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Using Urinary Biomarkers of Polycyclic Aromatic Compound Exposure to Guide Exposure-Reduction Strategies Among Asphalt Paving Workers

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Introduction: Paving workers are exposed to polycyclic aromatic compounds (PACs) while working with hot-mix asphalt (HMA). Further characterization of the source and route of these exposures is necessary to guide exposure-reduction strategies.

Methods: Personal air (n = 144), hand-wash (n = 144), and urine (n = 480) samples were collected from 12 paving workers over 3 workdays during 4 workweeks. Urine samples were collected at preshift, postshift, and bedtime and analyzed for 10 hydroxylated PACs (1-OH-pyrene; 1-, 2-, 3-, 4-OH-phenanthrene; 1-, 2-OH-naphthalene; 2-, 3-, 9-OH-fluorene) by an immunochemical quantification of PACs (I-PACs). The air and hand-wash samples were analyzed for the parent compounds corresponding to the urinary analytes. Using a crossover study design, each of the 4 weeks represented a different exposure scenario: a baseline week (normal conditions), a dermal protection week (protective clothing), a powered air-purifying respirator (PAPR) week, and a biodiesel substitution week (100% biodiesel provided to replace the diesel oil normally used by workers to clean tools and equipment). The urinary analytes were analyzed using linear mixed-effects models.

Results: Postshift and bedtime concentrations were significantly higher than preshift concentrations for most urinary biomarkers. Compared with baseline, urinary analytes were reduced during the dermal protection (29% for 1-OH-pyrene, 15% for I-PACs), the PAPR (24% for 1-OH-pyrene, 15% for I-PACs), and the biodiesel substitution (15% for 1-OH-pyrene) weeks. The effect of PACs in air was different by exposure scenario (biodiesel substitution > dermal protection > PAPR and baseline) and was still a significant predictor of most urinary analytes during the week of PAPR use, suggesting that PACs in air were dermally absorbed. The application temperature of HMA was positively associated with urinary measures, such that an increase from the lowest application temperature (121°C) to the highest (154°C) was associated with a 72% increase in ΣOH-fluorene and 1-OH-pyrene and an 82% increase in ΣOH-phenanthrene. Though PACs in hand-wash samples were not predictors of urinary analytes, the effects observed during the PAPR scenario and the week of increased dermal protection provide evidence of dermal absorption.

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Conclusions: Our results provide evidence that PACs in air are dermally absorbed. Reducing the application temperature of asphalt mix appears to be a promising strategy for reducing PAC exposure among paving workers. Additional reductions may be achieved by requiring increased dermal coverage of workers and by substituting biodiesel for diesel oil as a cleaning agent.

Keywords: asphalt; biodiesel; biomarkers; dermal; polycyclic aromatic compounds

INTRODUCTION

Asphalt (or bitumen) is used internationally as an industrial material and in the United States is predominantly used in the road paving industry [National Institute for Occupational Safety and Health (NIOSH), 2000]. Since asphalt contains a complex mixture of polycyclic aromatic compounds (PACs) that varies by crude oil source and application practices, paving workers are exposed to heterogeneous combinations of unsubstituted polycyclic aromatic hydrocarbons (PAHs), substituted PAHs, and PAH heterocyclic derivatives, many of which are either known or suspected to be carcinogenic (NIOSH, 2000).

Previous epidemiologic studies have described an excess risk of cancer among asphalt-exposed workers; however, despite a large literature that includes a study of almost 30 000 workers from eight different countries, limited information about the source of exposure has made it difficult to determine whether the excess cancer risk is attributable to asphalt or rather to coexposure to diesel oil, tobacco, and/or coal tar (Boffetta *et al*., 2003a,b; Burstyn *et al*., 2007; Schulte, 2007; Olsson *et al*., 2010). Further characterization of the source and routes of these exposures is also necessary to inform the design of new exposure-reduction strategies in the paving industry.

A previous investigation of paving workers found that urinary metabolites of PACs were associated with both inhalation and dermal exposure, though dermal exposure appeared to be the primary exposure route (McClean *et al*., 2004b; Sobus *et al*., 2009a,b). However, since the previous study was conducted with workers who used small paving machines (<16 000 pounds) without engineering controls, it is unclear whether the previous results are representative of workers who use larger, highway-class pavers that are equipped with engineering controls in accordance with guidelines developed by NIOSH in partnership with representatives from industry and labor (NIOSH, 1997). Additionally, there are remaining questions about the extent to which measured exposures were the result of contact with asphalt emissions and condensate versus the diesel oil commonly used by paving workers as a cleaning agent (McClean *et al*., 2004a; Weker *et al*., 2004).

Accordingly, a crossover study design was utilized to monitor a population of hot-mix asphalt (HMA) paving workers under four exposure scenarios: (i) normal working conditions, (ii) increased dermal protection, (iii) increased inhalation protection, and (iv) substitution of biodiesel for the diesel oil often used by workers to clean tools and equipment. The primary objectives were to use the repeated measures of urinary analytes collected during these four exposure scenarios to assess the impact of each on PAC absorption for the purpose of guiding future exposure-reduction strategies.

METHODS

Collaborative partnership

This study was conducted by a multidisciplinary team with representation from academia, government Centers for Disease Control and Prevention (CDC), industry (National Asphalt Pavement Association), and labor (Laborers' International Union of North America, International Union of Operating Engineers). The details of the study design and methods have been described previously and are summarized here (Kriech *et al*., 2011).

