COMPARING URINARY BIOMARKERS OF EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the department of Environmental Sciences and Engineering

Chapel Hill
2008

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ABSTRACT

JON RUSSELL SOBUS: Comparing Urinary Biomarkers of Exposure to Polycyclic Aromatic Hydrocarbons
(Under the direction of Stephen M. Rappaport)

Polycyclic aromatic hydrocarbons (PAHs), which consist of two or more fused aromatic rings, are ubiquitous products of combustion. These compounds are found in wood and tobacco smoke, engine (particularly diesel) exhausts, and in numerous workplaces, including those producing coke and aluminum and those processing hot asphalt. Because some PAHs are potent carcinogens, it is important to assess the levels of exposure to these compounds that are received by workers and the general public. However, PAHs are difficult to measure in the environment because they exist in both the gas phase and the particulate phase, and the carcinogenic particle-bound compounds are present at very low concentrations. In contrast, the smaller vapor-phase PAHs are more abundant in the environment and produce a host of urinary products that can be used as biomarkers of exposure. Here we consider the hypothesis that urinary biomarkers of naphthalene (a 2-ring compound) and phenanthrene (a 3-ring compound) can be used as measures of exposure to total PAHs. We test this hypothesis in three distinct phases of research. First, we determine the predictive value of airborne naphthalene and phenanthrene as measures of exposure to diesel exhaust in a controlled chamber study. Second, we determine the predictive value of urinary levels of naphthalene and phenanthrene as measures of occupational exposure to diesel exhaust, asphalt emissions, and coke-oven emissions. And finally, we develop statistical models of

urinary biomarkers of naphthalene and phenanthrene in asphalt-exposed workers to determine effects of particulate PAH levels in air and on the skin, job categories and tasks, and physiological factors that might affect the uptake and elimination of these compounds. Overall, this research demonstrates that urinary biomarkers of naphthalene and phenanthrene can be used as measures of exposure to total PAHs in air and on the skin.

This dissertation is dedicated to my parents, John and Doris Sobus, and my wife, Ryan.

ACKNOWLEDGEMENTS

I thank my advisor Steve Rappaport for his continued support, guidance, and friendship. Steve's influence on my professional growth over these past years is immeasurable. Thanks to my committee members, Dr. Lawrence Kupper, Dr. Leena Nylander-French, Dr. Ivan Rusyn, and Dr. James Swenberg, for their encouragement, helpful ideas and suggestions, and belief in my ability to succeed. Thanks to Joachim Pleil for his insight and expertise, sense of humor, generosity, and unwavering dedication to his friends and colleagues. Thanks to Suramya Waidyanatha for teaching me everything I know about analytical chemistry, and for setting a terrific example of excellence in our lab. Thanks to my labmates, fellow students, and colleagues, Bill Funk, Sung Kim, David Kim, Christina Kim, Yu-Sheng Lin, Renan Wu, Berrin Serdar, Alistair Henderson, Chris Trent, Russell Bowen, Andrew Lindstrom, Peter Egeghy, Melissa Troester, Heidi Hubbard, and Frank Onyemauwa, for their helpful conversations and friendships. Thanks to my collaborators, specifically Dr. Michael Madden, Dr. Michael McClean, Dr. Robert Herrick, Dr. Eric Garshick, Dr. Thomas Smith, and Dr. Yulix Zheng without whom this work would not have been possible. Thanks to Dr. Myriam Medina-Vera, Dr. Allan Vette, Dr. Hunter Daughtrey, Dr. Nicole Tulve, Dr. Linda Sheldon, Jon Berntsen, and Karen Oliver for their assistance with the controlled chamber study. Thanks to the faculty and staff of the Environmental Sciences and Engineering Department and the Biostatistics Department, specifically Jack

Whaley, Melody Levy, Robin Whitley, Vera Bennett, Debbie Quach, Dr. Michael Aiken, Dr. Donald Fox, Dr. David Leith, Dr. Michael Flynn, Richard Kamens, Dr. Howard Weinberg, Dr. Louise Ball, Dr. Gunnar Boysen, James Wallace, and Randy Goodman, for their advice and assistance. Finally, thanks to my Mom and Dad, my Wife, my Brother and Sister, my Aunt and Uncle, my Godfather, Dr. Elichia Venso, and the rest of my family and friends for always believing in me and supporting me along the way.

This work was supported by the National Institute of Environmental Health Sciences through training grant T32ES07018 and research grants P42ES05948 and P30ES10126, and by the Environmental Protection Agency through the Student Services Contracts Program.

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LIST OF ABBREVIATIONS AND SYMBOLS

Ace	acenaphthene
Anap	acenaphthalene
Ant	anthracene
ATSDR	Agency for Toxic Substances and Disease Registry
BaA	benz[a]anthracene
BaP	benzo[a]pyrene
BbF	benzo[b]fluoranthene
BgP	benzo[g,h,i]perylene
BkF	benzo[k]fluoranthene
Ben	benzene
BeP	benzo[e]pyrene
BSM	benzene soluble matter
ºC	degrees centigrade
Chr	chrysene
cm^2	square centimeter(s)
CTPV	coal tar pitch volatiles
CV	coefficient of variation
CYP	cytochrome P450
DaA	dibenz[a,h]anthracene
DE	diesel exhaust
DPM	diesel particulate matter
EI	electron impact ionization

