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Investigation of PAH Biomarkers in the Urine of Workers Exposed to Hot Asphalt

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Airborne emissions from hot asphalt contain mixtures of polycyclic aromatic hydrocarbons (PAHs), including several carcinogens. We investigated urinary biomarkers of three PAHs, namely naphthalene (Nap), phenanthrene (Phe), and pyrene (Pyr) in 20 road-paving workers exposed to hot asphalt and in 6 road milling workers who were not using hot asphalt (reference group). Our analysis included baseline urine samples as well as postshift, bedtime, and morning samples collected over three consecutive days. We measured unmetabolized Nap (U-Nap) and Phe (U-Phe) as well as the monohydroxylated metabolites of Nap (OH-Nap), Phe (OH-Phe), and Pyr (OH-Pyr) in each urine sample. In baseline samples, no significant differences in biomarker levels were observed between pavers and millers, suggesting similar background exposures. In postshift, bedtime, and morning urine samples, the high pairwise correlations observed between levels of all biomarkers suggest common exposure sources. Among pavers, levels of all biomarkers were significantly elevated in postshift samples, indicating rapid uptake and elimination of PAHs following exposure to hot asphalt (biomarker levels were not elevated among millers). Results from linear mixed-effects models of levels of U-Nap, U-Phe, OH-Phe, and OH-Pyr across pavers showed significant effects of work assignments with roller operators having lower biomarker levels than the other workers. However, no work-related effect was observed for levels of OH-Nap, apparently due to the influence of cigarette smoking. Biological half-lives, estimated from regression coefficients for time among pavers, were 8 h for U-Phe, 10 h for U-Nap, 13 h for OH-Phe and OH-Pyr, and 26 h for OH-Nap. These results support the use of U-Nap, U-Phe, OH-Phe, and OH-Pyr, but probably not OH-Nap, as short-term biomarkers of exposure to PAHs emanating from hot asphalt.

Keywords: asphalt; biomarker; exposure; PAH; urine

INTRODUCTION

Asphalt (also referred to as bitumen) is a by-product of petroleum refining that is widely used to pave roads. Road-paving workers are exposed to emissions from hot asphalt that contain polycyclic aromatic hydrocarbons (PAHs), some of which are carcinogens (IARC, 1987). Although epidemiologic evidence points to increased cancer risks among asphalt-exposed workers (Partanen and Boffetta, 1994; Boffetta *et al.*, 1997; Hooiveld *et al.*, 2002; Burstyn *et al.*, 2003; Randem *et al.*, 2004), the dose–response relationship is not well defined (Chiazze *et al.*, 1991; NIOSH, 2000). This inability to characterize the asphalt/PAH dose–response relationship stems, in part, from the lack of data related to levels of PAH exposures from airborne and dermal routes among road-paving workers (Chiazze *et al.*, 1991; NIOSH, 2000).

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To better understand asphalt exposures and related health effects, efforts have focused on biomarkers of exposure, such as unmetabolized PAHs and PAH metabolites in urine. These short-term biomarkers represent internal doses of PAHs received from all routes during the current work shift. Indeed, a urinary metabolite of pyrene (Pyr) [namely 1-hydroxypyrene (OH-Pyr)] has been shown to reflect recent asphalt exposures (Jongeneelen *et al.*, 1988; McClean *et al.*, 2004b; Campo *et al.*, 2006b) and is regarded as a 'gold standard' for a general assessment of PAH exposures. More recently, urinary levels of unmetabolized naphthalene (Nap) and phenanthrene (Phe) (designated as U-Nap and U-Phe, respectively) (Campo *et al.*, 2006a; Buratti *et al.*, 2007), and of hydroxylated Nap and Phe metabolites (Väänänen *et al.*, 2003; Väänänen *et al.*, 2006; Buratti *et al.*, 2007), have been shown to reflect recent exposures to asphalt emissions. (Hereafter, the sum of 1- and 2-hydroxynaphthalene will be referred to as 'OH-Nap' and the sum of 1-, 2-, 3-, 4-, and 9-hydroxyphenanthrene will be referred to as 'OH-Phe'). However, the rates of uptake and elimination of these biomarkers have not been well characterized, and the influences of covariates, such as job, smoking status, and hydration level, have not been thoroughly examined as potential confounders or modifiers of urinary analyte levels.

In a previous analysis of highway construction workers' exposures to polycyclic aromatic compounds (PACs, a group of aromatic hydrocarbons containing primarily PAHs and heterocyclic compounds with four or more rings), 26 workers in the Greater Boston area of the USA were monitored during a workweek to evaluate the extent of PAC exposure from the air and dermal routes and the impact of PAC exposures on urinary levels of OH-Pyr (McClean *et al.*, 2004a,b). Results showed that measurements of PACs in the air (representing inhalation exposure) and of dermal patch samples (representing dermal exposure) varied significantly according to the work assignment (McClean *et al.*, 2004a). Additionally, results showed that urinary OH-Pyr concentrations in workers were significantly affected by inhalation of and dermal contact with asphalt emissions (McClean *et al.*, 2004b). The purpose of this study was to measure the levels of U-Nap, U-Phe, OH-Nap, OH-Phe and OH-Pyr in these same urine samples to identify additional useful surrogates of PAC/PAH exposure and to clarify the effects of potential confounders on urinary analyte levels. Here, we pay particular attention to correlations across measured urinary analytes, the rates of elimination of analytes from the body, and the effects on the subjects' urinary analyte levels due to the workday, work assignment, level of hydration (as measured by urinary creatinine), and smoking status. Because repeated samples of urine were collected from the workers over time, we applied mixed-effects models for statistical analysis, given the advantages of these models for estimating effects with correlated data (i.e. urinary analyte levels measured at different times from the same workers are positively correlated) (Rappaport and Kupper, 2008).

