Characterizing and Biological Monitoring of Polycyclic Aromatic Hydrocarbons in Exposures to Diesel Exhaust

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · MAY 2007

Impact Factor: 5.33 · DOI: 10.1021/es062863j · Source: PubMed

9 AUTHORS, INCLUDING:

Wei Huang
Peking University Health Science Center
31 PUBLICATIONS **812** CITATIONS
SEE PROFILE

Thomas J Smith
Harvard University
235 PUBLICATIONS **5,543** CITATIONS
SEE PROFILE

Robert Herrick
Harvard University
109 PUBLICATIONS **2,879** CITATIONS
SEE PROFILE

David Christiani
Harvard University
470 PUBLICATIONS **18,271** CITATIONS
SEE PROFILE

Characterizing and Biological Monitoring of Polycyclic Aromatic Hydrocarbons in Exposures to Diesel Exhaust

WEI HUANG,*,† THOMAS J. SMITH,† LONG NGO,§ TONG WANG,‖ HONGQIAO CHEN,‖ FANGGU WU,‖ ROBERT F. HERRICK,† DAVID C. CHRISTIANI,† AND HUI DING‖

Harvard School of Public Health, Boston, Massachusetts 02115, Harvard Medical School, Brookline, Massachusetts 02446, and Beijing Municipal Institute of Labor Protection, Beijing 100054, P.R. China

Polycyclic aromatic hydrocarbons (PAHs) are one of the most important and carcinogenic components in diesel exhaust (DE). Therefore, ambient PAHs concentrations were measured and characterized for work areas in a locomotive engine inspection plant. Pre- and post-shift urine samples and concurrent air samples were collected on 17 workers to measure the concentration of urinary 1-hydroxypyrene (1-OHP), a metabolite of pyrene. Increased urinary 1-OHP concentrations were observed over at least three consecutive sampling days. The biological kinetics of pyrene metabolism was studied with a one-compartment pharmokinetic model. The conversion rate and elimination rate of 1-OHP were estimated using nonlinear mixed-effects model, and validated with multiple nonlinear regression models by assessing the pattern of elimination rates of each worker separately. Urinary 1-OHP was confirmed to be a sensitive marker of PAHs exposure with mean half-life of 29 h in this population of Chinese workers. The study results would be beneficial to future occupational and environmental studies of PAH exposure.

Introduction

An important class of organic compounds emitted from diesel engines are polycyclic aromatic hydrocarbon (PAHs), such as pyrene, benzo[a]pyrene, and nitropyrene, some of which are mutagenic and cause adverse health effects (*1*). The emission is composed of vapor and aerosol fraction, and the PAHs are distributed between these phases. It is clear that in ambient air, the 2- to 4-ring PAHs, and 2-ring nitro-PAHs primarily exist in the vapor phase, whereas the PAHs with 5 rings or more are found primarily in the particulate phase (*2*). Some gaseous PAHs emitted in diesel exhaust have been shown to undergo reactions with OH and NO_3 radicals in the gas phase to produce nitropolycyclic lactones, which are of particular concern to human health, as the nitro-PAH products are often more mutagenic and carcinogenic than the original PAHs (*3*−*4*).

Exposure assessment for health effects of exposure to diesel exhaust should focus on the measurement of specific PAHs that are known carcinogens, such as benzo[a]pyrene (BAP). However, these compounds are typically found at very low levels in the ambient environment and are not unique to diesel exhaust. Also, biological monitoring has been considered a more accurate approach to quantify exposures because biomarkers integrate exposures over longer time periods, and exposure from multiple routes, such as inhalation and dermal contacts. Thus, both air monitoring and biological monitoring were conducted in this study to quantify the occupational exposures to PAHs in diesel exhaust (DE) particulates and the corresponding markers and internal doses of metabolites.

As the metabolite of pyrene (one of the major PAH compounds in combustion emissions), urinary 1-hydroxypyrene (1-OHP) has been studied intensively in occupations with various exposure levels to PAHs (*5*−*9*). Compared with the metabolites of other PAHs that have also been studied as potential biomarkers, urinary 1-OHP has been shown to be a specific marker of pyrene and detectable at low levels (*10*−*11*). For example, Zhao et al. (*10*) reported elevated urinary 1-OHP among residents in highly polluted industrial cities (Shenyang and Anshan) of China, as well as detectable urinary 1-OHP levels among students, bank officers, and housewives in the Beijing. However, significant inter-individual variation of urinary 1-OHP has also been reported in previous studies, possibly resulting from different metabolism rates among individuals. The half-life of pyrene among human subjects varies from 4 to 35 h (*12*), which may be due to differences in cytochrome P-450 enzyme *CYP1A1* activity (*5*, *13*−*14*). This enzyme is involved in the initial metabolic conversion of most PAHs, and can also be produced by pyrene in cigarette smoke (*15*), consumption of smoked meat (*16*), and exposures to environmental chemicals. Thus, potential factors in evaluating person to person variability in 1-OHP excretion should be considered.