EPA	Environmental Protection Agency
Etb	ethylbenzene
Etol	4-ethyltoluene
eV	electronvolt
Fla	fluoranthene
Flu	fluorene
g	gram(s)
g/l	gram per liter
GC-MS	gas chromatography-mass spectrometry
GSH	glutathione
GST	glutathione s-transferase
h	hour(s)
He	helium
HPLC	high performance liquid chromatography
IARC	International Agency for Research on Cancer
ICC	intraclass correlation coefficient
IgA	Immunoglobulin A
IgG	Immunoglobulin G
Ind	indeno[1,2,3-c,d]pyrene
IRB	Institutional Review Board
k	rates constant
LC-MS/MS	liquid chromatography-tandem mass spectrometry
l/min	liters per minute

ln	natural logarithm
LOD	limit of detection
LOQ	limit of quantitation
mg	milligram(s)
min	minutes
ml	milliliter(s)
m^3/min	cubic meters per minute
mp-xyl	m,p-xylene
MS	mass spectrometer
m/z	mass to charge ratio
n	number
na	not available
Nap	naphthalene
NERL	National Exposure Research Laboratory
ng	nanogram(s)
ng/cm^2	nanogram per square centimeter
ng/m^3	nanogram per cubic meter
NHEERL	National Health and Environmental Effects Research Laboratory
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
ns	not significant
o-xyl	o-xylene

PAC	polycyclic aromatic compounds
PAH	polycyclic aromatic hydrocarbons
PDMS	polydimethylsiloxane
Phe	phenanthrene
PM	particulate matter
PM2.5	particulate matter with aerodynamic diameters less than 2.5 μm
PTFE	polytetrafluoroethylene
Pyr	pyrene
ROS	reactive oxygen species
SD	standard deviation
SE	standard error
SIM	selective ion monitoring
SPE	solid-phase extraction
SPME	solid phase-microextraction
Sty	styrene
SULT	sulfotransferase
$T_{1/2}$	half-time
Tmb	1,3,5-trimethylbenzene
Tmb*	1,2,4-trimethylbenzene
Tol	toluene
$\mu g/m^3$	microgram per cubic meter
UGT	uridine diphosphate glycosyltransferase
VOCs	volatile organic compound(s)

VAPS	versatile air pollutant sampler
yr	year

1. Background and Significance

1.1. PAHs

1.1.1. Occurrence and health effects

Polycyclic aromatic hydrocarbons (PAH) are a large class of chemicals composed of two or more fused aromatic rings. These chemicals are formed by the incomplete combustion of organic materials, and are therefore ubiquitous contaminants of the world's air, water, and soil. Sources of PAHs from the natural environment include forest fires and volcanoes; occupational PAH sources include coal tar, coal tar pitch, coke tars, coke-oven emissions, creosote, asphalt (also known as bitumen), and industrial smoke and soot; additional anthropogenic sources include motor vehicle exhaust derived from diesel-powered engines, tobacco smoke, and char-broiled foods. Considering the breadth and variety of environmental sources, human exposures to PAHs occur though inhalation of polluted air, ingestion of contaminated food and water, and dermal contact with PAH-containing products.

Although hundreds of individual PAHs have been identified (NIST 1997), the assessment of exposures to PAHs is often based on a subset of commonly occurring consitituent compounds (ATSDR 1995), including acenaphthene (Ace), acenaphthylene (Anap), anthracene (Ant), benz(*a*)anthracene (BaA), benzo(*a*)pyrene (BaP), benzo(*e*)pyrene (BeP), benzo(*b*)fluoranthene (BbF), benzo(*g,h,i*)perylene (BgP), benzo(*b*)fluoranthene (BbF), benzo(*k*)fluoranthene (BkF), chrysene (Chr), dibenz(*a,h*)anthracene (DaA), fluoranthene (Fla), fluorene (Flu), indeno(1,2,3-*c,d*)pyrene (Ind), naphthalene (Nap), phenanthrene (Phe),

and pyrene (Pyr). Of this group, only Nap, a 2-ring PAH, exists almost entirely in the gaseous phase. The 3-ring PAHs (e.g., Phe) exist both in gaseous and particulate forms, and the larger compounds with 4 or more rings (e.g., BaP) typically exist adsorbed to particulate matter.

Chronic exposure to PAHs in some work environments has been associated with human cancers, specifically those of the lung, skin, and bladder (Boffetta et al. 1997). Although studies have linked Nap with lung tumors in rats and mice (Abdo 1992; NTP 1992; NTP 2000; Abdo et al. 2001), the larger 4-6 ring PAHs (Chr, BaA, BbF, BkF, BaP, DaA, Ind, and BgP) appear to posses the greatest carcinogenic potency (IARC 1983; IARC 1987). Of these 4-6 ring compounds, BaP is a known human carcinogen (IARC Vol. 92 in preparation) is used as an index of the health hazards associated with PAH exposure (EPA 1993).

Noncancer health effects of PAH exposure include decreased ventilatory function (as evidenced by breathing problems, bloody vomit, chest pains and irritation, and pleural effusions) and reduced serum immunoglobulins (specifically serum IgG and IgA) (ATSDR 1995). Recent studies have identified an association between adverse reproductive outcomes and exposures to PAHs during pregnancy. Specifically, studies have shown that elevated exposure to PAHs can result in reduced fetal growth, birth weight, gestational duration, and head circumference (Dejmek et al. 2000; Lederman et al. 2004; Perera et al. 2005b). Also, it is suggested that a developing fetus has increased susceptibility to DNA damage from PAH exposure, perhaps leading to a disproportionate cancer risk (Perera et al. 2005a).