METHODS

Study population and design

Many details of this study have been published (McClean *et al.*, 2004a,b). In the current investigation, we consider 26 male highway construction workers residing in the Greater Boston area of the USA. Workers were recruited with informed consent according to a protocol approved for the protection of human subjects. Twenty pavers were involved with the application of hot asphalt on road surfaces and six millers were involved in removal of old road materials including asphalt. (Since the millers did not work with hot asphalt, they were included in this analysis as a reference group). The pavers were subdivided according to their work assignments, namely, paver operators (three workers), screedmen (five workers), rakers (eight workers), and roller operators (four workers). Paver operators drove the paving machines, screedmen were positioned on a platform at the rear of the paving machines, rakers followed closely behind the paving machines to fill holes, and rolling operators drove rolling machines to smooth and compact the asphalt.

Over each of two or three consecutive days, starting at the beginning of the workweek, urine samples were collected before work (morning), after work (postshift), and at bedtime. Morning samples from the first day of the workweek were treated as baseline samples. Overall, seven urine samples were collected from each miller (two sampling days), and 10 urine samples were collected from each paver (three sampling days). Urine was collected in sterilized polypropylene containers and stored at $-20°C$ for up to 7 years prior to analysis. Smoking status was obtained by questionnaire (6 smokers; 20 nonsmokers). Creatinine levels were determined using a colorimetric procedure (Sigma, 1984).

Measurement of urinary naphthalene (U-Nap) and phenanthrene (U-Phe)

Naphthalene and Phe were extracted from 0.7 ml of urine using head space solid-phase microextraction and analyzed using gas chromatography–mass spectrometry, as previously described (Waidyanatha *et al.*, 2003; Sobus *et al.*, 2009). Standard curves were prepared by spiking Nap, Phe, ($^2H_8$)naphthalene, and ($^2H_{10}$)phenanthrene (internal standards) into pooled urine collected from nonsmoking volunteer subjects from our laboratory. Quantitation of U-Nap and U-Phe in the samples was based on response ratios of analytes with respect to the corresponding internal standards.

The estimated limit of detection for both U-Nap and U-Phe was 0.40 ng l^{-1} (Sobus *et al.*, 2009). The estimated coefficients of variation for U-Nap and U-Phe were 0.25 and 0.26, respectively (Sobus *et al.*, 2009).

Analysis of urinary PAH metabolites

The hydroxylated metabolites, OH-Nap, OH-Phe, and OH-Pyr, were measured as described elsewhere (Onyemauwa *et al.*, 2009). Briefly, 3-ml aliquots of urine were digested using β-glucuronidase/arylsulfatase (Roche Diagnostics, Indianapolis, IN, USA), and the hydroxylated metabolites were purified using solid-phase extraction (EnvirElute PAH cartridges, Varian, Palo Alto, CA, USA) and analyzed via liquid chromatography–tandem mass spectrometry with a Surveyor LC system (ThermoElectron, San Jose, CA, USA) and a Finnigan TSQ Quantum Ultra triple quadrupole mass spectrometer (ThermoElectron). A ThermaGold C18 column (3 μm, 150 mm × 2.1 mm) (ThermoFisher Scientific, Waltham, MA, USA) was used for separation with a flow rate of 250 μl min^{-1}. Methanol and water were used as the mobile phase with a gradient of 55–81.8% methanol over 25 min. The following parent-to-product ion transitions were monitored: *m/z* 143.02–115.09 for 1- and 2-OH-Nap, *m/z* 193.02–165.08 for 1-, 2-, 3-, 4-, and 9-OH-Phe, and *m/z* 217.02–189.08 for 1-OH-Pyr. Calibration curves were prepared by adding standards and a mixture of isotopically labeled internal standards [($^{2}H_8$)1-hydroxynaphthalene, ($^{13}C_6$)3-hydroxyphenanthrene, and ($^{2}H_8$)1-hydroxypyrene] into pooled urine collected from nonsmoking laboratory volunteer subjects. Quantitation was based on the response ratio of each analyte with respect to the corresponding internal standard. The limits of quantitation (based on a signal-to-noise ratio of 10) for the urinary PAH metabolites were 0.001 μg l^{-1} for 2-OH-Nap; 0.002 μg l^{-1} for (2 + 3)-OH-Phe (these isomers could not be chromatographically separated and were quantified together); 0.005 μg l^{-1} for 1-OH-Phe, 4-OH-Phe, 9-OH-Phe, and 1-OH-Pyr; and 0.010 μg l^{-1} for 1-OH-Nap (Onyemauwa *et al.*, 2009). The estimated coefficients of variation for these analytes ranged from 0.053 to 0.27 (Onyemauwa *et al.*, 2009).