Even though 1-OHP appeared to be a useful biological indicator of exposure to pyrene, few data are available on its pharmarcokinetic mechanism which is important in understanding the association between exposure and physiological response of exposed individuals to diesel exhaust particulates. In order to study the conversion and elimination of 1-OHP that results from exposure to pyrene, we collected ambient particulate samples of PAHs (including pyrene) and workers' pre- and post-shift urine samples over consecutive working days to estimate the inhaled PAH exposure and corresponding urinary 1-OHP levels in a locomotive engine inspection plant. The study objectives of this paper are (1) to characterize the indoor PAH compounds from samples collected in work area; (2) to quantify the formation rate of internal 1-OHP from inhaled pyrene among diesel-exposed Chinese workers; and (3) to estimate the excretion rate of 1-OHP and its corresponding half-life among Chinese workers.

Materials and Methods

Aerosol Sample Collection. A series of work area and outdoor measurements of PM_{10} and its PAH compounds were collected during working shifts at a locomotive engine inspection plant in both summer and winter in Beijing in 2000. Sampling was performed during two periods: from mid-May to early June (temperature 28 ± 5 °C, relative humidity 75 ± 15%), representing summer; and from early

November to mid-November (temperature 5 ± 5 °C, relative humidity 45 ± 15%), representing winter. During the two sampling periods, Beijing was under usual meteorological and seasonal conditions. No dust storms occurred during the summer sampling period, and the heating season had not yet started during the winter sampling period.

The locomotive plant is located about 10 km southwest of the Beijing urban area. There is a total workforce of 44 workers currently working on diesel locomotive engine inspection and repair in this plant. About 150 new and used locomotive engines are inspected in this plant annually. The testing cycle for each engine is between 7 and 10 work days. All of the working activities have been carried out in one principal plant (100 m × 60 m × 60 m) which is well-insulated but equipped with doors and windows. Four stacks and roof fans were built inside the plant to remove indoor pollutants. Workers take breaks in the adjacent control rooms while an engine is running under tests and work on the engine inspection in the plant when engines are shut off and cooled down. The testing of engines is not synchronized and some engines are running while others are under inspection. Smoking is strictly forbidden in the work areas; however, workers can smoke in the control rooms. Environmental tobacco smoke (ETS) is generally present in the control rooms, because most of the workers are smokers.

Air samples were collected at one fixed diesel engine area in the plant on a daily basis for PM_{10}, representing occupational exposure, in both summer and winter. PM_{10} and its particulate polycyclic aromatic hydrocarbons (PAHs) were collected on 200 mm × 250 mm glassfiber membrane filters using a high-volume pump (XH1000-TSP/PM_{10}, Hebei, P.R. China) at a sampling flowrate of 1050 L/min for 24 h. The filters were heated to 500 °C in a muffle furnace for 2 h before use to remove the organic compounds on filters. Both blank and sampled filters were weighed with an analytical balance with 10 μg resolution in room temperature. The high-volume gravimetric samples of PM_{10} were analyzed to determine concentrations of PAHs over daily 24-h sampling periods. Sampled filters were wrapped in aluminum foil and stored at −20 °C before analysis.

Urine Sample Collection. Multiple pre-shift and post-shift urine samples were collected on 17 workers over consecutive sampling days to determine the urinary 1-hydroxypyrene (1-OHP) content in winter only. Each study participant was sampled for 4 consecutive days to examine the variation in exposures between workers and over time for individual. Daily indoor PAH concentration was assigned as individual exposures on workers sampled on that day. During the sampling period, about 5−6 workers working inthe same shift were sampled simultaneously, and two shifts (from 8:00−16:00 and 16:00−24:00) were sampled each day. All workers were sampled for two 8-h morning shifts first, then they were switched to night shifts and followed for another two 8-h shifts, or vice versa.

Each worker was asked to provide the pre-shift urine sample upon arrival at work on the day, and to provide the post-shift urine sample upon leaving work for the day. Immediately after urine samples were collected, a 1-mL aliquot was separated from each sample and sent to an accredited hospital laboratory at the end of sampling day to determine the creatinine contents. The rest of the samples were stored at −20 °C until analysis.

At the beginning of the study, participants were asked to fill out a baseline questionnaire, providing information on age, height, weight, years at current job, and current smoking and drinking status. An approved human subject protocol was followed for the collection of all human data and samples. No personal identifiers were collected. Subject information was linked by a random code number assigned when the questionnaire was administrated. On each sampling day, each participant was asked to fill out a daily questionnaire on grilled or broiled food before the sampling days and number of cigarettes smoked during the sample days.

Chemical Analysis. PAH compounds analysis was performed in an accredited chemistry analysis laboratory using the U.S. EPA method (*17*). The soluble organic fraction was analyzed using a TuboMass GC/MS (Perkin-Elmer, NY). Sixteen PAH compounds in PM_{10} filters were identified and measured: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(b)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)-pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene, and benzo(ghi)-perylene.

