA and BaP were expressed in $\mu\text{g m}^{-3}$.

Discussion

PAHs are not present in the environment as a single component, but as mixtures of several hundred compounds, including either carcinogenic or non-carcinogenic components. It is, therefore, not feasible to measure all components of PAHs routinely in environmental or biological samples. It has been reported that the relative contribution of each component of PAHs might vary from source to source, but was relatively constant as long as the air samples collected were from the same source (Jongeneelen 1992, 1997, Ny et al. 1993). Therefore, the measurement of one of the major PAH compounds can be used to predict the exposure to total PAHs. In the present study, the PAHs sampled from gardeners, residents and workers may not come from the same source. However, the levels of pyrene monitored were still correlated very well with corresponding BaA and BaP. Furthermore, the levels of urinary 1-OHP were observed to associate strongly not only with its parent compound, but also with BaA and BaP. These findings further support the fact that 1-OHP can serve as an exposure marker to predict the exposures to the mixtures or other components of PAHs.

It has been suggested that many confounding factors might affect the levels of 1-OHP or 1-OHP-G in urine (Strickland & Kang 1999). In addition to environmental exposures, tobacco smoke and diet are also sources of PAHs uptake. For example, mainstream smoke of filter cigarettes were reported to yield about 10 ng BaP per cigarette, leading to an intake of about 200 ng day^{-1} for a pack-a-day cigarette smoker (Grimmer et al. 1987, Hoffmann & Hoffmann 1997). Therefore, the potential effect of cigarette smoke on the excretion of 1-OHP-G and total 1-OHP in urine was examined in this study. As expected, significant differences were detected in urinary 1-OHP-G and total 1-OHP between heavy smokers and non-smokers among recruited residents and gardeners (Table II). In addition, the level of urinary 1-OHP-G was significant higher in heavy smokers than in moderate smokers. The levels of

environmental exposures to PAHs were in the same range without significant differences between smokers and non-smokers ($p > 0.05$). This result was consistent with previous study (Sithisarankul et al. 1997) that cigarette smoke makes a major contribution to the formation of 1-OHP-G in populations with similar environmental exposures to PAHs. Further evidence to support this finding came from the multiple regression analyses conducted with the entire set of participating subjects including those workers exposed to PAHs at high levels. Cigarette smoke was again identified as a confounding factor to the increase of 1-OHP-G, even though the significant levels for the effects of environmental exposures to PAHs on the formation of 1-OHP-G were not significantly changed by controlling for cotinine levels (Table III). This suggests that the contribution of cigarette smoke to 1-OHP-G may be relatively small compared with the high levels of occupational exposure to PAHs. However, this may not be true for the low levels of environmental exposure. As shown in Table IV, when the multiple regression analyses were conducted with the residents and gardeners only, the magnitude and the significant levels for the effects of environmental exposure on 1-OHP-G excretion were dramatically changed by adjustment of cotinine levels (the coefficient of BaP exposure reduced from 13.57 to 10.11 with p increased from 0.0008 to 0.0115, respectively). Therefore, the individual variations in cigarette smoking need to be properly adjusted while using 1-OHP-G as a biomarker to estimate environmental exposures to PAHs, especially at low ambient levels.

PAHs were reported to be present in barbecued meat at levels up to 164 ppb with BaP levels as high as 30 ppb (Panalaks 1976). The daily intake of BaP from diet is estimated to range from 120 to 2800 ng day⁻¹ (Hattermer-Frey & Travis 1991). This contribution may mask the influence of the inhaled PAHs from air on urinary 1-OHP, but this was unlikely to be a problem in the present study. First, the present study indicated that after cessation of exposures to PAHs the estimated half-life of 1-OHP-G was 15.4 h similar to 18 and 20 h reported in other early studies (Jongeneelen et al. 1990, Buchet et al. 1992). Compared with inhalation exposure, the clearance of 1-OHP-G appeared to be much faster for dietary exposure, with an estimated half-life of 4.4 h ranging from 3.9 to 6 h (Buckley & Lioy 1992). Therefore, dietary intake of PAHs may interfere with environmental exposure to PAHs on urinary 1-OHP-G less than cigarette smoking. Second, as indicated in a recent volunteers study, 1-OHP might not be a reliable bioindicator of ingested pyrene (PAHs) under normal feeding conditions since the biliary excretion and enterohepatic cycling may affect urinary excretion of 1-OHP derived from dietary intake of PAHs (Viau et al. 2002). Finally,

Table IV. Summary of multiple regression analyses of 1-OHP-G on BaP exposure with or without controlling for cotinine.