Study population

The study population included a total of 12 HMA workers, 4 workers from each of 3 paving crews (3 different companies) based in Wisconsin and Indiana, USA. Each crew included a paver operator who controlled the speed and direction of the paving machine, a screedman who controlled the depth and width of the asphalt mat, and a raker who used hand tools (e.g. rake, lute, shovel) for detailed work around street castings or curbs. The job of the fourth worker varied by crew (foreman, laborer, shuttle buggy operator). The foreman spent approximately half the time performing the duties of a screedman, while the other half was divided among raking and general foreman duties. The laborer spent over half of the time away from the paver (spray painting and driving the tack truck) and the rest of the time

performing the duties of a raker. The shuttle buggy operator controlled a machine used to remix and store the asphalt mix during transfer from the truck to the paver. Study protocols were reviewed and approved by the NIOSH Human Subjects Review Board, and written informed consent was obtained from each volunteer prior to participation.

Study design

The study was conducted in August, September, and October of 2008. Personal air, hand-wash, and urine samples were collected from each worker over 3 consecutive workdays during 4 workweeks (12 workers × 12 workdays = 144 workerdays), with each of the 4 weeks designed to evaluate a different exposure scenario: (i) normal operating conditions (also known as baseline scenario), (ii) providing gloves, hats with neck cloth, pants, and long-sleeved shirts to reduce dermal exposure (also known as dermal protection scenario), (iii) using powered air-purifying respirators (PAPR) to reduce inhalation exposure (also known as PAPR scenario), and (iv) substituting the biodiesel product B-100 (100% monoalkyl esters of long-chain fatty acids, containing no PACs) for the diesel oil normally used by workers to clean tools and equipment (also known as biodiesel substitution scenario). The order of the week-long exposure scenarios was randomized among the three crews, the personal protective equipment (PPE) was standardized across workers, and the crossover design allowed each worker to serve as his own control.

The dermal protection scenario was designed to assess the impact of an inexpensive and feasible approach that included string-knit gloves with a double-dipped latex coating, hats with cotton neck guards, cotton painter's pants, and cotton long-sleeved shirts. The biodiesel scenario was designed to assess the contribution of diesel fuel as a source of PAC exposure among asphalt workers. The PAPR scenario was not included as a possible intervention strategy, but rather to minimize inhalation exposure for the purpose of providing information about dermal exposure using an approach that is not limited by dermal exposure assessment methods. The PAPR included an airstream helmet and face shield (i.e., loose fitting) with a 3M organic vapor/high-efficiency cartridge (R.S. Hughes Company, Inc. Indianapolis, IN; #051131-07196). Units were calibrated pre- and postshift and monitored midday to ensure airflow of at least 6 cfm. Workers were permitted to lift their face shield while smoking and/or eating but were asked to smoke and/or eat away from the paving operation to minimize inhalation of asphalt-related PACs.

Participant characteristics including sex, age, height, weight, and smoking status were obtained at the start of the study. Highway-class pavers with engineering controls (NIOSH, 1997) were used on all but one occasion when a paver equipped with a widener was used to accommodate worksite-related logistical issues. Asphalt binder types also varied by crew and project and included the following Superpave performance grades (PG): PG 64-22, PG 58-28, and PG 76-22. Throughout each workday, a minimum of six readings of HMA application temperature were collected from the asphalt mat at the back of the screed using a HMA Lab Supply 8" Stainless Steel Dial Stem thermometer (Catalog no. TM-4500). Additionally, four measurements of wind speed, air temperature, and humidity were collected each workday using a Kestrel® 4000 Pocket Weather Tracker.

Collection and analysis of urine samples

A single void urine specimen was collected from each worker at preshift, postshift, and bedtime during each of the first 3 workdays of each week. An additional preshift sample was collected the morning after the third day of sampling (10 samples/worker/week × 12 workers × 4 weeks = 480 urine samples). The pre- and postshift samples were obtained onsite, while the bedtime samples were refrigerated overnight and transported to the site by the worker the following morning. Each urine sample was aliquoted in the field as follows: 10 ml urine to a glass tube for the analysis of hydroxylated PACs (OH-PACs), 3 ml urine and 1 ml methanol to a glass tube for the immunochemical quantification of PACs (I-PACs) and their metabolites, 1.25 ml urine to a cryovial for the analysis of cotinine, and 1.8 ml urine to a polypropylene vial for the analysis of creatinine. The aliquots were stored on ice in the field and transferred to a −20°C freezer at the end of each workday. At the end of each sampling week, urine samples were transported on dry ice to the NIOSH laboratory in Cincinnati, OH, for storage at 20°C (glass) or 50°C (plastic).

Aliquots were shipped on dry ice to the CDC laboratory in Atlanta, GA, for the analysis of the following 10 OH-PACs: 1-OH-pyrene; 1-, 2-, 3-, and 4-OH-phenanthrene; 1- and 2-OH-naphthalene; and 2-, 3-, and 9-OH-fluorene. Overnight enzymatic deconjugation using glucuronidase/arylsulfatase derived from Helix Pomatia was conducted to cleave the glucuronic acid and/or sulfate-conjugated OH-PACs. The resulting free OH-PACs were isolated with automated liquid–liquid extraction into pentane using a Gilson 215 Liquid Handler. Once

the pentane evaporated, the sample was reconstituted in toluene and derivatized to the trimethylsiloxane derivatives. The OH-PACs were quantified using gas chromatography (GC) isotope dilution high-resolution mass spectrometry (ThermoFinnigan MAT95XP) as described previously (Li *et al*., 2006).