PAHs were quantified against the calibration curves generated from the 16 PAHs standard mixtures (Chem. Service, Inc.) at three concentration levels. Filters were subjected to 18 h extraction with 130 mL methylene chloride in a Soxhlet apparatus. The extracts were concentrated to 3 mL in Kuderna-Danish concentrators, and then transferred for analysis into 5-mL vials using cyclohexane. A 0.4 μL aliquot of each sample was analyzed using a TuboMass GC/MS (Perkin-Elmer, NY) with a DB-5 fused silica capillary column (30 cm × 0.32 mm i.d.). The column temperature was set at 150 °C for 5 min, then was increased to 300 °C at 10 °C/min to be held for 10 min for the analysis. Helium was used as the carrier gas running at 60−80 psi. The PAHs recovery rate was 74.6%, and the limit of detection for PAH compounds was 1−5 ng for the 16 measured PAH compounds.

Urinary 1-OHP Analysis. *Sample Preparation.* Urine samples underwent hydrolysis before 1-OHP extraction and analysis. Phosphoric acid (5 mL) was added to 10 mL urine samples to adjust pH to 6.8. The buffer contained β-glucuronidase/sulfatase (450 000 units/g and 22 000 units/g) from Sigma Chemical Co. (St. Louis, MO). The buffer solution was mixed completely, and then was incubated at 37 °C for 1 h before instrumental analysis. It was found there was no difference on hydrolysis efficiency of urine between 1-hr incubation and 4-hr incubation (*10*).

Extraction and Separation. A SEP-PAK C_{18} cartridge from Waters (MA) was used to enrich and purify the extract of 1-OHP. The cartridge was primed with 5 mL of methanol, followed by 10 mL of distilled water, and then the hydrolyzed sample was passed through the cartridge at ∼5 mL/min. Subsequently, the cartridge was washed with 8 mL of methanol to elute the 1-OHP. High-performance liquid chromatography (HP 1050) with a fluorescence spectrophotometer (HP 1046A) was used for 1-OHP detection. The optimal excitation and emission wavelengths obtained by scanning different excitation and emission wavelengths (345 and 388 nm) for optimal sensitivity and specificity were used to quantify 1-OHP. Hydrolyzed urine sample (50 μL) wasinjected into to a 150 mm × 4.6 mm Phenomenex Luna C_{18} column. The column was running at flowrate of 1 mL/min at room temperature (22 ± 3 °C). The retention time of 1-OHP was 6.1 min. The limit of detection (LOD) of 1-OHP was 0.012 ng.

To minimize the effect of hydration states of subjects, the urinary 1-OHP concentrations were calibrated against their corresponding creatinine concentrations measured in an independent hospital laboratory and were expressed as μmol/mol creatinine. At extremely low or high creatinine levels, the correction may be incomplete.

Statistical Analysis. All field measurements and laboratory data were manually entered and stored in a spreadsheet and double-entered by the same person. Statistical analysis was performed using Statistical Analysis Software (SAS Release 8.0 for Windows, SAS Institute, Inc., Cary, NC). Statistical significance was reported at the 0.05 level.

Histograms, stem-and-leaf plots, and box plots were used to examine the distributions of all exposure variables, and

the results indicated that the exposure data were slightly skewed but reasonably near normal. Therefore, normally distributed data were not transformed in order to make the model results more interpretable. Sample data were excluded when there were equipment failures (e.g., pump sampling failure).

Arithmetic means (AMs) and geometric means (GMs) and the corresponding standard deviations (SDs and GSDs) were calculated. AMs are sensitive to large outlier values of measurements, while GMs are closely related to the median of lognormal data in many occupational situations. Box plots were used to illustrate the distribution of measurements by plotting the 10th, 25th, median, 75th, 90th, and outliers of observations.

Nonlinear Mixed-Effects Model. Calculations of conversion rates and elimination rates were based on a nonlinear mixed-effects pharmarcokinetic model with zero-order formation rates and first-order elimination rates (eq 1). The mean rates of all workers with repeated measurements on multiple days were estimated as follows (See Supporting Information):

$$OHP_{i,j+1} = (k_{ci} + k_c)Pyrene_{internal} + OHP_{ij} \times \exp^{-k_e(t_{i,j+1}-t_{ij})} + \epsilon_{ij} \quad (1)$$

$$Pyrene_{internal} = If \times Pyrene_{ij}(t_{i,j+1} - t_{ij}) + \epsilon_{ij} \quad (2)$$

In eq 1, $OHP_{i,j+1}$ represents the urinary 1-OHP concentration for the i-th worker at $(j+1)$-th hour, and OHP_{ij} represents the urinary 1-OHP concentration for the i-th worker at j-th hour. $Pyrene_{internal}$ is the internal dose of pyrene from inhalation exposure, which could be estimated using eq 2. k_c is the population parameter for conversion rate of internal pyrene into internal 1-OHP, and k_{ci} is the conversion rate for the i-th worker, distributed with an approximately normal, $\sim N(0, \sigma_c^2)$, random-effect. k_e is the elimination rate of internal 1-OHP, which equals ln2 divided by the half-life. Attempts were made to estimate individual random effects for elimination rate by including k_{ei}; however, convergences were not obtained. The term $(t_{i,j+1} - t_{ij})$ is the time intervals (in hrs) between either pre- and post-shift samples on same day or post-shift and next pre-shift sample on the next day, which are 8, 16, or 24 h in this paper.