Statistical methods

For all statistical analyses, levels of 1- and 2-OH-Nap and levels of 1-, 2-, 3-, 4-, and 9-OH-Phe were summed and are presented here as OH-Nap and OH-Phe, respectively. Urinary analyte data were evaluated using SAS statistical software (v. 9.1, SAS Institute, Cary, NC, USA) after natural log-transformation to satisfy normality assumptions and to remove heteroskedasticity. Due to the repeated measures sampling design, linear mixed-effects models (Proc MIXED) were used to estimate correlation coefficients between analytes according to Hamlett *et al.* (2003). To test the effects of job (pavers and millers) and urine collection time (baseline, postshift, bedtime, and morning) on analyte levels, baseline data (one measurement per subject) were analyzed using general linear models (Proc GLM), and postshift, bedtime, and morning data (repeated measures) were assessed using mixed models (Proc MIXED). A P-value < 0.05 was considered significant (two-tailed test). Mixed models were also used to assess covariate influence [creatinine (g l^{-1}), time (h postshift), work assignment, workday, and smoking status (current smoker versus nonsmoker)] on analyte levels. Rather than dividing each analyte concentration by the corresponding creatinine concentration prior to statistical analysis, the creatinine concentration was included as an independent variable in the multivariable models to allow analyte levels and other covariates to be simultaneously adjusted for variations in urine dilution (Barr *et al.*, 2005). To assess time effects, postshift measurements were assigned values of 0 h, and bedtime and morning measurements were assigned values representing the times after the postshift urine samples were collected [bedtime median = 6.00 h (range: 2.50–14.3 h), morning median = 15.3 h (range: 11.819.1 h)]. Elimination rate constants (k) and biological half-lives ($T_{1/2} = -0.693/k$) were estimated using the regression coefficients for time after adjusting for significant covariates. Multivariable mixed models were evaluated using manual backwards stepwise elimination at a significance level of $\alpha = 0.10$. Bayesian Information Criterion (BIC) diagnostic values were used to select between competing models. A compound symmetry covariance matrix was used in all mixed-effects models. This covariance structure generally yielded the lowest BIC values compared to those from other homogeneous autoregressive and heterogeneous autoregressive matrices.

RESULTS

Descriptive statistics and correlation analysis for urinary analytes

Table 1 lists geometric means (GMs), geometric standard deviations (GSDs), and numbers of observations for each analyte measured in urine specimens collected at different times from pavers and millers. As expected, the GMs of urinary analyte levels were greater for pavers than for millers. Among pavers, OH-Nap was the most abundant biomarker (GM = 17 600 ng l^{-1}) followed by OH-Phe (GM = 7930 ng l^{-1}), OH-Pyr (GM = 2110 ng l^{-1}), U-Nap (GM = 92.0 ng l^{-1}), and finally U-Phe (GM = 70.8 ng l^{-1}). Figure 1 shows a correlation matrix for all analytes (in log scale). Significant pairwise correlations were observed in all cases, suggesting common exposure sources for these urinary PAH

Table 1. Descriptive statistics for urinary analytes (ng l^{-1}) measured in road pavers and millers

Analyte	Job	Baseline		Postshift		Bedtime		Morning	
		n	GM (GSD)	*n*	GM (GSD)	*n*	GM (GSD)	*n*	GM (GSD)
U-Nap	Pavers	20	29.6 (2.31)	58	92.0*** (2.13)	54	48.7** (2.28)	56	36.8 (2.14)
	Millers	6	36.1 (1.43)	11	34.4 (2.13)	9	31.2 (1.70)	9	26.4 (1.51)
U-Phe	Pavers	20	9.16 (3.12)	58	70.8*** (2.61)	54	32.4*** (3.46)	56	13.5 (3.40)
	Millers	6	14.9 (3.12)	11	24.7 (3.34)	9	12.8 (3.51)	9	6.56 (2.11)
OH-Nap	Pavers	19	9200 (3.42)	55	17600*** (2.45)	52	12500* (2.94)	54	10500 (2.87)
	Millers	6	11000 (2.16)	11	9510 (2.93)	9	4580 (3.11)	9	3830 (2.86)
OH-Phe	Pavers	19	1640 (2.26)	55	7930*** (2.14)	52	4750*** (2.73)	54	2400** (2.20)
	Millers	6	1380 (1.89)	11	1850 (2.39)	9	1690 (2.68)	9	1240 (2.36)
OH-Pyr	Pavers	19	375 (3.43)	55	2110*** (2.63)	52	1130*** (4.02)	54	708** (3.63)
	Millers	6	323 (3.79)	11	536* (3.97)	9	484 (3.01)	9	367 (3.64)

n, number of observations; U-Nap, urinary naphthalene; U-Phe, urinary phenanthrene; OH-Nap, sum of monohydroxylated metabolites of naphthalene; OH-Phe, sum of monohydroxylated metabolites of phenanthrene; OH-Pyr, 1-hydroxypyrene.
***Significantly different than baseline level (*P*-value < 0.0001); **Significantly different than baseline level (*P*-value < 0.05); *Marginally different than baseline level (*P*-value < 0.10).