1.1.2. Metabolism of PAHs

PAHs are lipophilic compounds that can penetrate cellular membranes and be stored in adipose tissue. Metabolic transformation via Phase-I and Phase-II enzymatic activity renders PAHs more water soluble and suitable for excretion. While the metabolism of PAHs has been studied both *in vitro* (cultured human cells) and *in vivo* in several species of animals (ATSDR 1995), little data exist as to the *in vivo* metabolism of PAHs in humans. Despite these data gaps, it is thought that the structural similarities in many PAH compounds lead to similarities in their metabolism (ATSDR 1995). As BaP is used to gauge the relative toxicity of other PAHs, it is also frequently used to model PAH metabolism. As shown in Figure 1.1., BaP is first oxidized by cytochrome P-450 (1A1, 1A2, 1B1, 3A4, 2C), forming several PAH epoxides (Daly et al. 1972; Bauer et al. 1995). It is hypothesized that phenols may also be formed from the parent compound in the liver by direct oxygen insertion (Gelboin 1980; Levin et al. 1982). PAH epoxides can: 1) spontaneously rearrange to phenols, which are then either oxidized to form quinones, or conjugated (via UGT1A6 or SULT1A1) and excreted in the urine, 2) react covalently with glutathione as catalyzed by glutathione-*S*-transferases (GSTM1, GSTT1, GSTP1), 3) react with macromolecules to form adducts (Viau and Carrier 1995; Boysen and Hecht 2003; Brandt and Watson 2003), or 4) undergo hydration to the corresponding trans-dihydrodiols via microsomal epoxide hydrolase (Boyland and Levi 1935; Booth and Boyland 1949). Dihydrodiols can be: 1) eliminated in the urine (either free or conjugated via UGT2B7), 2) converted to catechols (via dihydrodiol dehydrogenase), which are then either oxidized to *ortho*-quinones (Smithgall et al. 1986; Smithgall et al. 1988; Flowers-Geary et al. 1995) or conjugated and eliminated in the urine, or 3) oxidized (via cytochrome P-450 1A1, 1A2, 1B1, 3A4, 2C) to diolepoxides (Sims et al. 1974; Kim et

al. 1998). Diolepoxides can be: conjugated with glutathione (GSTM1, GSTT1 or GSTP1), hydrolyzed to tetraols via epoxide hydrolase (Simpson et al. 2000), or form macromolecular adducts with either protein, RNA, or DNA (Koreeda et al. 1978; Szeliga and Dipple 1998; Dipple et al. 1999). Bay-region diolepoxides (e.g., the 7,8-dihydrodiol-9,10-epoxide of BaP) are recognized as promutagenic tumorigenic metabolites of several PAHs (ATSDR 1995). In addition, *ortho*-quinones formed during the metabolic process are known to undergo redox cycling, thereby forming reactive oxygen species (ROS), such as the superoxide anion, hydrogen peroxide, and hydroxyl radicals. These ROS can cause oxidative stress within cells, thus damaging cellular macromolecules and activating signaling pathways (Bolton et al. 2000). Therefore, production of *ortho*-quinones in PAH metabolism offers a mechanism of carcinogenesis other than the production of promutagenic DNA adducts by PAH metabolites.

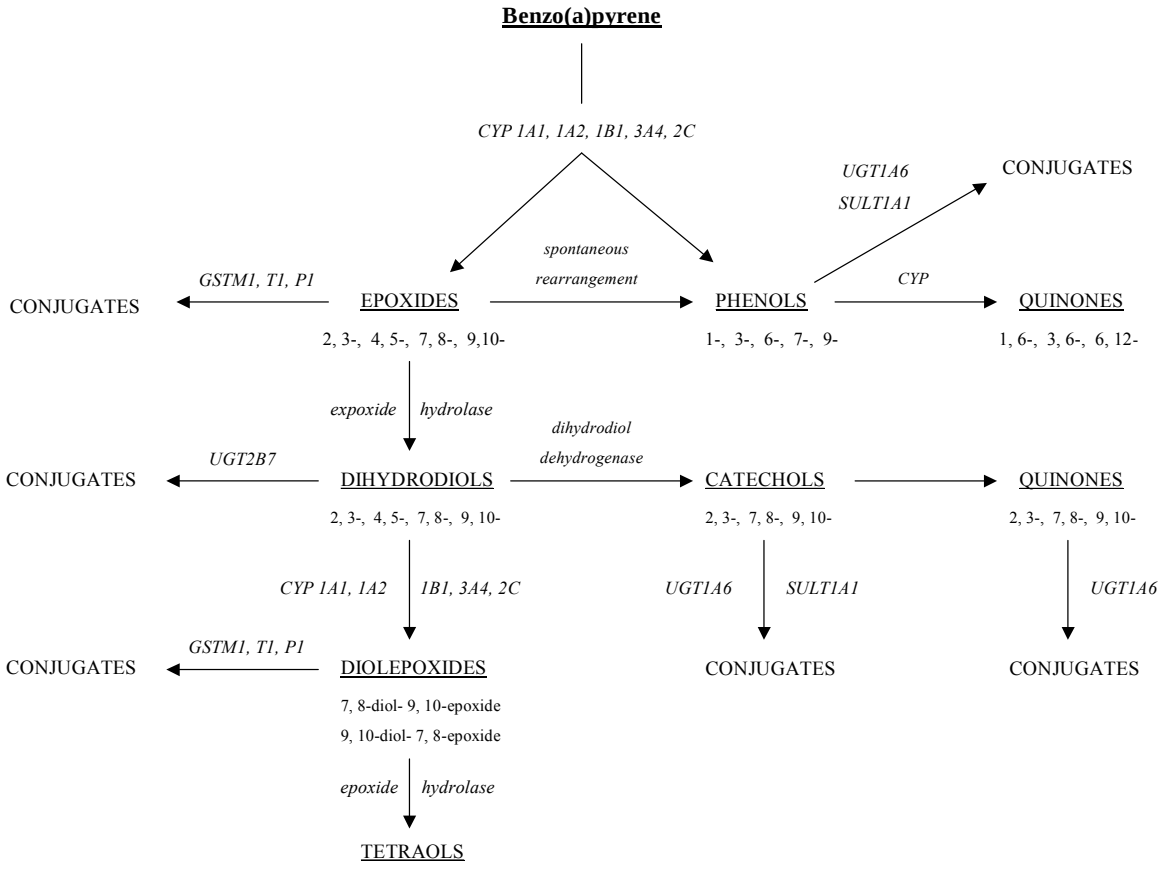

Figure 1.1. Proposed metabolic scheme for PAHs using BaP as a prototype [adopted from (ATSDR 1995)].