The internal dose of pyrene was estimated using eq 2, which describes the process of internal pyrene formation from inhalation exposure. I represents the inhalation rate (equal to 10 L/min) for an average person, f represents the deposition fraction of particle-phase pyrene in the lungs, equal to 0.309 for pyrene (*18*).

In the pharmarcokinetic model (eq 1), the first pre-shift urinary 1-OHP concentration after 2 days of no exposure was considered as the background level, which resulted from the residual left over from occupational exposure from the previous week and the personal lifestyle (diet and smoking). During the work with exposure to PAHs, a portion of the pyrene was metabolized to 1-OHP and excreted on a daily basis, thus the corresponding formation and elimination rates could be calculated, and the half-life ($t_{1/2}$) of 1-OHP excretions could be calculated from the elimination rate.

Nonlinear Regression Models. Nonlinear regression models were also used to estimate the individual conversion rate and elimination rate of each individual separately:

$$OHP_{i,j+1} = k_{ci} \times Pyrene_{internal} + OHP_{ij} \times \exp^{-k_{ei}(t_{i,j+1}-t_{ij})} + \epsilon_{ij} \quad (3)$$

In eq 3, the deposited internal dose of pyrene was estimated also using eq 2. Fourteen nonlinear regression models were fitted and fourteen k_c's and fourteen k_e's were estimated. The mean, standard deviation, and median of the distributions of k_c and k_e were reported and used as descriptive information to check the result of the nonlinear mixed-effects model (eq 1).

TABLE 1. Concentration of Pyrene, Total PAHs (in ng/m^3), and PM_{10} (in μg/m^3)[a]

	summer ($N = 6$)		winter ($N = 6$)	
	AM ± SD	GM (GSD)	AM ± SD	GM (GSD)
pyrene	146 ± 27	148 (1.22)	114 ± 37	106 (1.48)
total PAHs	1798 ± 275	1780 (1.17)	1731 ± 567	1650 (1.41)
PM_{10}	156 ± 60	143 (1.62)	185 ± 72	174 (1.45)

[a] *N*: Number of sampling days; AM: arithmetic mean; GM: geometric mean; SD: standard deviation; GSD: geometric standard deviation.

Results and Discussion

Subjects Demographic Data. The major job in this plant is to conduct new diesel engine performance inspection before it is assembled into a locomotive unit, and to repair or inspect used engines as well. Workers with job titles of mechanics, electrical line inspector, and fuel line inspector work on each locomotive engine. A total of seventeen workers were recruited in this study for multiple sampling days. The age (mean ± SD) of the sampled workers was 30 ± 9 years. The working years at the current position for each worker was 7 ± 7 years; younger workers tended to have fewer years and older workers had more. Seventy three percent of the sampled workers were smokers. The average Body Mass Index (BMI) of workers was 24.2 ± 2.9.

Indoor PAHs Exposure. *PAH Compounds Concentration Levels.* Table 1 summarizes the indoor concentrations of pyrene, total PAHs, and PM_{10} sampled in summer and winter 2000. The concentrations of total PAHs were the sum of the 16 measured PAH compounds. In Beijing, strong northwest wind and dry weather characterize the winter season, whereas hot and humid weather with occasional showers are typical in summer season.

Although the high flow rates of sampling and long sampling period might have increased the volatilization resulting in the loss of lower molecular weight PAHs from samples in both seasons, the measurements were still significantly higher than reported ambient PAHs levels in an industrial area in Beijing (*19*). Huang et al. (*19*) reported the concentrations of the most carcinogenic PAH, BAP, were 44 and 65 ng/m^3 in the industrial area in Beijing during non-heating and heating seasons, respectively. The concentrations of the most carcinogenic PAH, BAP, in the occupational setting in this study varied between 138 and 382 ng/m^3 indoors and 93 and 724 ng/m^3 outdoors in winter, which are much higher than reported ambient BAP concentrations in Beijing using a similar sampling and analysis method.

Biological Monitoring of PAHs Exposure. *Pre- and Post-Shift Urinary 1-OHP Concentrations.* The mean concentration variation of pre- and post-shift urinary 1-OHP among 17 workers during 4 consecutive sampling days is presented in Figure 1. The first pre-shift urinary 1-OHP levels are close to the reported baseline levels among Chinese workers (*20*–*21*). The mean urinary 1-OHP concentration in the post-shift samples of the fourth day was 0.51 μmol/mol creatinine, which was two times higher than the mean concentration in the first pre-shift sample after 2 days off from work, 0.23 μmol/mol creatinine. As shown in Figure 1, the level of urinary 1-OHP generally decreased in the pre-shift and increased in the post-shift samples, and rose gradually over the 4 days. The differences between pre- and post-shift samples were not statistically significant, likely due to the small sample size. A few observations of high level of urinary 1-OHP in pre-shift samples were checked with the daily food ques-