The analyses of I-PACs, cotinine, and creatinine were conducted at the NIOSH laboratory in Cincinnati, OH. The analysis of I-PACs was conducted using an enzyme-linked immunosorbant assay kit that was developed for total PACs using phenanthrene standards (RaPID Assay PAH Test kit Product A00156/A00157; Strategic Diagnostics). The I-PAC measure was included as a biomarker of total PACs to complement OH-PACs as biomarkers of individual PACs. Additional details of this validated method have been described previously (Smith *et al*., 2011). Cotinine was determined using the Immulite® 2000 (Siemens Medical Diagnosis) analytical platform that uses solid-phase competitive chemiluminescent immunoassay. Creatinine was determined using the Vitros Autoanalyzer (Ortho Clinical Diagnosis).

Collection and analysis of air samples

Personal air samples were collected daily from each worker using a sampling train that included a 37-mm poly-tetrafluoroethylene membrane filter (2-μm pore size; Catalog. no. 225-27-07; SKC, Inc.; Eighty Four, PA) housed in a cassette and a XAD-2 polymeric resin/charcoal tube connected in series in accordance with NIOSH Method 5042 (NIOSH, 1998). The inlet was fastened to the outer layer of clothing near the breathing zone and attached to an AirChek 2000 personal air-sampling pump operating at 2 l min^{-1}. The sampler was located in the same position during all exposure scenarios, such that during the PAPR scenario, the inlet was positioned to sample unfiltered air (attached below and to the side of the face shield with the inlet pointing down). At the end of the work shift, samples were collected from workers, transported on ice, and stored at –20ºC.

The air samples were analyzed by the Heritage Research Group (HRG) in Indianapolis, IN. The filter and XAD tube were extracted together and analyzed for pyrene, phenanthrene, naphthalene, and fluorene using GC and time of flight mass spectrometry (GC/TOFMS) following the guidelines of EPA SW-846 8270C (Kriech *et al*., 2002). Total organic matter (TOM), defined as the amount of C6–C42 organics, was analyzed by GC equipped with a flame ionization detector (GC/FID) performed using a modification of EPA Method SW846-8015B (Kriech *et al*., 2002). Since the sample extracts were used for multiple analyses, no surrogates were added. Additional analytes were measured in air samples and have been described previously (Cavallari *et al*., 2012a).

Collection and analysis of hand-wash samples

Dermal exposures to PACs were quantified at the end of each worker's shift using a hand-washing method (Vaananen *et al*., 2005). Briefly, 3 ml of sunflower oil was added to the palm of one hand, and after rubbing the hands together for 1 min, the workers wiped the oil from their own hands using a crepe material (DuPont™ Sontara®; creped by Micrex Corporation). The hand-wash wipes were transported on ice and stored at –20°C until analyzed by HRG (Indianapolis, IN). Each wipe was extracted with dichloromethane and analyzed for TOM by GC/FID using a modification of EPA Method SW846-8015B, and for pyrene, phenanthrene, fluorene, and naphthalene by GC/TOFMS. Analytical results for hand-wash samples were normalized for each individual by dividing by the surface area of the hand, as measured in the field (Kriech *et al*., 2011). Additional analytes were measured in hand-wash samples and have been described previously (Cavallari *et al*., 2012b).

Statistical analysis

For all statistical analyses, the OH-PACs were analyzed and presented as follows: OH-Pyr (1-OH-pyrene), ΣOH-Phen (sum of 1-, 2-, 3-, and 4-OH-phenanthrene), ΣOH-Nap (sum of 1- and 2-naphthalene), and ΣOH-Fluor (sum of 2-, 3-, and 9-OH-fluorene). Urinary analyte data were evaluated using SAS statistical software (v. 9.1, Cary, NC) after natural log-transformation to satisfy normality assumptions. Since multiple urine samples were collected from each worker, linear mixed-effects models (Proc MIXED) were used to evaluate predictors of urinary OH-PACs and I-PACs using a compound symmetry covariance matrix structure. The air and hand-wash data were blank-corrected when the mean field blank was significantly different from zero. For all analytes in air, hand-wash, and urine samples, the limit of detection (LOD) divided by the square root of 2 was substituted for values less than the LOD.

The study was designed to determine whether (i) urinary OH-PACs and I-PACs vary by exposure scenario (baseline, biodiesel substitution, dermal protection, PAPR), (ii) urinary OH-PACs and I-PACs were associated with PACs measured in personal breathing-zone air and hand-wash samples, and (ii) the relationship between airborne PACs and urinary

biomarkers was different by exposure scenario. First, we evaluated how urinary analytes varied by exposure scenario (four-level categorical variable). Next, we evaluated continuous air and dermal measurements as predictors of urinary analytes, utilizing an interaction term that allowed the effects of air and dermal exposure to vary by exposure scenario (i.e. air models included terms for air, scenario, and air × scenario).

Cotinine, creatinine, and time of urine collection were included as predictors in all statistical models. Cotinine, a metabolite of nicotine, was included as a covariate to control for smoking. Creatinine, a waste product of muscle metabolism, was included as a covariate to control for water content of urine samples. The time variable was calculated by assigning postshift measurements a value of 0 h, while measurements obtained at bedtime and at preshift the next morning were assigned values representing the hours since collection of the previous postshift sample. Controlling for time in this way allowed the predictors associated with each workshift to be linked with a set of three urinary measures (i.e. exposures during day 2 linked to postshift on day 2, bedtime on day 2, and preshift on day 3), using all urine measurements with the exception of preshift on the first day of each week. Additional job factors evaluated as predictors included task (paver operator, screedman, raker, other), HMA application temperature, air temperature, work rate (tons of asphalt applied per hour), asphalt grade, crew, wind speed, and relative humidity.