1.1.3. PAH exposure assessment

Beginning in 1775, when Sir Percival Pott recognized an increased incidence of scrotal cancer in chimney sweeps (Pott 1775), causal associations have been made between occupational exposures to PAHs and human cancers, notably in the production of steel, iron, and aluminum, where exposure levels are very high (Boffetta et al. 1997). However, in work environments containing low to moderate levels of PAHs, (e.g., those involving exposure to diesel exhaust and asphalt emissions), causal associations between PAH exposure and cancer incidence are more uncertain (NIOSH 2000; EPA 2002). This uncertainty stems, in part, from the general lack of quantitative data regarding levels of PAH exposures in these workplaces.

In a typical workplace, volatile and semivolatile PAHs (i.e., 2- and 3- ring PAHs) are more abundant than those bound to particulate matter (Bjorseth and Lunde 1977; Bjorseth et al. 1978a; Petry et al. 1996; Kuusimaki et al. 2002; Strunk et al. 2002; Kuusimaki et al. 2003; Rappaport et al. 2004). Nontheless, most assessments of exposures to PAHs focus upon the particulate fraction because the 4-6 ring compounds tend to be more potent carcinogens. These measurements of airborne particulate matter require cumbersome and expensive sampling equipment, such as battery-operated pumps that are worn by study participants. While volatile and semivolatile PAHs are not as strongly associated with health effects, their relatively large abundance allows them to be quantified more easily in air. In fact, since smaller 2- and 3- ring PAHs (e.g., Nap and Phe, respectively) are often observed primarily in the gas phase, air concentrations can be measured using passive sampling techniques that do not require portable pumps (Egeghy et al. 2003). These more affordable sampling techniques increase the ability to obtain more air measurements at a given cost for studies of health

effects (Rappaport 1991). Since air levels of Nap have been shown to be highly correlated with those of total measured PAHs in studies of workers in the steel and aluminum industries, the use of Nap and Phe as surrogates for exposures to all PAHs (hereafter 'total PAHs') is an appealing possibility (Rappaport et al. 2004).

1.1.4. Biomarkers of PAH exposure

Given potential contributions to total PAH exposure from inhalation, ingestion, and dermal contact, and the use of personal protective equipment (primarily respirators and gloves), there is a need for good biomarkers of PAH exposure. A biomarker of exposure is generally considered to be either the parent compound or its metabolic product(s) in exhaled breath or a bodily fluid (NRC 1987). Biomarkers of effect, on the other hand, include measures of DNA or tissue damage, including DNA adducts, mutagenicity, chromosome damage, etc. In fact, the first biomarkers applied to PAH-exposed subjects were based on mutagenic activity in urine samples (Moller and Dybing 1980; Recio et al. 1984; Venier et al. 1985). These methods lacked the sensitivity and specificity to attribute mutagenic activity to exposures to particular PAHs, and thus, had limited applications.

A more useful biomarker of PAH exposure involves measurement of 1-hydroxypyrene (OH-Pyr) in urine (Keimig et al. 1983; Jongeneelen et al. 1985; Jongeneelen et al. 1987). While other Pyr metabolites have been described (Harper 1957; Boyland and Sims 1964; Jacob et al. 1982), OH-Pyr is the main urinary metabolite of Pyr. Studies in many different working environments have linked OH-Pyr levels to PAH exposure [reviewed in (Jacob and Seidel 2002; Hansen et al. 2008)]. While Pyr, a 4-ring PAH, is a useful surrogate for particle-bound PAHs, certain disadvantages are associated with using OH-Pyr

more generally as a biomarker of total PAH exposure. Since only one metabolite of Pyr is measured in the urine, little insight is gained regarding variability in PAH metabolism as a function of exposure and the influence of covariates such as gender, race, age, and genetic factors. In addition, Pyr is not carcinogenic (IARC 1983; IARC 1987), nor does it produce a bay-region diolepoxide via biotransformation, a feature closely associated with the tumorigenic potential of PAHs (ATSDR 1995).

To more accurately assess exposures to carcinogenic PAHs and their subsequent metabolism, BaP metabolites have been investigated as biomarkers of PAH exposure. However, this strategy is problematic due to the very low levels of BaP metabolites in urine from all but the most highly exposed workers, and the fact that BaP metabolites are predominantly excreted in the feces rather than the urine [reviewed in (Jacob and Seidel 2002)].

Taking into consideration their high relative abundance, several smaller PAHs have recently been considered as biomarkers of PAH exposure. Although Nap does not produce a bay-region diolepoxide, it is metabolized and excreted as phenolic and dihydrodiol conjugates in human urine (Serdar et al. 2003b; Serdar et al. 2004; Wu et al. 2005). Additionally, protein adducts of the naphthoquinones (metabolites of Nap) have been measured in human serum albumin (Waidyanatha et al. 2004). The availability of these biomarkers of exposure, coupled with the ability of Nap to cause respiratory tumors in rodents, has increased interest in this analyte and its biomarkers as potential surrogates for total PAH exposure.

In addition to Nap, studies have demonstrated the utility of Phe metabolites as biomarkers of PAH exposure (Carmella et al. 2004; Kim et al. 2005). This stems in part

from the high relative levels of Phe in air samples, the predominant excretion of Phe in urine rather than feces, and the ability of Phe to be biotransformed and excreted in urine as a number of different metabolites (i.e., phenols, dihydrodiols, and tetraols). Phenanthrene is the simplest PAH to contain a bay region, and, despite the fact that it is not a human carcinogen (IARC 1983; IARC 1987), Phe metabolism closely mimics that of BaP (Shou et al. 1994; Carmella et al. 2004). Moreover, as with BaP, Phe is oxidized by various cytochrome P450s that may exhibit different regiospecificities (Jacob and Grimmer 1996). The ability to quantify various urinary metabolites of Phe may allow a better understanding of human metabolism of carcinogenic PAHs *in vivo*.