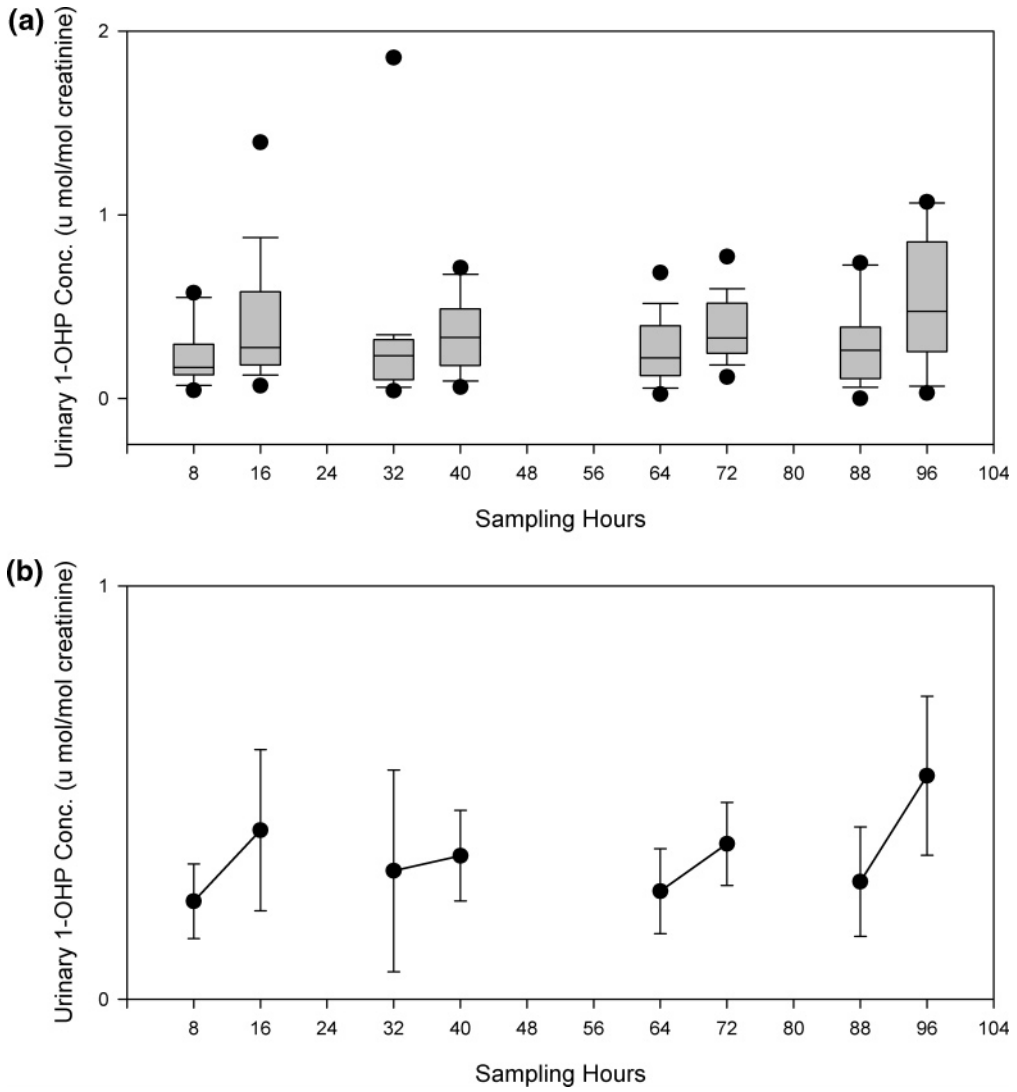

FIGURE 1. (a) Distribution of pre- and post-shift urinary 1-OHP concentrations over sampling hours. Each box plot shows the 10th, 25th, median, 75th, 90th percentiles. The outliers are also shown in the figures as dots. (b) Mean levels (95% confidence interval) of pre- and post-shift urinary 1-OHP over sampling hours.

tionnaire, and were determined to be associated with grilled or broiled food intake. However, the observed urinary 1-OHP concentrations were much lower in this study than in other occupations with similar environmental exposure levels (*22*–*23*). This is probably because workers spent less than 50% of work time in the diesel engine area during a full work shift (*24*), therefore the true personal exposures to PAHs were likely to be overestimated by using the ambient exposure values.

Pharmarcokinetics of Pyrene Metabolism. There was significant correlation between pre- and post-shift urinary 1-OHP concentration (the Pearson's correlation coefficient was 0.408 with p-value <0.01), and the majority of post-shift values were larger than pre-shift values. This suggested that the post-exposure urinary 1-OHP concentration might be better explained by the nonlinear metabolism kinetics among individuals. Therefore it is necessary to examine the conversion rate of 1-OHP from internal pyrene and urinary excretion rate of internal 1-OHP through a simple pharmarcokinetic model. This can also examine the observed urinary 1-OHP concentration variation between workers and over days.