RESULTS

The 12 workers who participated in this study were all male, with a mean age of 36 years (range of 24–59). Five of the workers were nonsmokers, six smoked cigarettes, and one was a smoker during only part of the study period (i.e. quit during the investigation). One nonsmoker chewed tobacco. Over the 36 sampling days, HMA application temperatures ranged from 121 to 154°C, and the crews primarily used asphalt grade PG 58-28 (61%) compared to PG 64-22 (33%) and PG 76-22 (6%).

Table 1 presents the geometric means (GMs), geometric standard deviations, and range for all urinary analytes (ng ml^{-1}) measured at preshift, postshift, and bedtime. All urinary analytes were detected above the LOD in >99% of samples, with the exception of 4-OH-Phen (96% > LOD). Among the postshift samples, concentrations of OH-PACs were highest for metabolites of naphthalene ($GM_{\Sigma OH\text{-}Nap}$ = 17.8 ng ml^{-1}), followed by fluorene ($GM_{\Sigma OH\text{-}Fluor}$ = 4.3 ng ml^{-1}), phenanthrene ($GM_{\Sigma OH\text{-}Phen}$ = 1.6 ng ml^{-1}), and pyrene ($GM_{OH\text{-}Pyr}$ = 0.7 ng ml^{-1}). The GM postshift and bedtime concentrations were significantly ($P < 0.05$) higher than preshift concentrations for most analytes, while adjusting for creatinine and cotinine. Though I-PACs were 29% higher at postshift than at preshift, the difference was not statistically significant. The comparisons of postshift and bedtime to preshift were similar irrespective of whether the preshift values on day 4 of each work were included or excluded in the analysis (*P*-value designations identical using either approach).

Table 1. Summary of urinary OH-PAC and I-PAC data (ng ml^{-1}).

Analyte	Preshift (n = 192)		Postshift (n = 144)		Bedtime (n = 144)	
	GM (GSD)	Range	GM (GSD)	Range	GM (GSD)	Range
ΣOH-Fluor	2.1 (2.2)	0.28–10.7	4.3 (2.3)**	0.4–33.4	2.8 (2.2)**	0.3–40.8
2-OH-Fluor	1.1 (2.2)	0.12–6.4	2.1 (2.3)**	0.17–13.0	1.4 (2.2)**	0.1–24.4
3-OH-Fluor	0.5 (2.9)	0.03–3.9	0.7 (2.8)**	0.04–5.4	0.5 (2.8)*	0.04–9.1
9-OH-Fluor	0.5 (2.1)	0.07–4.4	1.3 (2.7)**	0.005–18.7	0.7 (2.3)**	0.16–8.9
ΣOH-Nap	13.2 (2.4)	0.97–121	17.8 (2.5)*	1.1–117	13.2 (2.5)	2.2–266
1-OH-Nap	5.3 (2.7)	0.42–49.5	7.2 (2.8)*	0.63–49.9	5.7 (2.9)	0.5–121
2-OH-Nap	7.4 (2.3)	0.55–71.8	10.0 (2.5)*	0.50–67.6	6.9 (2.5)	0.9–145
ΣOH-Phen	0.9 (2.1)	0.12–5.9	1.6 (2.3)**	0.2–15.3	1.2 (2.2)**	0.2–18.5
1-OH-Phen	0.3 (2.2)	0.04–2.9	0.6 (2.4)**	0.08–8.4	0.4 (2.3)**	0.09–6.1
2-OH-Phen	0.2 (2.0)	0.03–1.1	0.3 (2.4)**	0.04–2.6	0.2 (2.2)**	0.05–3.1
3-OH-Phen	0.3 (2.1)	0.04–2.0	0.6 (2.4)**	0.08–5.9	0.5 (2.3)**	0.06–8.2
4-OH-Phen	0.04 (2.5)	0.005–0.3	0.08 (2.8)**	0.005–0.5	0.06 (2.8)*	0.005–1.1
OH-Pyr	0.4 (2.6)	0.05–5.3	0.7 (2.8)**	0.05–12.8	0.6 (2.8)**	0.07–12.8
I-PACs	143.2 (2.1)	17.0–1188	184.9 (2.4)	6.5–1453	137.9 (2.5)	5.0–1454

ΣOH-Nap, sum of 1- and 2-OH-naphthalenes; ΣOH-Phen, sum of 1-, 2-, 3-, 4-OH-phenanthrenes; OH-Pyr, 1-OH-Pyrene; I-PACs, immunochemical quantification of PACs.
*$P < 0.05$; **$P < 0.0001$. Comparison with preshift while controlling for creatinine and cotinine.

Table 2 presents the summary statistics for the five analytes measured in personal air (μg m^{-3}) and hand-wash (ng cm^{-2} for all but TOM in μg cm^{-2}) samples that were selected to correspond with the urinary analytes. In air samples, all analytes were commonly measured above detection limits, ranging from 78% to 99%. The frequency of detection was lower in hand-wash samples, such that only phenanthrene (98%), pyrene (73%), and TOM (54%) were included in additional statistical analyses. Detailed assessments of the air and dermal data have been published previously but are summarized here as key predictors of urinary analytes (Cavallari *et al*., 2012a,b).

Figure 1 shows the GM concentrations of phenanthrene metabolites during the workweek, where each time point summarizes data from 48 workerdays (12 workers × 4 exposure scenarios). The figure shows the four individual OH-phenanthrene metabolites (1-, 2-, 3-, 4-) and the sum of the four (ΣOH-Phen). There was a significant increase in ΣOH-Phen from pre- to postshift during each of the 3 workdays, followed by a subsequent decrease to bedtime and preshift the following morning. However, there was no evidence of an increase throughout the workweek, which was confirmed by an assessment of 'day' in regression models (data not shown). Though concentrations were higher for 1- and 3-OH-Phen than for 2- and 4-OH-Phen, the temporal patterns were consistent across metabolites, confirming that ΣOH-Phen provides a useful summary measure for use in statistical analyses. Similar temporal patterns and correspondence among related metabolites were observed for the other urinary analytes as well (data not shown).