1.2. Diesel exhaust

Diesel exhaust (DE) is a complex mixture of gaseous and particulate species, the exact composition of which depends on the type of engine (light-duty vs. heavy duty), the type of fuel (high vs. low sulfur fuel), and engine operating conditions (idle vs. acceleration). Vapor-phase constituents of DE include atmospheric gases (e.g., nitrogen, oxygen, carbon dioxide, water vapor), oxides of sulfur and nitrogen, and volatile organic compounds (e.g., benzene and small PAHs). Diesel particulate matter (DPM) consists of an elemental carbon core to which organic compounds (including PAHs) and inorganic compounds (e.g., nitrates, sulfates, and metals) are adsorbed. DPM includes fine (< 2.5 μm) and ultrafine (< 0.1 μm) particles; these small particles penetrate to the deep lung following inhalation. Consequently, following inhalation both gaseous and particle-adsorbed PAHs can diffuse into the bloodstream.

DE exposure in controlled human studies has been associated with cardiopulmonary changes including lung inflammation, altered vascular response, and cardiac ischemia (Stenfors et al. 2004; Mills et al. 2005; Mills et al. 2007). In animal studies, DE exposure has been associated with respiratory tumors in rats (HEI 1995; EPA 2002). Epidemiological evidence has repeatedly shown associations between occupational DE exposure and lung cancer (Bhatia et al. 1998; Lipsett and Campleman 1999). Considering this evidence, DE is considered to be a probable human carcinogen (IARC 1989; EPA 2002).

However, the mechanism whereby DE might cause cancer is unclear, and the relationship between DE exposure and response is clouded by the complexity of gaseous and particulate DE emissions, and the lack of quantitative exposure data (EPA 2002). Measures of DE exposure that have been used in health effects studies include organic carbon (OC), elemental carbon (EC), and fine particulate matter (PM2.5 = particulate matter with aerodynamic diameters less than 2.5 μm) (Davis et al. 2006; Smith et al. 2006; Davis et al. 2007). Epidemiological studies of DE have also been complicated due to potential confounding by smoking (as well as other covariates) and the inability to discriminate diesel particles from those of non-diesel origins.

There is also the lingering question as to whether DE exerts a carcinogenic effect via PAHs or by deposition of insoluble soot particles and reactive gases in the respiratory tract (EPA 2002). Traditional measures of DE exposure (i.e., OC, EC, and PM2.5) have not clarified this uncertainty, mainly because they are not specific to DE, and don't produce any identifiable biomarkers of exposure. Recently, urinary PAH biomarkers (including OH-Pyr and metabolites of Nap and Phe) have been used to assess DE exposures (Nielsen et al. 1996; Hara et al. 1997; Seidel et al. 2002; Adonis et al. 2003; Kuusimaki et al. 2004). While the

application of these PAH biomarkers has been promising, studies have been limited by small sample sizes, lack of analytical sensitivity and specificity, influence of PAH exposures from the diet and other unknown sources, and influence of smoking.

1.3. Asphalt

Asphalt is produced by the nondestructive distillation of crude oil during petroleum refining (NIOSH 2000). Due to its adhesive properties, flexibility, durability, and water resistance, asphalt has been widely used in the road-paving industry as a binding agent for aggregate (i.e., sand, gravel, crushed stone, slag, and recycled concrete). During road surfacing, workers are exposed to asphalt vapors (a product of heating), and particulate matter containing asphalt fumes (condensed vapor), as well as other constituents, via inhalation and dermal contact. Although the composition of asphalt is variable, asphalt is a known source of occupational PAH exposure.

Acute exposure to asphalt emissions is associated with irritation of the eyes, nose, throat, skin, and respiratory tissues, along with headaches, dizziness, fatigue, insomnia, nausea, and stomach discomfort (NIOSH 2000). Additionally, occupational asphalt exposure has been associated with bronchitis, emphysema, and asthma (Hansen 1991). Epidemiological evidence suggests an increased risk of cancer among asphalt-exposed workers (Partanen and Boffetta 1994; Hooiveld et al. 2002; Burstyn et al. 2003; Randem et al. 2004; Boffetta et al. 1997). However, the relationship between asphalt exposure and cancer risk is uncertain (Chiazze et al. 1991; NIOSH 2000). This uncertainty stems from a lack of quantitative exposure measurements and the potential effects of covariates that influence asphalt exposures from airborne and dermal routes (NIOSH 2000).

In order to better characterize exposure to asphalt emissions, numerous exposure surrogates have been employed including air measurements of total particulate matter, the benzene-soluble fraction of total particulates, polycyclic aromatic compounds (PACs, which include unsubstituted PAHs and alkylated or otherwise substitiuted PAHs), and individual PAHs. Some investigators have used dermal patches to quantify deposition of PAH-containing particulate matter on the skin of asphalt-exposed workers (Jongeneelen et al. 1988; McClean et al. 2004a; Vaananen et al. 2005). Biomarkers of PAH exposure from asphalt emissions include Nap, Phe, and Pyr metabolites in urine (Vaananen et al. 2003; McClean et al. 2004b; Campo et al. 2006a; Campo et al. 2006b; Vaananen et al. 2006; Buratti et al. 2007; Campo et al. 2007). Using urinary measurements, combined with measurements of the parent compounds in air and on the skin, investigators have demonstrated increased PAH exposure among asphalt workers. Yet, uncertainty remains regarding the predominant exposure routes, rates of uptake and elimination of analytes, and the overall suitability of individual analytes as biomarkers of exposure to asphalt emissions.