In estimating the formation rate of internal 1-OHP and the elimination rate of internal 1-OHP, a pharmarcokinetic model was developed (see the appendix for model development methodology). A Nonlinear Mixed-Effects Model was used in analysis, with the urinary 1-OHP concentrations at different time points as the dependent variable. The model included two parts for the accumulation and elimination of internal 1-OHP: conversion of new OHP from internal pyrene during a given time interval, and the residual OHP after a period of removal. The conversion and elimination rates were estimated based upon the repeated measurements for each subject. Nonlinear regression models were also applied to estimate the conversion and elimination rates for each worker separately to validate the results obtained from linear mixed-effects model.

TABLE 2. Nonlinear Mixed-Effects Analysis of the Conversion Rate (k_c), Elimination Rate (k_e), and Half-Life ($t_{1/2}$) of 1-OHP among All Workers

k_c		k_e		$t_{1/2}$
estimate[a]	standard error	estimate (h^{-1})	standard error	half-life (h)
0.66	0.23	0.0239	0.0066	29.00

[a] In unit of (μmol OHP/mol creatinine)/(μg pyrene).

Nonlinear Mixed-Effects Model. Table 2 summarizes the parameter estimates (±SD) obtained in nonlinear mixed-effects analysis for all workers (eq 1). The urinary 1-OHP concentration after exposure was treated as the dependent

TABLE 3. Nonlinear Regression Analysis of the Conversion Rate (k_c), Elimination Rate (k_e), and Half-Life ($t_{1/2}$) of 1-OHP of Each Worker

	k_c		k_e		$t_{1/2}$	approximate
worker	estimate[a]	std error	estimate (h^{-1})	std error	half-life (h)	correlation k_c vs k_e
1	2.92	1.96	0.0449	0.0301	15.43	0.40
2	0.65	0.15	0.0688	0.0331	10.07	0.30
3	1.39	0.67	0.0179	0.0156	38.72	0.26
4	1.00	0.65	0.0064	0.0178	108.28	0.38
5	0.63	0.33	0.0150	0.0141	46.20	0.37
6	1.17	0.40	0.0180	0.0118	38.50	0.35
7	1.07	0.37	0.0571	0.0291	12.14	0.41
8	0.53	0.35	0.1087	0.1044	6.38	0.45
9	0.57	0.26	0.0143	0.0116	48.46	0.30
10	0.68	0.18	0.0272	0.0145	25.48	0.36
11	2.06	0.45	0.0501	0.0281	13.83	0.29
12	1.82	0.67	0.0301	0.0187	23.02	0.31
13	1.02	0.52	0.0211	0.0147	32.84	0.27
14	0.10	0.18	0.0561	0.0865	12.35	0.36
mean ± SD	1.10 ± 0.76		0.0385 ± 0.0286		33.07 ± 28.98	0.34 ± 0.06
median	1.00		0.0286		24.26	0.36
25% quantile	0.60		0.0165		12.24	
75% quantile	1.60		0.0566		42.46	

[a] In unit of (μmol OHP/mol creatinine)/(μg pyrene).

variable. Both the internal dose of pyrene, and the prior-exposure urinary 1-OHP concentrations were treated as covariates in the model. The conversion rate is treated as a random effect in the model with normal distribution $(0, \sigma^2)$. The initial conversion and elimination rates in nonlinear mixed-effects model (eq 1) were 0.18 (μmol OHP/mol creatinine) from 1 μg internal pyrene dose given to exposed subjects and 0.07 h^{-1}, respectively.

The interpretation for estimated conversion rate (standard error) is that 0.66 ± 0.23 μmol OHP/mol creatinine would result from 1 μg internal pyrene given subjects with exposure to pyrene. With this estimation, it would be feasible to determine the possible inhaled dose of pyrene for workers by measuring the urinary 1-OHP concentrations. Such an application would also provide information on reducing the work environment PAHs exposure to the baseline level for workers by measuring urinary metabolites levels.

Although the observed urinary 1-OHP concentrations in this study were much lower than the observations in other studies, the estimated elimination rate (equal to 0.0239/hr) and correspondingly derived half-life (equal to 29.00 h) after exposures are comparable with the observed mean values in most other studies showing the range of 5−35 h (*12*, *25*−*26*). The result suggested that Asian may have comparable metabolic oxidation of PAHs through cytochrome P-450 enzyme *CYP1A1* in comparison with other races. The findings also indicated an overnight accumulation in the body over a 4-day work schedule; however, the 2-day work break after each work week would be sufficient to reduce the internal dose back to baseline levels.

Nonlinear Regression Model. Table 3 shows the parameter estimates (standard error) obtained in nonlinear regression analysis for each individual worker, and the statistical summary of the parameter estimates. The urinary 1-OHP concentration after exposure was treated as the dependent variable. Both the internal dose of pyrene, and the prior-exposure urinary 1-OHP concentrations were treated as covariates in the model. Among a total of 17 sampled workers, the nonlinear regression results for 3 workers were either negative or not estimable. Therefore, the estimates for these three workers were excluded for statistical summary of all parameter estimates.