Table 3 shows the results of the multivariate mixed-effects models evaluating predictors of urinary biomarkers. Compared with the baseline scenario, the urinary metabolite concentrations were consistently reduced during the three exposure scenarios (biodiesel substitution, PAPR, and dermal protection), though the reductions were only statistically significant for OH-Pyr and I-PACs. Since the urinary analyte data were log-transformed for statistical analysis, estimates of the percent reduction can be obtained by exponentiating the parameter estimates presented in Table 3. Accordingly, the dermal protection scenario was associated with reductions of 29% in OH-Pyr (i.e. $0.29 = 1 - e^{-0.34}$, 95% CI: 18–38%) and 15% in I-PACs (95% CI: 2–26%), the PAPR scenario was associated with reductions of 24% in OH-Pyr (95% CI: 12–33%) and 15% in I-PACs (95% CI: 2–26%), and the biodiesel substitution scenario was associated with reductions of 15% in OH-Pyr (95% CI: 0.3–27%).

The application temperature of the HMA was positively associated with concentrations of ΣOH-Fluor ($P < 0.0001$), ΣOH-Phen ($P < 0.0001$), and OH-Pyr ($P = 0.0002$). In fact, using the parameter estimates for HMA application temperature, an increase from the lowest application temperature (121°C) to the highest application temperature (154°C) was associated with a 72% increase in ΣOH-Fluor and OH-Pyr and an 82% increase in ΣOH-Phen. Air temperature also showed a consistently positive association with urinary metabolites but association was only significant for ΣOH-Phen ($P = 0.003$) and ΣOH-Pyr ($P = 0.002$). As expected, urinary cotinine and creatinine showed a consistently positive association with all urinary analytes, indicating that increased exposure to tobacco smoke and decreased hydration were associated with elevated concentrations of urinary analytes. Additional job factors such as task, work rate, asphalt grade, crew, wind speed, and relative humidity were evaluated but not found to be associated with urinary analytes and were therefore excluded from final models.

A significant negative effect of time (hours) was observed for each analyte, indicating a decrease in urinary metabolites from the time of postshift, to bedtime, to preshift the next morning. The one exception was I-PACs, which is not surprising given

Table 2. Summary of personal air and dermal exposure data.

Analyte	Air (μg m^{-3})			Postshift hand-wash (ng cm^{-2})		
	>LOD (%)	GM (GSD)	Range	>LOD (%)	GM (GSD)	Range
Fluorene	78	0.06 (6.5)	0.01–1.6	48	*(a)*	0.03–5.0
Naphthalene	87	0.83 (4.5)	0.30–13.8	13	*(a)*	0.23–1.2
Phenanthrene	99	0.38 (2.8)	0.05–4.4	98	1.4 (3.9)	0.04–9.8
Pyrene	92	0.06 (3.5)	0.009–0.9	73	0.3 (7.9)	0.03–6.1
TOM[b]	99	864 (2.3)	0.14–5395	54	*(a)*	5.4–264

LOD, limit of detection; GM, geometric mean; GSD, geometric standard deviation.
[a]Not shown due to low percentage of detection above the LOD.
[b]Units for TOM (total organic matter) in hand-wash samples are μg cm^{-2}.

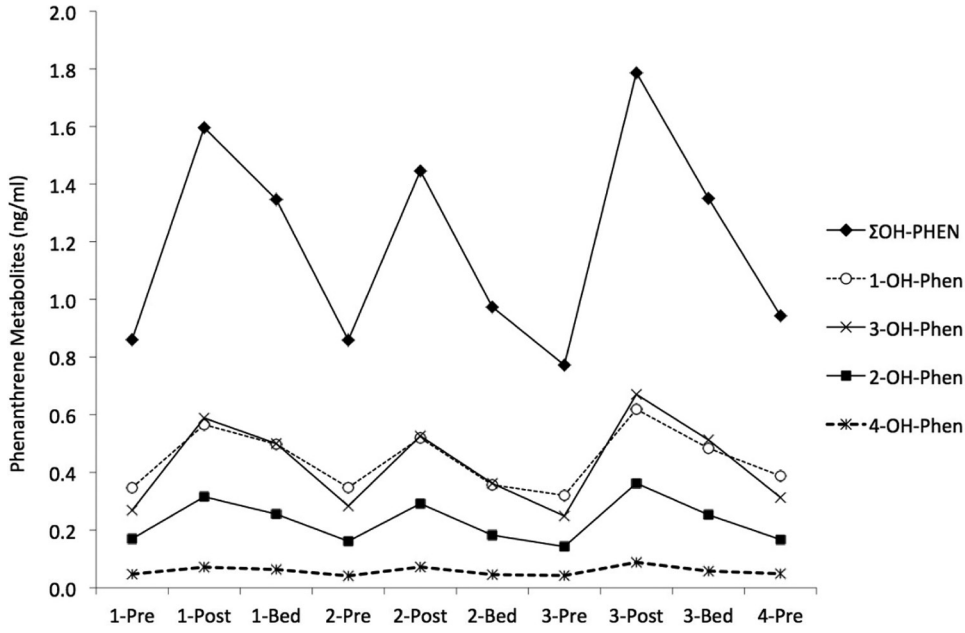

Fig. 1. Geometric mean concentrations of urinary phenanthrene metabolites at preshift, postshift, and bedtime throughout workweek during all exposure scenarios. Concentrations shown are not adjusted for creatinine or cotinine.
Note: ΣOH-PHEN, sum of 1-, 2-, 3-, 4-OH-phenanthrenes.

that I-PACs is an immunochemical quantification of many PACs and metabolites. The half-life estimates that correspond to the elimination rate constants for each urinary analyte (negative regression coefficient of time) are not presented since the exposures in this population were relatively low compared with other studies of PAC-exposed workers (in part due to the study design) and would therefore result in inflated estimates.