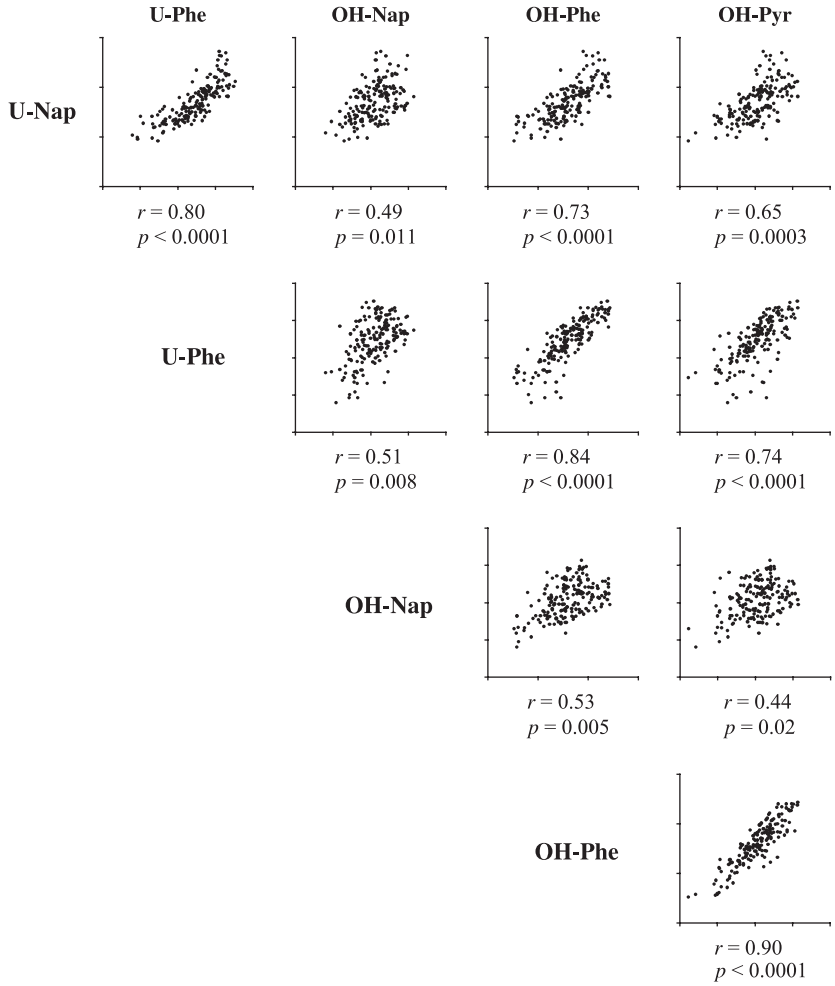

Fig. 1. Pairwise correlations between urinary analytes (in log scale) for milling and paving workers. *n* (number of paired observations) = 223 for U-Nap versus U-Phe and 215 for all other comparisons. (Legend: U-Nap, urinary naphthalene; U-Phe, urinary phenanthrene; OH-Nap, sum of monohydroxylated metabolites of naphthalene; OH-Phe, sum of monohydroxylated metabolites of phenanthrene; and OH-Pyr, 1-hydroxypyrene).

biomarkers. The strongest correlations were observed between OH-Phe and OH-Pyr ($r = 0.90$), U-Phe and OH-Phe ($r = 0.84$), and U-Nap and U-Phe ($r = 0.80$). Weaker correlations were observed when comparing OH-Nap to all other analytes ($r \leq 0.53$). We note that the levels of OH-Pyr measured here were highly correlated with the original OH-Pyr measurements reported by McClean *et al.* (2004b) ($r = 0.89$). Furthermore, a regression coefficient of 1.02 (standard error = 0.044) was observed when OH-Pyr measurements from this study were regressed on those from the original study in log scale (McClean *et al.*, 2004b). These results, combined with the fact that the overall GM OH-Pyr levels were nearly identical between studies [this study: GM = 1130 ng l^{-1} versus original study: GM = 1100 ng l^{-1} (based upon 194 common observations)], indicate good agreement between laboratories and that the prolonged period of storage (7 years) prior to our laboratory analyses did not appreciably affect measurements.

Effects of job and time of urine collection

In baseline urine samples, results from general linear models showed no significant effect of job on urinary analyte levels (P-value ≥ 0.37). When we considered all other collection times (i.e. postshift, bedtime, and morning), significant effects of job were observed after adjusting for time of urine collection (P-value < 0.05 for U-Nap, U-Phe, OH-Phe, and OH-Pyr; P-value = 0.07 for OH-Nap). Table 1 displays job-specific GM levels of urinary analytes for each urine collection time. Among millers, postshift, bedtime, and morning analyte levels were not significantly higher than baseline levels (P-value ≥ 0.09). However, among pavers, levels of OH-Phe and OH-Pyr were significantly higher than baseline levels in postshift samples (P-value < 0.0001), bedtime samples (P-value < 0.0001), and morning samples (P-value ≤ 0.02), and levels of U-Nap and U-Phe were significantly elevated in postshift samples (P-value < 0.0001) and bedtime samples (P-value ≤ 0.005). For OH-Nap, the increase in postshift levels over baseline levels was highly significant (P-value < 0.0001), while the increase in bedtime levels over baseline levels was marginally significant (P-value = 0.07).