The statistical summary of estimated conversion and elimination rates was consistent with nonlinear mixed-effects analysis. The between-subjects variation was found to be significant; the conversion rate varied between 0.10 and 2.92 (μmol OHP/mol creatinine)/(μg pyrene), and the elimination rate and corresponding half-life varied widely between 0.0141 and 0.1044/h and 6.38 and 108.28 h. However, the approximate correlation matrixes for conversion rate and elimination were quite consistent among workers, in the range between 0.26 and 0.45 with a mean level of 0.34. This result indicated a quantitative and consistent relationship between internal transformation of exposed compound and corresponding elimination speed.

The significant between-worker variation of estimated conversion rates and elimination rates may be explained by the following: (1) The use of an exposure surrogate, limited number of fixed-location indoor measurements collected consistently over a 24-hr sampling period does not include the personal exposure variation between workers and probably caused less variation in estimating internal dose and more variability of parameter estimation. Huang et al. (*24*) found the between-worker variations of exposures were substantial. (2) Unmeasured vapor-phase PAHs and absorbed PAHs through dermal exposure could also substantially increase true exposure levels and internal doses, as well as the PAH exposure through diet, smoking, and exposure to second-hand smoke. (3) Inter-individual differences of metabolism through cytochrome P-450 enzyme *CYP1A1* and excretion kinetics of PAHs.

The results obtained from this study will be useful to assess the roles of both environmental and biological monitoring on diesel engine inspection workers' exposures to PAHs. Since the internal exposure of pyrene could be estimated using measured urinary 1-OHP and the calculated conversion and elimination rates of internal 1-OHP, the environmental exposure level of pyrene could be estimated based on internal dose estimation. Furthermore, the environmental exposure to total PAHs could also be estimated, if the relative portion of pyrene in total PAHs is known in a specific working environment. The mean conversion and elimination rates of 1-OHP estimated using two different statistical approaches yielded similar results. Although the estimation could be better evaluated if personal exposures were measured, the nonlinear mixed-effects model proved to be an efficient analysis method of repeated measurement study design.

Acknowledgments

This research was supported in part by the Fogarty International Institute Training Grant TW00828 to the Harvard School of Public Health (HSPH, USA) and by the Beijing Center for City Hazardous Source Control Technology at Beijing Municipal Institute of Labor Protection (BMILP, China).

Supporting Information Available

The pharmarcokinetic model development method is provided. The material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Lipsett, M.; Campleman, S. Occupational Exposure to Diesel Exhaust and Lung Cancer: A Meta-Analysis. *Am. J. Public Health* **1999**, *89* (7), 1009−17.

(2) Pankow, J. F.; Bidleman, T. F. Interdependence of the Slopes and Intercepts from Log-Log Correlations of Measured Gas-Particle Partitioning and Vapor Pressure: I. Theory and Analysis of Available Data. *Atmos. Environ.* **1992**, *26A*, 1071−80.

(3) IARC. *Monographs on the Evaluation of Carcinogenic Risks to Humans. 46. Diesel and Gasoline Engine Exhausts and Some Nitroarenes*; IARC: Lyon, France 1989.

(4) Tokiwa, H.; Ohnishi, Y. Mutagenicity and Carcinogenicity of Nitroarenes and Their Sources in the Environment. *Crit. Rev. Toxicol.* **1986**, *17*, 23−60.

(5) Wu, M. T.; Huang, S.; Ho, C. Cytochrome *P450 1A1 MspI* Polymorphism and Urinary 1-Hydroxypyrene Concentrations in Coke-Oven Workers. *Cancer Epidemiol., Biomarkers Prev.* **1998**, *7*, 823−829.

(6) Jongeneelen, F. J.; Scheepers, P. T. J.; Groenendijk, A. Airborne Concentrations, Skin-Contamination, and Urinary Metabolite Excretion of Polycyclic Aromatic Hydrocarbons among Paving Workers Exposed to Coal Tar Derived Road Tars. *Am. Ind. Hyg. Assoc. J.* **1988**, *49* (12), 600−607.

(7) McClean, M. D.; Rinehart, R. D.; Ngo, L.; Eisen, E. A.; Kelsey, K. T.; Wiencke, J. K.; Herrick, R. F. Urinary 1-hydroxypyrene and polycyclic aromatic hydrocarbon exposure among asphalt paving workers. *Ann. Occup. Hyg.* **2004**, *48* (6), 565−78.

(8) Tsai, P. J.; Shih, T. S.; Chen, H. L.; Lee, W. J.; Lai, C. H.; Liou, S. H. Urinary 1-Hydroxypyrene as An Indicator for Assessing the Exposures of Booth Attendants of a Highway Toll Station to Polycyclic Aromatic Hydrocarbons. *Environ. Sci. Technol.* **2004**, *38*, 56−61.

(9) Unwin, J.; Cocker, J.; Scobbie, E.; Chambers, H. An Assessment of Occupational Exposure to Polycyclic Aromatic Hydrocarbons in the UK. *Ann. Occup. Hyg.* **2006**, *50* (4), 395−403.

(10) Zhao, J. H.; Quan, W. Y.; Tian, D. H. Biological Monitoring of PAHs: Detection Method of Urinary 1-OHP. *China Environ. Monit.* **1990**, *6* (4), 37−40.