Table 4 presents the results from models investigating the measured concentrations in air and hand-wash samples as predictors of the corresponding urinary metabolites as follows: fluorene for ΣOH-Fluor, naphthalene for ΣOH-Nap, phenanthrene for ΣOH-Phen, pyrene for OH-Pyr, and TOM for I-PAC. Each model included an interaction term to determine whether the exposure–dose relationship (i.e. relationship between PACs in air and the corresponding urinary analytes, or between PACs in dermal samples and the corresponding urinary analytes) differed by exposure scenario. The effect estimates are, therefore, presented by scenario in Table 4.

Concentrations in air were consistently associated with urinary analytes, though the relationship between the airborne PAC and corresponding urinary analyte was different by experimental scenario for ΣOH-Fluor (interaction $P = 0.049$), ΣOH-Phen (interaction $P < 0.0001$), and OH-Pyr (interaction $P < 0.0001$). The observed effect of PACs in air was largest during the biodiesel substitution week, with parameter estimates that were approximately three to six times higher than during the baseline scenario (e.g. $\beta = 3.98$ compared with $\beta = 0.64$ for OH-Pyr), suggesting that the same 1 unit increase in airborne pyrene would have a significantly larger effect on urinary OH-Pyr during the biodiesel substitution week than during the baseline week. The observed effect of air was next highest during the dermal protection week, with parameter estimates that were approximately two to four times higher than during the baseline scenario (e.g. $\beta = 2.84$ compared with $\beta = 0.64$ for OH-Pyr). Interestingly, PACs in air were found to be significantly associated with urinary analytes during the PAPR week.

The concentrations of PACs in hand-wash samples were not observed to be significant predictors of the urinary analytes. The largest effect estimate was observed for phenanthrene as a predictor of ΣOH-Phen during the biodiesel substitution week; however, even this association was not statistically significant ($P = 0.1$).

DISCUSSION

This study was designed to evaluate the contribution of inhalation and dermal PAC exposure to

Table 3. Exposure scenario and job factors as predictors of urinary metabolites using multivariate mixed-effects models.

	ΣOH-Fluor		ΣOH-Nap		ΣOH-Phen		OH-Pyr		I-PACs	
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
Fixed effects										
Intercept	4.4 (0.7)	<0.0001	8.8 (0.7)	<0.0001	2.5 (0.6)	0.002	2.3 (0.8)	0.01	3.1 (0.7)	0.0006
Exposure scenario		0.9		0.1		0.4		0.0003		0.06
Baseline	Reference		Reference		Reference		Reference		Reference	
Dermal protection	–0.02 (0.07)	0.8	0.13 (0.07)	0.07	–0.01 (0.06)	0.9	–0.34 (0.07)	<0.0001	–0.16 (0.07)	0.02
PAPR	–0.06 (0.07)	0.4	0.02 (0.07)	0.8	–0.09 (0.06)	0.2	–0.27 (0.07)	0.001	–0.16 (0.07)	0.02
Biodiesel substitution	–0.03 (0.08)	0.7	–0.05 (0.07)	0.5	–0.08 (0.07)	0.3	–0.16 (0.08)	0.05	–0.10 (0.07)	0.2
Time	–0.05 (0.005)	<0.0001	–0.01 (0.005)	0.01	–0.04 (0.004)	<0.0001	–0.03 (0.005)	<0.0001	–0.006 (0.005)	0.2
HMA temperature	0.009 (0.002)	<0.0001	–0.001 (0.002)	0.8	0.01 (0.002)	<0.0001	0.009 (0.002)	0.0002	0.003 (0.002)	0.1
Air temperature	0.004 (0.004)	0.3	0.002 (0.004)	0.6	0.01 (0.004)	0.003	0.01 (0.004)	0.002	0.0003 (0.004)	0.9
Cotinine	0.0001 (0.00001)	<0.0001	0.0001 (0.00001)	<0.0001	0.00003 (0.00001)	0.004	0.0001 (0.00002)	0.001	0.0001 (0.00001)	<0.0001
Creatinine	0.01 (0.0004)	<0.0001	0.004 (0.0004)	<0.0001	0.005 (0.0003)	<0.0001	0.005 (0.0004)	<0.0001	0.005 (0.0004)	<0.0001
Random effects										
Between-worker σ^2	0.08 (0.04)	0.04	0.15 (0.07)	0.05	0.10 (0.05)	0.04	0.33 (0.1)	0.02	0.10 (0.05)	0.03
Within-worker σ^3	0.24 (0.02)	<0.0001	0.23 (0.02)	<0.0001	0.19 (0.01)	<0.0001	0.26 (0.02)	<0.0001	0.21 (0.02)	<0.0001

SE, standard error; ΣOH-Fluor, sum of 2-, 3-, 9-OH-fluorenes; ΣOH-Nap, sum of 1- and 2-OH-naphthalenes; ΣOH-Phen, sum of 1-, 2-, 3-, 4-OH-phenanthrenes; OH-Pyr, 1-OH-Pyrene; I-PACs, immunochemical quantification of PACs; PAPR, powered air-purifying respirator; HMA, hot-mix asphalt.

urinary biomarkers, explore the role of diesel oil as a source of PAC exposure, and identify other job characteristics that may also affect the total dose of PACs among HMA paving workers. Concentrations of PACs in air were consistently associated with urinary analytes, even during the week of PAPR use, which provides evidence that PACs in air are dermally absorbed. Our findings also show that the application temperature of HMA was a strong predictor of PAC metabolites, while increased dermal protection and the substitution of biodiesel for diesel oil as a cleaning agent were also associated with decreased urinary biomarkers. PACs measured in hand-wash samples were not associated with urinary analytes.