Elimination rates and effects of hydration level, work assignment, workday, and smoking status

Biomarker levels among pavers were investigated with linear mixed-effects models to estimate elimination rate constants and to test for differences associated with hydration level (as indicated by levels of urinary creatinine), work assignments, workday, and smoking status. [Observations from millers were not included in this analysis because preliminary results showed no effect of occupational exposure on analyte levels (see Table 1). Additionally, as morning measurements of U-Nap, U-Phe, and OH-Nap from pavers were not significantly elevated above baseline levels (see Table 1), only postshift and bedtime measurements were included in the final models]. Table 2 shows estimated parameters from final models for each analyte. In each case, a significant positive effect of urinary creatinine was observed (P-value < 0.0001), indicating increased analyte levels with decreased urine volume. After adjusting for creatinine concentration, a significant negative effect of time was observed for each analyte (P-value ≤ 0.01), indicating a decrease in analyte levels from the time of postshift urine collection to bedtime. Using estimated elimination rate constants (as indicated by the negative regression coefficients for time in the models), the corresponding half-lives ($T_{1/2}$ values) and 95% confidence intervals were estimated to be 7.70 h (5.17 h, 15.1 h) for U-Phe, 10.2 h (7.07 h, 17.8 h) for U-Nap, 13.3 h (7.79 h, 46.2 h) for OH-Pyr, 13.6 h (9.00 h, 27.7 h) for OH-Phe, and 25.7 h (14.1 h, 116 h) for OH-Nap.

After adjusting for time, significant or marginally significant effects of work assignment were observed for U-Nap, U-Phe, OH-Phe, and OH-Pyr (P-value < 0.09). These results (shown in Table 2) indicate that analyte levels were lower in roller operators compared to paver operators, screedmen, and rakers. Specifically, results from the models suggest that U-Phe and OH-Phe levels in roller operators were significantly lower than in paver operators, screedmen, and rakers (P-value < 0.05), and that U-Nap and OH-Pyr levels in roller operators were significantly lower than in screedmen and rakers (P-value < 0.05) and marginally lower than in paver operators (P-value < 0.15). When roller operators were removed from the models, no significant effect of work assignment was observed across paver operators, screedmen, and rakers (P-value ≥ 0.05). Figure 2A–E shows baseline analyte levels along with postshift and bedtime analyte levels over three consecutive days. Because analyte levels did not differ significantly across paver operators, screedmen, and rakers, the mean (logged) values of these combined work groups, along with the mean values of roller operators, are displayed. The differences in analyte levels between roller operators and all other pavers are apparent for all analytes except OH-Nap (Fig. 2C). Increases in analyte levels over each workday were also observed (P-value < 0.10; Table 2) for U-Nap (Fig. 2A), OH-Phe (Fig. 2D), and OH-Pyr (Fig. 2E). While no work assignment or workday effect was observed for OH-Nap, a highly significant smoking effect was found on levels of OH-Nap, with smokers having higher analyte levels than nonsmokers (Table 2). No other analytes were significantly affected by smoking status.

DISCUSSION

Although some relationships between airborne exposures to PAHs and urinary PAH biomarkers have

Table 2. Results from the linear mixed-effects models evaluating urinary analytes in paving workers

Parameters	U-Nap		U-Phe		OH-Nap		OH-Phe		OH-Pyr	
	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value
Fixed effects										
Intercept	3.04 (0.331)	<0.0001	2.03 (0.413)	0.0002	9.68 (0.317)	0.0001	6.91 (0.288)	<0.0001	5.25 (0.457)	<0.0001
Creatinine (g l^{-1})	0.550 (0.111)	<0.0001	0.652 (0.159)	<0.0001	0.554 (0.085)	<0.0001	0.639 (0.100)	<0.0001	0.830 (0.145)	<0.0001
Time (h)	−0.068 (0.015)	<0.0001	−0.090 (0.022)	0.0001	−0.027 (0.011)	0.01	−0.051 (0.013)	0.0002	−0.052 (0.019)	0.007
Work assignment		0.09		0.003		0.3		<0.0001		0.02
Paver operator	0.724 (0.379)		0.918 (0.440)		0.141 (0.360)		0.960 (0.316)		0.857 (0.537)	
Screedman	0.782 (0.335)		1.33 (0.390)		0.565 (0.324)		1.25 (0.280)		1.21 (0.475)	
Raker	0.648 (0.306)		1.26 (0.356)		0.343 (0.303)		1.29 (0.257)		1.39 (0.435)	
Roller operator	0 (ref.)		0 (ref.)		0 (ref.)		0 (ref.)		0 (ref.)	
Workday		0.004		NS		NS		0.02		0.09
Day 1	−0.398 (0.125)						−0.305 (0.117)		−0.362 (0.164)	
Day 2	−0.065 (0.127)						−0.033 (0.119)		−0.170 (0.168)	
Day 3	0 (ref.)						0 (ref.)		0 (ref.)	
Smoker		NS		NS		<0.0001		NS		NS
No					−0.160 (0.256)					
Yes					0 (ref.)					
Random effects										
Between-subject variance component (σ^2_{bY})	0.194 (0.093)		0.210 (0.118)		0.190 (0.083)		0.127 (0.060)		0.406 (0.177)	
Within-subject variance component (σ^2_{wY})	0.279 (0.043)		0.648 (0.098)		0.148 (0.023)		0.223 (0.035)		0.440 (0.069)	