1.4. Specific goals of this project

The primary aim of this study is to evaluate the suitability of Nap and Phe and their metabolites as urinary biomarkers of exposure to total PAHs. This objective is broken down into the following specific goals:

Specific Goal 1: Determine the predictive value of Nap and Phe as measures of exposure to diesel exhaust based on air measurements of diesel exhaust in a controlled chamber study (Paper 1).

Specific Goal 2: Determine the predictive value of urinary levels of Nap and Phe as surrogates for exposures to total PAHs in workers exposed to diesel exhausts, asphalt emissions (road paving and milling), and coke-oven emissions (steel industry) (Paper 2).

Specific Goal 3: Use statistical models to evaluate the effects of work groups, tasks, timing of urine sampling, and other covariates on urinary levels of Nap and Phe and their metabolites among asphalt exposed workers (road paving) (Papers 3 and 4).

2. Paper I. Identification of Surrogate Measures of Diesel Exhaust Exposure in a Controlled Chamber Study [Sobus, J.R., Pleil, J.D., Madden, M.C., Funk, W.E., Hubbard, H.F., Rappaport, S.M., submitted to Environmental Science and Technology, April, 2008.]

2.1. Abstract

Exposure to diesel exhaust (DE) has been associated with acute cardiopulmonary and vascular responses, chronic noncancer health effects, and respiratory cancers in humans. To better understand DE exposures and eventually their related health effects, we established a controlled chamber experiment wherein human volunteer subjects were exposed to approximately 100 μg/m^3 of DE. In general, human exposure assessment for DE is based on ambient air measurements of surrogates such as elemental carbon (EC) or total organic carbon (OC) collected on filters. As specific health effect mechanisms and dose response are obscured by the complex composition of DE, the linkage from exposure to internal dose can presumably be improved using specific biomarkers and metabolites in blood, breath, or urine. Because EC or OC are not suitable as biomarkers, in this study, we focus on identifying compounds that are demonstrated indicators of DE and can also be found in biological fluids. We measured an assortment of volatile, semi-volatile, and particle-bound aromatic compounds in the chamber air and report their airborne concentrations in DE and purified air, as well as the estimated values of the corresponding exposure ratios (mean DE air concentration: mean purified air concentration). These estimated exposure ratios were used to identify naphthalene (Nap) and phenanthrene (Phe) as potentially useful surrogates for DE exposure that could also serve as biomarkers. Estimated mean levels of Nap and Phe

associated with the nominal 100 μg/m^3 of DE were 2600 ng/m^3 and 765 ng/m^3 with estimated exposure ratios of 252 and 92.4, respectively. Nap levels were significantly correlated with OC, and total particle-bound PAHs; Phe levels were significantly correlated with total volatile+semivolatile PAHs. These results suggest that Nap and Phe may be particularly useful surrogates for DE concentrations. While Nap and Phe are not validated here as internal biomarkers of DE exposure, we are currently assessing human biological specimens collected during this study, and will discuss those results in ensuing articles.

2.2. Introduction

Diesel exhaust (DE) is comprised of a mixture of gaseous and particulate contaminants, the exact composition of which depends upon the type of fuel, engine operating conditions, and other factors. Vapor-phase constituents of DE include atmospheric gases, oxides of sulfur and nitrogen, and volatile organic compounds. Particulate matter emitted from diesel engines consists of an elemental carbon core to which a variety of organic and inorganic compounds are adsorbed. Controlled human exposures to DE have been associated with cardiopulmonary changes including lung inflammation, altered vascular response, and cardiac ischemia (Stenfors et al. 2004; Mills et al. 2005; Mills et al. 2007). Chronic DE exposure has been associated with respiratory tumors in rats (HEI 1995; EPA 2002) and lung cancers in occupational epidemiological studies (Bhatia et al. 1998; Lipsett and Campleman 1999). Thus, DE is considered to be a probable human carcinogen (IARC 1989; EPA 2002).

Health effect mechanisms and dose-response relationships remain unclear due to the chemical complexity of DE and limited quantitative exposure data (EPA 2002).

Additionally, identification of susceptible subpopulations is not yet clear, further clouding the issue of effective dose (Gilliland et al. 2004). To simplify the process of assessing exposures to DE, investigators have employed surrogate measures of particulate diesel emissions, including organic carbon (OC), elemental carbon (EC), and fine particulate matter (PM2.5 = particulate matter with aerodynamic diameters less than 2.5 μm) mass (Davis et al. 2006; Smith et al. 2006; Davis et al. 2007). These particulate measures may not reflect the many potentially toxic aromatic constituents of DE, notably benzene (Ben) and a host of PAHs in the volatile fraction [e.g., naphthalene (Nap)], the semivolatile fraction [e.g., phenanthrene (Phe) and fluorene (Flu)], and the particle-bound fraction [e.g., benzo(*a*)pyrene (BaP) and chrysene (Chr)]. Therefore, the goal of this study was to characterize volatile, semivolatile, and particle-bound aromatic compounds in DE to identify useful exposure surrogates. Here we present our sampling design and measurement techniques, descriptive statistics, and a correlation analysis to evaluate selected DE surrogates.