	Parameter, estimate $\pm$ SE	p
Intercept	0.2184 $\pm$ 0.2791	0.4364
Sex	-0.0322 $\pm$ 0.1321	0.8083
Age	0.0095 $\pm$ 0.0075	0.2071
BaP exposure	13.5723 $\pm$ 3.8906	0.0008
Intercept	-0.0486 $\pm$ 0.2818	0.8636
Sex	0.1580 $\pm$ 0.1422	0.2703
Age	0.0083 $\pm$ 0.0072	0.2513
BaP exposure	10.1179 $\pm$ 3.8989	0.0115
Cotinine	0.1564 $\pm$ 0.0543	0.0052

during the study period, the participants did not eat food known to be rich in PAHs according to a week-long daily food consumption recorded by the participating subjects before urine sample collection. We collected the dietary information from workers and gardeners. The following criteria proposed in the literature were used for estimating BaP intake ($\mu\text{g kg}^{-1}$) from their food consumption: cereals and bread, 0.2; leafy vegetables, 5.0; root vegetables and fruits, 0.2; meat and sausage, 3.0; barbecued meat, 30; fish, 1; and dietary products, 0.5 (Panalaks 1976, Lo & Sandi 1978, Hattemer-Frey & Travis 1991, Waldman et al. 1991, Scherer et al. 2000). The multiple regression analyses with adjustment for age, dietary intake, and cotinine levels showed that PAHs intake from food consumption did not interfere with environmental exposures in relation to 1-OHP-G excretion in urine (data not shown).

In addition to examining the potential confounding factors, the major purpose of this study is to determine the sensitivity and usefulness of 1-OHP-G in human populations with exposure to PAHs. As a biomarker, 1-OHP-G will be useful only if it can detect exposures at the levels likely encountered in the ambient air. As shown in Figure 1, however, 1-OHP-G accounts for only 75% of total urinary 1-OHP excreted in gardeners. This suggests that 1-OHP-G may be less sensitive compared with 1-OHP measured in urine with pretreatment of glucuronidase, especially for those subjects with low ambient exposure. In fact, this may not be true since 1-OHP-G was reported to be approximately three- to fivefold more fluorescent than free 1-OHP and, therefore, could be used to detect or predict much lower exposures of PAHs (Strickland et al. 1994, Giessing et al. 2003). As expected in our study, an excellent exposure-response relationship and strong correlation between PAHs exposure and 1-OHP-G excretion were observed in all recruited subjects with broad range of exposures. It was also found that the levels of 1-OHP-G in residents ($0.94 \pm 0.37 \mu\text{mol mol}^{-1}$ creatinine) were significantly higher than that in gardeners ($0.53 \pm 0.58 \mu\text{mol mol}^{-1}$ creatinine, $p < 0.0001$, data not shown). This finding demonstrates that 1-OHP-G is sensitive enough to distinguish the difference between various environmental settings. Furthermore, the personal exposure monitoring data indicated that 26 subjects out of the 78 participating residents and gardeners were exposed to BaP at the levels above 20 ng m^{-3} , which are not likely encountered in the developed countries. Therefore, further comparison was conducted between subgroups with BaP exposures either below 10 ng m^{-3} ($6.1 \pm 0.14 \text{ ng m}^{-3}$) or below 20 ng m^{-3} ($16.2 \pm 2.8 \text{ ng m}^{-3}$). A significant difference was still detected in 1-OHP-G between the two groups (Figure 3). Since, there were no significant differences between the groups in the potential confounding factors, such as age and cotinine levels, this finding provides strong evidence that 1-OHP-G is a reliable and sensitive biomarker for estimating PAHs exposures at the current ambient levels.

In conclusion, 1-OHP-G is a predominant form of pyrene metabolites and shows strong correlations with exposures to not only pyrene, but also BaA and BaP. It can serve as a sensitive and reliable biomarker for PAHs exposures at the levels currently encountered in the ambient air. Moreover, the potential confounding effects of cigarette smoke should be carefully considered and adjusted for while using 1-OHP-G as an exposure marker for risk assessment of PAHs. Overall, 1-OHP-G is an excellent indicator for internal dose of PAHs absorbed through all potential routes.

Acknowledgements

The authors thank all their Chinese colleagues who participated in the study for their excellent work; and they are grateful for the participation of the workers and the cooperation of the management in Taiyuan Iron and Steel Co., China. Work was supported in part by grants from the CDC/NIOSH, NIEHS; Grant Nos R21 OH07632 and ES00260.

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