SE, standard error; NS, not significant; ref., reference group; U-Nap, urinary naphthalene; U-Phe, urinary phenanthrene; OH-Nap, sum of monohydroxylated metabolites of naphthalene; OH-Phe, sum of monohydroxylated metabolites of phenanthrene; OH-Pyr, 1-hydroxypyrene; σ^2_{bY}, between-person variance component (log scale); σ^2_{wY}, within-person variance component (log scale).

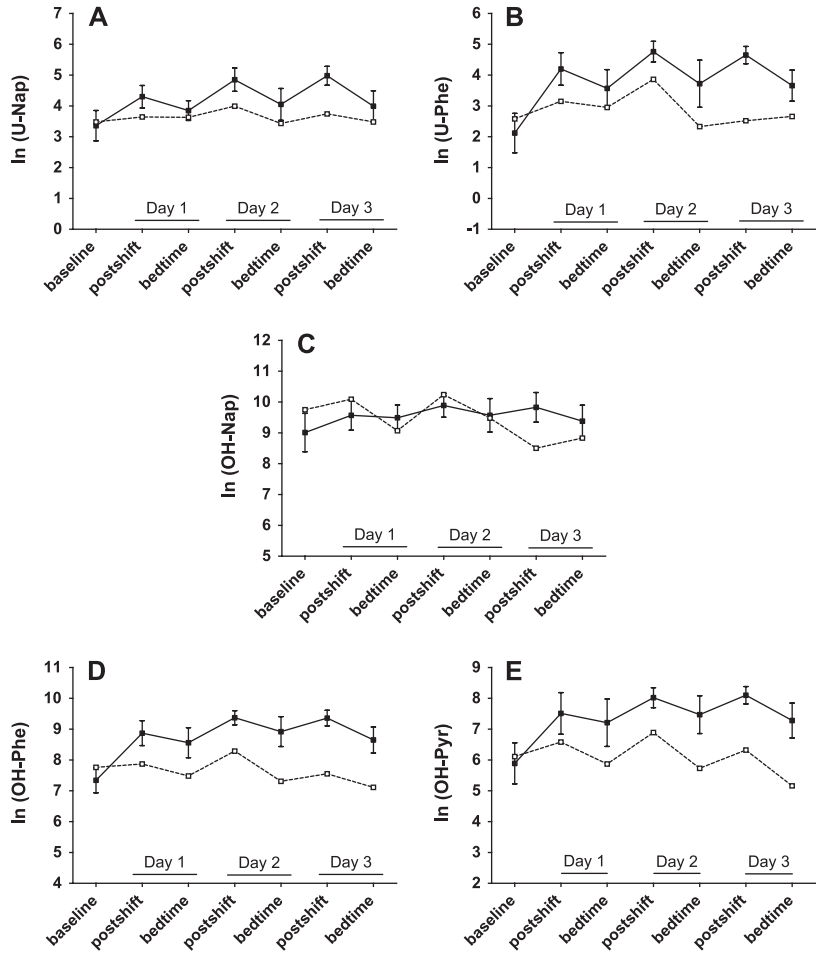

Fig. 2. Estimated means of logged concentrations (ng l^{-1}) and 95% confidence intervals of urinary analytes measured among paving workers at different times. Closed symbols represent paver operators, screedmen, and rakers; open symbols represent roller operators. Urinary analytes: (A) naphthalene, (B) phenanthrene, (C) hydroxynaphthalene, (D) hydroxyphenanthrene, (E) hydroxypyrene.

been examined in workers exposed to hot asphalt (Väänänen *et al.*, 2003; McClean *et al.*, 2004b; Campo *et al.*, 2006a,b; Väänänen *et al.*, 2006; Buratti *et al.*, 2007), questions remain regarding the rates of uptake and elimination for individual PAHs and the influence that jobs, hydration level, and smoking status might have on biomarker levels. To address these questions, we applied linear models to levels of five PAH biomarkers, measured in the urine of 26 road construction workers using a repeated measures sampling design (McClean *et al.*, 2004a,b).