2.3. Methods

2.3.1. Study design

All controlled chamber exposures took place at the EPA National Health and Environmental Effects Research Laboratory (NHEERL) Human Studies Facility in Chapel Hill, NC, with approval from the University of North Carolina at Chapel Hill Biomedical Institutional Review Board (IRB# 99-EPA-283 Title: Physiological, Cellular, and Biochemical Effects of Diesel Exhaust in Healthy Young Adults). Ten volunteer subjects were exposed once to purified air and once to DE in a random and double-blind fashion, with two-hour exposure sessions separated by a minimum of three weeks. Purified air used in

control exposures was drawn across activated charcoal to remove gaseous organic constituents, and high-efficiency particle absolute (HEPA) filters to remove particulates. For DE exposures, exhaust was introduced into the chamber after an approximate 1:30 dilution with purified air. The DE was generated from an idling six-cylinder, 5.9L-displacement diesel engine (Cummins, Columbus IN), mounted in a vehicle located outside of the human studies facility, which burned a certified diesel fuel (Chevron Phillips Chemical Company, Borger TX, 0.05 LS Certification Fuel, type II). DE particulate levels were feedback-controlled via an exhaust dilution manifold using real-time measurements given by a Tapered Element Oscillating Microbalance (ThermoFisher Scientific, Franklin, MA) and monitored using a DataRAM™ aerosol monitor (ThermoFisher Scientific, Franklin, MA). Particle size was measured using a Scanning Mobility Particle Sizer™ (TSI Inc., Shoreview, MN), and PM2.5 exposure concentration was determined using a Versatile Air Pollutant Sampler (VAPS) (URG, Chapel Hill, NC). The volume median diameter (± SD) particle size over ten DE exposure periods was 0.10 ± 0.02 μm, and the estimated mean PM2.5 concentration was 106.3 ± 8.6 $\mu g/m^3$. This concentration is comparable to levels encountered at busy intersections in large urban areas (EPA 2002).

2.3.2. Collection and analysis of volatile aromatic compounds and 2-4 ring PAHs

Volatile aromatic compounds and 2-4 ring PAHs were sampled with custom-made 89 mm x 6.4-mm (¼ in.) o.d. aluminum tubes containing 350 mg of 60-80 mesh Tenax®TA (Scientific Instrumentation Specialists, Inc., Ringoes, NJ) that was held in place with stainless steel screens. The day prior to each chamber experiment, all adsorbent cartridges were cleaned by thermal desorption at 290ºC for 60 minutes with a constant helium flow

(99.999% purity) of 100 mL/min. After cleaning, tubes were sealed using metal Swagelok® fittings and were stored overnight at room temperature. Three adsorbent tubes were sampled in parallel inside the chamber for each two-hour exposure period at an air flow of 100 mL/min. At the conclusion of each experiment, adsorbent tubes were removed from the chamber, sealed with metal Swagelok® fittings, and stored at -20ºC prior to analysis (up to two weeks). Air flow rates were calibrated both before and after air sampling, using a DryCal® DC-1 flow calibrator (Bios International Corp., Butler, NJ).

Adsorbent tubes were thermally desorbed using a Markes Unity Thermal Desorber coupled to an Ultra Autosampler (Markes International, Ltd., Llantrisandt, UK), and analyzed using an Agilent 6890N gas chromatograph (GC) coupled to a 5973I mass spectrometer (MS) (Agilent, Santa Clara, CA). Sample batches included two reagent blanks, three chamber samples, three field blanks, and two external standards containing all analytes of interest. Each sample tube was thermally desorbed at 260ºC for ten minutes, focused on a secondary trap at 0ºC for three minutes, and ballistically desorbed at a maximum temperature of 310°C for GC injection. A 0.7-mm i.d. injector liner was used in the injection port of the GC, which was held at 250ºC and operated in the splitless mode with a pulse pressure of 103 kPa (15 psi). An RTX-5SILMS (Restek Corp., Bellefonte, PA) fused silica capillary column (60 m, 0.25-mm i.d., 0.25-µm film thickness) was used with helium as the carrier gas at a flow rate of 1.1 mL/min. After injection of the sample into the GC, the oven was held at 40°C for two minutes, and then ramped at a rate of 12°C/min to a final temperature of 300°C, where it was held for eight minutes. The MS transfer line was held at 280°C, the source temperature at 200°C, and the quadrupoles at 100°C. The MS operated with electron ionization (EI) at an ionization voltage of 70 eV. Analytes were identified using the selective-ion monitoring

(SIM) mode at *m/z* values summarized in Table 2.1. (first 17 analytes). Quantitation was based on conversion of raw area counts into ng/sample values using external calibration standards while correcting for field blanks. Air concentrations (ng/m^3) were determined using the volume of air sampled by each tube. Limits of quantitation (LOQ) were defined as three times the standard deviation of field-blank levels for each analyte.

Table 2.1. List of measured analytes with respective analytical parameters.

chemical name	abbreviation	Method[a]	SIM ion (*m/z*)	LOQ (ng/sample)[b]	LOQ (ng/m^3)[c]
benzene	Ben	TD GC-MS	78	1.48	123
toluene	Tol	TD GC-MS	91	3.19	264
ethylbenzene	Etb	TD GC-MS	106	0.63	52.3
m,p-xylene	mp-xyl	TD GC-MS	106	4.93	408
styrene	Sty	TD GC-MS	104	2.36	195
o-xylene	o-xyl	TD GC-MS	106	1.86	154
4-ethyltoluene	Etol	TD GC-MS	120	2.04	169
1,3,5-trimethylbenzene	Tmb	TD GC-MS	120	2.28	189
1,2,4-trimethylbenzene	Tmb*	TD GC-MS	120	10.7	885
naphthalene	Nap	TD GC-MS	128	0.32	26.6
acenaphthalene	Anap	TD GC-MS	152	0.09	7.09
acenaphthene	Ace	TD GC-MS	154	0.12	9.79
fluorene	Flu	TD GC-MS	166	0.25	20.4
phenanthrene	Phe	TD GC-MS	178	0.24	20.0
anthracene	Ant	TD GC-MS	178	0.18	15.0
fluoranthene	Fla	TD GC-MS	202	0.10	8.31
pyrene	Pyr	TD GC-MS	202	0.12	9.56
benz[*a*]anthracene	BaA	LE GC-MS	228	0.00004	0.00002
chrysene	Chr	LE GC-MS	228	0.00012	0.00006
benzo[*b*]fluoranthene	BbF	LE GC-MS	252	0.00147	0.00076
benzo[*k*]fluoranthene	BkF	LE GC-MS	252	0.00138	0.00072
benzo[*e*]pyrene	BeP	LE GC-MS	252	0.00157	0.00081
benzo[*a*]pyrene	BaP	LE GC-MS	252	0.00027	0.00014
indeno[1,2,3-*cd*]pyrene	Ind	LE GC-MS	276	0.00266	0.00138
benzo[*g,h,i*]perylene	BgP	LE GC-MS	276	0.00303	0.00157
dibenz[*a,h*]anthracene	DaA	LE GC-MS	278	0.00003	0.00002