(11) Hu, Y.; Zhou, Z.; Xue, X.; Li, X.; Fu, J.; Cohen, B.; Melikian, A. A.; Desai, M.; Tang, M. S.; Huang, X.; Roy, N.; Sun, J.; Nan, P. Sensitive biomarker of polycyclic aromatic hydrocarbons (PAHs): urinary 1-hydroxyprene glucuronide in relation to smoking and low ambient levels of exposure. *Biomarker* **2006**, *11* (4), 306−18.

(12) Jongeneelen, F. J.; van Leeuwen, F. E.; Oosterink, S.; Anzion, R. B.; van der Loop, F.; Bos, R. P.; van Veen, H. G. Ambient and biological monitoring of cokeoven workers: determinants of the internal dose of polycyclic aromatic hydrocarbons. *Br. J. Ind. Med.* **1990**, *47* (7), 454−61.

(13) Perera F. P.; Mooney L. A.; Whyatt R. M. Molecular Epidemiology in Environmental Carcinogenesis. *Environ. Health Perspect.* **1996**, *104 Suppl 3*, 441−3.

(14) Adonis, M.; Martinez, V.; Riquelme, R.; Ancic, P.; Gonzalez, G.; Tapia, R.; Castro, M.; Lucas, D.; Berthou, F.; Gil, L. Susceptibility and Exposure Biomarkers in People Exposed to PAHs from Diesel Exhaust. *Toxicol. Lett.* **2003**, *144*, 3−15.

(15) Conney, A. H. Induction of Microsomal Enzymes by Foreign Chemicals and Carcinogensis by Polycyclic Aromatic Hydrocarbons. *Cancer Res.* **1982**, *42*, 4875−4917.

(16) Buckley, T. J.; Lioy, P. J. An Examination of the Time Course from Human Dietary Exposures to Polycyclic Aromatic Hydrocarbons to Urinary Elimination of 1-Hydroxypyrene. *Br. J. Ind. Med.* **1992**, *49*, 113−124.

(17) Winberry, W. T., Jr.; Forehand, L.; Murphy, N. T.; Ceroli, A.; Phinney, B.; Evans, A. *Methods for Determination of Indoor Air Pollutants (EPA Methods)*; Engineering Science, Inc.: Cary, NC, 1993.

(18) Volckens, J.; Leith, D. Partitioning Theory for Respiratory Deposition of Semivolatile Aerosols. *Ann. Occup. Hyg.* **2003**, *47* (2), 157−164.

(19) Huang, C. L.; Xu, W. J.; Zhao, G. D.; Zhong, J. X.; Jiang, K. The Composition of Polycyclic Aromatic Hydrocarbons in the Ambient Particulates in Beijing. *Environ. Sci.* (in Chinese). **2001**, *22* (4), 16−20.

(20) Lu, P. L.; Chen, M. L.; Mao, I. F. Urinary 1-Hydroxypyrene Levels in Workers Exposed to Coke Oven Emissions at Various Locations in a Coke Oven Plant. *Arch. Environ. Health* **2002**, *57* (3), 255−261.

(21) Pan, G.; Hanaoka, T.; Takahashi, K. A Study of Multiple Biomarkers in Coke Oven Workers-A Cross-Sectional Study in China. *Carcinogenesis* **1998**, *19* (11), 1963−1968.

(22) Lafontaine, M.; Payan, J. P.; Delsaut, P.; Morele, Y. Polycyclic Aromatic Hydrocarbon Exposure in an Artificial Shooting Target Factory: Assessment of 1-Hydroxypyrene Urinary Excretion as a Biological Indicator of Exposure. *Ann. Occup. Hyg.* **2000**, *44* (2), 89−100.

(23) Tsai, P. J.; Shieh, H. Y.; Shih, T. S. Urinary 1-Hydroxypyrene as a Biomarker of Internal Dose of Polycyclic Aromatic Hydrocarbons in Carbon Black Workers. *Ann. Occup. Hyg.* **2002**, *46* (2), 229−235.

(24) Huang, W. Air and Biological Monitoring of Occupational Exposure to Diesel Exhaust; Doctoral Thesis, Harvard School of Public Health, Boston, MA, 2003; pp 12−50.

(25) Buchet, J. P.; Gennart, J. P.; Lauwerys, R. Evaluation of Exposure to Polycyclic Aromatic Carbons in a Coke Production and a Graphite Electrode Manufacturing Plant: Assessment of Urinary Excretion of 1-Hydroxypyrene as a Biological Indicator of Exposure. *Br. J. Ind. Med.* **1992**, *49*, 761−768.

(26) Boogaard, P. J.; Van Sittert, N. J. Exposure to Polycyclic Aromatic Hydrocarbons in Petrochemical Industries by Measurement of Urinary 1-Hydroxypyrene. *Occup. Environ. Med.* **1994**, *51*, 250−258.

Received for review December 3, 2006. Revised manuscript received February 6, 2007. Accepted February 9, 2007.

ES062863J