Urinary concentrations of U-Nap, U-Phe, OH-Nap, OH-Phe, and OH-Pyr were comparable to those reported in other occupational studies of asphalt exposure (Campo *et al.*, 2006a,b; Buratti *et al.*, 2007). Overall, OH-Nap was the most abundant urinary analyte, followed by OH-Phe, OH-Pyr, U-Nap, and finally U-Phe. Our results indicate that the concentration of U-Nap was 0.35% of OH-Nap, and that the concentration of U-Phe was 0.85% of OH-Phe, based upon the overall median values of these analytes. These values are an about order of magnitude lower than those observed in a previous study of coke oven workers, where the mean levels of U-Nap and U-Phe were ∼4 and 13%, respectively, of the corresponding phenolic metabolite levels (Waidyanatha *et al.*, 2003). Since these coke oven workers are believed to have had higher exposures to Nap and Phe than the asphalt workers in the current study (Sobus *et al.*, 2009), these results suggest that higher amounts of unmetabolized Nap and Phe are excreted in urine at elevated exposure levels. These observations may indicate metabolic saturation for Nap and Phe in work environments where PAH levels are particularly high. However, additional research is needed to support this conjecture.

Overall, significant pairwise correlations between analyte levels suggest common exposure sources for Nap, Phe, and Pyr in paving and milling workers (see Fig. 1). In baseline samples collected on the

morning of the first day of the workweek, no differences in analyte levels were observed between pavers and millers. This indicates that PAHs arose from similar background sources over the preceding weekend, i.e., from the ambient environment and the diet, in both groups of workers. No significant increase above baseline levels was observed for any analyte in the urine of the millers (see Table 1), as expected because millers did not work with hot asphalt (McClean *et al.*, 2004a,b). For pavers, the largest departures from baseline levels were observed in postshift samples (Table 1). A comparison of GM levels showed that U-Phe was the most sensitive biomarker, with postshift levels being ~7-fold greater than baseline levels; increases above baseline were ~5-fold for OH-Phe and OH-Pyr, 3-fold for U-Nap, and 2-fold for OH-Nap.

Using linear mixed-effects models, we investigated effects of time, hydration level (based on measurements of urinary creatinine), work assignments, workday, and smoking status on levels of PAH biomarkers measured in postshift and bedtime urine samples from the 20 paving workers. Results showed that urinary creatinine was positively associated with each urinary analyte (P-value <0.0001), indicating higher analyte concentrations with reduced urine volume (see Table 2). These results for U-Nap and U-Phe were surprising, considering that unmetabolized organic compounds tend to enter urine via diffusion and, therefore, concentration should be independent of urine output (Boeniger *et al.*, 1993). Further research is warranted to determine the effects of creatinine on levels of U-Nap and U-Phe relative to those on OH-Nap, OH-Phe, and OH-Pyr. We note that the final statistical models for each analyte were nearly identical whether or not creatinine was included.

By including a fixed effect for time in our models, we were able to estimate elimination rate constants for each analyte. We first examined work assignment-specific elimination rates by including a 'time × assignment' interaction term in each model. Results showed that each model fit the data well using a single elimination rate constant, suggesting that analytes were eliminated at the same rate regardless of the work assignment. The estimated $T_{1/2}$ values and 95% confidence intervals were 7.70 h (5.17 h, 15.1 h) for U-Phe, 10.2 h (7.07 h, 17.8 h) for U-Nap, 13.3 h (7.79 h, 46.2 h) for OH-Pyr, 13.6 h (9.00 h, 27.7 h) for OH-Phe, and 25.7 h (14.1 h, 116 h) for OH-Nap. Although uncertainty was associated with these estimates (as indicated by the 95% confidence intervals), the unmetabolized compounds (i.e. U-Nap and U-Phe) appeared to be eliminated more quickly that the hydroxylated analytes; the rapid removal of U-Nap and U-Phe from the body has been observed elsewhere (Campo *et al.*, 2007). Slightly longer elimination half-lives were observed for OH-Phe and OH-Pyr. The estimates for these analytes (~13 h) were very similar and were within the range of estimates found in the literature for OH-Pyr (4–48 h) (Jacob and Seidel, 2002; Brandt and Watson, 2003; Hansen *et al.*, 2008). While not evaluated here, some assessments of the urinary excretion rate of OH-Pyr support a biphasic excretion process, including a rapid phase of elimination (hours), followed by a slower phase (days) (Heikkilä *et al.*, 1995; Buratti *et al.*, 2007). Additional evidence supports biphasic excretion of OH-Nap, with a very rapid initial phase (1.2–1.9 h), followed by a slower phase similar to that of OH-Pyr (14–46 h) (Heikkilä *et al.*, 1995). For this investigation, the estimate of 26 h for the half-life for OH-Nap is likely to be positively biased due to the highly significant effect of smoking.

Considering the estimated elimination rates of the PAH biomarkers in our study, we incorporated a fixed effect for 'workday' into our models to investigate bioaccumulation effects during consecutive workdays. No workday effects were observed for U-Phe, which had the shortest estimated elimination half-life or for OH-Nap, which was highly affected by smoking status. However, a significant workday effect was observed for U-Nap, OH-Phe, and OH-Pyr. For each analyte, levels were lowest on the first day of the workweek and increased over workdays two and three. While these significant workday effects point to accumulation of analyte levels from previous workdays, the contributions were relatively modest in relation to those associated with exposures on the current day (see Fig. 2).