The PAC exposures observed in this population were lower than those previously reported (McClean *et al*., 2004a,b). For example, airborne pyrene (GM = 0.06 μg m^{-3}) was three times higher in the previous investigation (GM = 0.18 μg m^{-3}), while urinary OH-Pyr (0.55 ng ml^{-1}) was approximately twice as high (1.1 ng ml^{-1}). Additionally, measurements of air, dermal, and urinary analytes were previously found to vary by task, but no task-related differences were observed in the present study (McClean *et al*., 2004a,b; Cavallari *et al*., 2012a,b). The discrepancies between studies may be attributable to differences in paving machines and job types since the previous work was done on secondary roads using small paving machines (<16 000 pounds) that were not equipped with engineering controls, while the current work was done on larger primary roads and a parking lot using highway-class pavers that were equipped with engineering controls. Additionally, the previous work was done during warmer months (ambient temperatures ranging from 18 to 24ºC), whereas this study was conducted in early fall (11 of 36 sampling days with ambient temperatures between 8 and 18ºC) such that workers generally wore more clothing in this study.

Table 4. Personal air and dermal exposures as predictors of urinary metabolites by exposure scenario using multivariate mixed-effects models.[a]

	ΣOH-Fluor		ΣOH-Nap		ΣOH-Phen		OH-Pyr		I-PACs	
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
Analyte in air										
Baseline	0.37 (0.18)	0.05	–0.03 (0.05)	0.6	0.18 (0.06)	0.004	0.64 (0.23)	0.005	0.10 (0.05)	0.04
Dermal protection	0.82 (0.18)	<0.0001	0.03 (0.02)	0.2	0.51 (0.08)	<0.0001	2.84 (0.62)	<0.0001	0.06 (0.03)	0.08
PAPR	0.36 (0.16)	0.02	0.02 (0.09)	0.8	0.15 (0.05)	0.002	0.98 (0.44)	0.03	0.08 (0.06)	0.20
Biodiesel substitution	1.10 (0.32)	0.001	0.05 (0.06)	0.5	0.76 (0.14)	<0.0001	3.98 (0.72)	<0.0001	0.31 (0.12)	0.01
	Interaction: 0.049		Interaction: 0.6		Interaction:	<0.0001	Interaction: <0.0001		Interaction: 0.2	
Analyte on hands										
Baseline	*(b)*		*(b)*		–0.003 (0.02)	0.9	0.02 (0.06)	0.7	*(b)*	
Dermal protection	*(b)*		*(b)*		–0.09 (0.06)	0.1	–0.11 (0.24)	0.7	*(b)*	
PAPR	*(b)*		*(b)*		0.03 (0.02)	0.1	0.02 (0.05)	0.7	*(b)*	
Biodiesel substitution	*(b)*		*(b)*		0.07 (0.04)	0.1	–0.07 (0.10)	0.6	*(b)*	
					Interaction:	0.1	Interaction: 0.9			

SE, standard error; PAPR, powered air-purifying respirator.

[a]Analytes in personal air and hand-wash samples correspond to log-transformed analytes as follows: fluorene for ΣOH-Fluor (sum of 2-, 3-, 9-OH-fluorenes), napthalene for ΣOH-Nap (sum of 1- and 2-OH-naphthalenes), phenanthrene for ΣOH-Phen (sum of 1-, 2-, 3-, 4-OH-phenanthrenes), pyrene for OH-Pyr (1-OH-pyrene), and total organic matter for I-PACs (immunochemical quantification of PACs). Each model includes time, cotinine, and creatinine as covariates.

[b]Not analyzed due to low percentage of detection above the LOD in hand-wash samples.

The purpose of the PAPR scenario was to minimize inhalation of PACs so that we could explore the role of dermal exposure. To address the question of whether dermal exposure occurs primarily via direct contact with contaminated surfaces or via transfer from air to skin (deposition of particulate and/or condensation of vapor), our goal was to minimize the inhalation route and determine whether PACs measured in air were still associated with urinary metabolites. For ΣOH-Fluor, ΣOH-Phen, and OH-Pyr, the corresponding parent compounds in air were indeed significant predictors of these urinary metabolites during the PAPR week, evidence in support of the hypothesis that workers dermally absorb PACs as a result of air-to-skin transfer.

These findings are consistent with those of Walter and Knecht (2007), who investigated the potential for dermal absorption of asphalt fume in human volunteers using an experimental chamber. A comparison of urinary metabolites in two subjects who were exposed to laboratory-generated asphalt emissions both with and without a PAPR suggested that dermal absorption accounted for ~53% of ΣOH-Phen and 58% of OH-Pyr (Walter and Knecht, 2007). While it seems likely that our results are due to the transfer of PACs from air to skin, the observed association could also be due, in part, to worker compliance (i.e. lifting the face shield of the PAPR while working) or if the air sampler's position on the lapel was influenced by air flow from the face shield. However, the impact of lifting the face shield was likely minimal since such instances were most common when workers were standing away from the paving machine. Similarly, the air flow from the face shield did not appear to affect PAC measurements in air because PACs in air were not significantly reduced during the week when the PAPRs were worn.