After adjusting for time and workday effects, a significant effect of work assignment was observed for U-Nap, U-Phe, OH-Phe, and OH-Pyr among paving workers, with roller operators having the lowest urinary analyte levels. When roller operators were removed from the models, no significant differences were observed for any analyte between paver operators, screedmen, and rakers. Prior assessments of this study population showed that the rank order of exposures to PACs varied across work assignments according to the exposure type (air measurement or dermal patch measurement) and in the case of dermal patch measurements, the particular exposure surrogate (total dermal PACs versus dermal Pyr) (McClean *et al.*, 2004a,b). However, roller operators were shown to have experienced both the lowest air levels (measured by both total airborne PACs and airborne Pyr) and the lowest dermal patch levels (measured by both total dermal PACs and dermal Pyr) when compared to paver operators, screedmen, and rakers. Since results of our urinary biomarkers follow the same patterns, we have evidence that U-Nap, U-Phe, OH-Phe, and OH-Pyr are probably good surrogates for occupational exposure to PACs (including PAHs) in jobs employing hot asphalt.

Results from the final model for OH-Nap indicate that smoking status had a highly significant effect on analyte levels. Similar effects of smoking on OH-Nap have been observed in other studies of asphalt-exposed workers (Väänänen *et al.*, 2006; Buratti *et al.*, 2007). This smoking effect probably explains the lower correlation coefficients for OH-Nap compared to the other analytes (see Fig. 1). After adjusting for the smoking effect, no significant effect of work assignment was observed for OH-Nap in contrast to U-Nap, U-Phe, OH-Phe, and OH-Pyr. This effect of smoking reduces the utility of OH-Nap as a measure of PAH exposure among asphalt-exposed workers compared to the other urinary biomarkers measured in our study.

Since Nap is present in cigarette smoke (Rustemeier *et al.*, 2002), an effect of smoking status on both OH-Nap and U-Nap was expected. While a strong smoking effect was observed for OH-Nap, no smoking effect was observed for U-Nap (Table 2). Similar observations of the effects of smoking on OH-Nap and U-Nap have been observed in studies of workers exposed to jet fuel (JP-8) and coke oven emissions (Serdar *et al.*, 2003a,b; Waidyanatha *et al.*, 2003). Serdar *et al.* (2004) suggested that detoxification enzymes may be induced by cigarette smoke at low Nap exposure levels, causing smokers to produce higher levels of OH-Nap than nonsmokers at low exposure levels and smokers to produce lower levels of OH-Nap than nonsmokers at high exposure levels (Serdar *et al.*, 2004). A similar mechanism may explain the inconsistencies observed between OH-Nap and U-Nap in this study.

Levels of OH-Pyr in the urine have often been associated with smoking status in studies of PAH exposure (Van Rooij *et al.*, 1994; Hansen *et al.*, 2008). Moreover, the effects of smoking on OH-Pyr have been demonstrated in studies of occupational exposure to PAHs emanating from asphalt (Campo *et al.*, 2006b; Marczynski *et al.*, 2006; Väänänen *et al.*, 2006; Buratti *et al.*, 2007). In our analysis of 20 workers applying hot-mix asphalt, no smoking effect (P-value > 0.10) was observed for OH-Pyr after adjusting for creatinine concentration, time of sample collection, work assignment, and workday. This result was not entirely surprising, given that only a marginal effect (P-value < 0.10) of smoking on OH-Pyr was observed in a previous analysis of this population, after adjusting for age, body mass index, and inhalation and dermal Pyr exposure (McClean *et al.*, 2004b). The fact that milling workers (i.e. workers that did not work with hot-mix asphalt) were not included in our final statistical models may have reduced our ability to observe a significant smoking effect on OH-Pyr. Overall, the effects of smoking on urinary biomarkers of PAH exposure, particularly U-Nap, OH-Nap, and OH-Pyr, require further investigation, where PAH exposure levels (both through inhalation and dermal routes) and smoking intensity (rather than smoking status) are well characterized for all study participants.

Results from this study indicate that U-Nap, U-Phe, and OH-Phe, as well as the gold standard OH-Pyr, are useful biomarkers of exposure to PAHs/PACs emanating from hot asphalt sources. The short half-lives of these analytes, which ranged from ~8 to 14 h, indicate that urinary biomarker levels reflect occupational exposures to PAHs in the short term and suggest that representative samples should be collected immediately after the workshift. The effect of hydration level on analyte concentrations should be carefully considered when analyzing biomarker levels, even for unmetabolized PAHs in the urine, which are predominantly eliminated by passive diffusion. Significant differences in the levels of U-Nap, U-Phe, OH-Phe, and OH-Pyr across work assignments indicate that workers are differentially exposed to PAHs during the workshift, depending on the specific tasks that they perform; this information should be considered in future assessments of health risks for paving workers. Finally, our results indicate that OH-Nap is not a useful PAH exposure biomarker, given the confounding effect of smoking, and the lack of a work assignment effect on analyte levels.

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