The week of increased dermal protection was associated with consistent reductions in urinary metabolites, but reduction was only significant for OH-Pyr (29% reduction) and I-PACs (15% reduction). A larger reduction was expected given previous findings that urinary OH-Pyr appeared to be primarily affected by dermal exposure (McClean *et al*., 2004b), and because our analysis of the hand-wash data indicated that 'sometimes glove use' and 'frequent glove use' were associated with pyrene concentrations that were 57% and 90% lower than 'no glove use,' respectively (Cavallari *et al*., 2012b). The contrast between the dermal protection week and the other three weeks was likely reduced due to the cool ambient temperatures that prompted workers to wear more clothing on the nondermal protection weeks than is typical during most of the work season.

The substitution of biodiesel for the diesel oil as a cleaning agent was associated with consistent reductions in urinary metabolites, but reduction was only significant for OH-Pyr (15% reduction), which is lower than the significant reductions observed for pyrene in the air (51% reduction) and hand-wash (73% reduction) samples when diesel oil was replaced with biodiesel (Cavallari *et al*., 2012a,b). The decreased PAC emissions were expected given that the biodiesel product contains no PACs (Bajpai and Tyagi, 2006). The effect of PACs in air on urinary metabolites was observed to be highest during the biodiesel substitution week, suggesting that the substitution of biodiesel for diesel oil resulted in reduced dermal exposure such that inhalation became the primary route of exposure to PACs and therefore a stronger predictor of urinary biomarkers. A possible but less likely explanation is that biodiesel may facilitate dermal absorption and/or increase the extent to which particulate adheres to the skin. If true, the benefit of substituting biodiesel for diesel oil would likely increase as PAC emissions from other sources decrease and could be useful in combination with reduced HMA application temperatures and increased dermal protection.

The positive associations between HMA application temperature and urinary metabolites were similar to those observed for air and dermal samples (Cavallari *et al*., 2012a,b). The application temperature of HMA was evaluated in the range of 121–154ºC. In a field-based study of mastic asphalt workers, Spickenheuer *et al*. (2011) found that a 10ºC increase in mastic asphalt application temperature resulted in a 20% increase in the breathing zone concentrations of mastic vapors and aerosols (temperature range of 216–270ºC). In a lab-based study, Lange and Stroup-Gardiner (2007) found that PAC emissions increased as temperatures increased from 130 to 290ºC and that the emission of four-, five-, and six-ringed PACs was highest at the highest temperatures. However, it should be noted that both studies included temperatures that exceeded the range in our study (Lange and Stroup-Gardiner, 2007; Spickenheuer *et al*., 2011).

The current study was limited in the extent to which the range of HMA application temperatures could be evaluated. The extent to which further reductions in exposure could be achieved at even lower application temperatures remains unclear. While the mean HMA application temperatures in the three crews were 131, 139, and 149ºC, warm mix asphalt (WMA) is generally laid and compacted at temperatures between 100 and 140ºC. Our results suggest that the use of WMA instead of HMA could

further reduce PAC exposure among paving workers, but additional investigation is required.

The fact that hand-wash PACs were found to be associated with biodiesel substitution, glove use, HMA application temperature, asphalt grade, and nicotine suggests that the hand-wash method provides a useful measure of dermal contact (Cavallari *et al*., 2012b). However, PACs in hand-wash samples were not observed to be predictors of the urinary analytes. Given the other evidence of dermal absorption in this study (i.e. association between PACs in air and urinary metabolites during the week of PAPR use, reduced urinary metabolites during the week of increased dermal protection), our results suggest that the hand-wash method may provide a useful measure of dermal contamination but not a useful measure of the biologically relevant portion that is absorbed. Additionally, by cleaning the skin of the workers at the end of each shift, we likely reduced the potential effect of dermal absorption.

The measurements of ΣOH-Fluor, ΣOH-Phen, OH-Pyr, and I-PAC in urine were generally consistent in the extent to which they were associated with the various job characteristics and PACs in air. Urinary biomarkers specific to the higher molecular weight PACs (the fraction more likely to be carcinogenic) were not evaluated because this fraction is more likely to be excreted in feces; however, pyrene was the highest molecular weight PAC that was frequently detected in air or dermal samples (Cavallari *et al*., 2012a,b). Unlike the other urinary markers, ΣOH-Nap was not found to be associated with job characteristics or PACs in air and was primarily associated with urinary cotinine. This finding for ΣOH-Nap compared with other urinary biomarkers is consistent with previous investigations (Serdar *et al*., 2003a,b; Sobus *et al*., 2009a).

Though this crossover study was designed to evaluate four exposure scenarios, our ability to interpret a comparison of the four scenarios was limited because the study was not conducted in a controlled environment. For instance, though we asked workers to wear gloves, a hat with neck cloth, clean pants, and long-sleeved shirts during the dermal protection week, we could not ask them to refrain from using such items during the other weeks. Because the ambient temperatures decreased as the study extended into early fall, workers tended to wear more clothing as temperatures decreased during the study period. Similarly, workers periodically raised the face shield of their PAPR during their shift (though primarily while standing away from the paving machine). Given these practical limitations, the effect of each exposure scenario compared with baseline was likely underestimated due to deviations from the desired protocol.

CONCLUSION

Our results provide evidence that PACs in air are dermally absorbed. Reducing the application temperature of asphalt mix appears to be a promising strategy for reducing inhalation exposure, dermal exposure, and the total absorbed dose of PACs among paving workers. Additional reductions in PAC exposure may be achieved by substituting biodiesel for diesel oil as a cleaning agent and requiring increased dermal coverage.

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