[a] TD GC-MS = thermal desorption coupled with gas chromatography-mass spectrometry.
LE GC-MS = liquid extraction coupled with gas chromatography-mass spectrometry.
[b] Limit of quantitation in ng/sample calculated as three times the standard deviation of field-blank values.
[c] Limit of quantitation in ng/m^3 assuming a 0.012 m^3 air sample (avg. vol.) for TD GC-MS samples, and a 1.9 m^3 air sample (avg. vol.) for LE GC-MS samples.

2.3.3. Collection and analysis of particle-bound PAHs

A VAPS was used to sample the chamber inflow airstream at an average rate of 15 L/min for each two-hour exposure period. Particulate matter, for analysis of particle-bound PAHs, was collected using a preweighed (±10% mass accuracy) 47-mm o.d. 2-µm polytetrafluoroethylene (PTFE) filter (Pall Life Sciences, Ann Arbor, MI). At the end of each exposure period, the filter was reweighed to determine total mass. Samples were stored at -20°C for up to six months prior to liquid extraction and GC-MS analysis. Particle-bound PAHs were analyzed according to a previous method (Pleil et al. 2004) with minor modifications. Each sample filter was excised from its polypropylene ring and placed in an 8-mL extraction vial to which a 4-mL solution of HPLC-grade dichloromethane (Burdick & Jackson, Morristown, NJ) was added containing 4 ng of both ($^2H_{10}$)pyrene (Sigma-Aldrich, St. Louis, MO) and ($^2H_{12}$)benzo(*e*)pyrene (Cambridge Isotope Laboratories, Andover, MA) as internal standards. Vials were capped, vortexed for 20 seconds, and then agitated at 300 rpm for 90 minutes on an orbital shaker. After agitation, filters were removed from the vials, and the extracts were reduced to 1 mL under high purity nitrogen gas (National Speciality Gases, Durham, NC). The extracts were transferred into conical high-recovery autosampler vials (Agilent, Santa Clara, CA), and further reduced to 50 µL. For increased storage stability, a solvent exchange was performed; 50 µL of HPLC-grade methanol (Fisher Scientific, Pittsburgh, PA) was added and the remaining dichloromethane was removed under nitrogen gas purge leaving approximately 50 µL final volume. Samples were sealed with Teflon-lined septum crimp caps and were stored at -20°C under aluminum foil (light sensitivity) for up to one month prior to analysis by GC-MS. Two µL of each sample extract was injected into an Agilent 6890N GC via a 7683 autoinjector connected to a 5973 MS

(Agilent, Santa Clara, CA). Samples were injected using splitless mode with pulse pressure of 138 kPa (20 psi); the injection port contained a gooseneck liner with glass wool (Restek Corp., Bellefonte, PA, USA) held at 275°C. An RTX-5SILMS (Restek Corp., Bellefonte, PA) fused silica capillary column (60 m, 0.25 mm-i.d., 0.25-μm film thickness) was used with helium as the carrier gas at a flow of 1.0 mL/min. After injection of the sample into the GC, the oven was held at 50°C for three minutes, ramped at a rate of 25°C/min to a temperature of 150°C, and then ramped at a rate of 10°C/min to 300°C where it was held for 24 minutes. The MS transfer line was held at 280°C, the source temperature at 200°C, and the quadrupoles at 100°C. The MS operated with EI at an ionization voltage of 70 eV. Nine 4-6 ring PAHs were detected using the SIM mode as summarized in Table 2.1. Quantitation was based on peak areas of selected ions relative to the deuterated internal standard closest in molecular weight to the analyte of interest. LOQs for each compound were defined as three times the standard deviation of field-blank levels

2.3.4. Collection and analysis of OC, EC, and PM2.5

During each exposure period, a VAPS configured with PTFE filters sampled PM2.5 in the chamber inlet airstream. Filters were weighed before and after sampling to determine total mass loadings. The VAPS sampler operated at a flow rate of 15 L/min. The average chamber concentration determined from duplicate filters was used for statistical analyses. One quartz fiber filter (Sunset Laboratory, Hillsborough, NC) sample was also collected during each exposure period and analyzed for OC and EC using NIOSH method 5040 (NIOSH 1998a).

2.3.5. Statistical Methods

All statistical analyses were performed using SAS statistical software (v. 9.1, SAS Institute, Cary, NC), and all graphs were generated using GraphPad Prism software (v. 4, GraphPad Software, Inc., San Diego, CA). For observations below detection limits (purified air exposures only), a value of LOD/$\sqrt{2}$ was imputed (Hornung and Reed 1990; Pleil and Lorber 2007). A total of 73 out of 1,148 total measurements were below detection limits, mainly for 4- to 6-ring PAHs. These values were used to generate descriptive statistics for control chamber measurements only, and were not used in subsequent statistical analyses. Restricted maximum likelihood estimates of within- and between-day variance components were determined for selected analytes via Proc MIXED of SAS. Values used in mixed-effects modeling satisfied assumptions of normality (Shapiro-Wilks *W* test). Spearman correlation coefficients, determined via Proc CORR of SAS, were used to estimate pairwise correlations among analytes. For all tests, $p < 0.05$ (two-tailed) was determined to be statistically significant.

2.4. Results and discussion

2.4.1. Volatile aromatics and PAHs in DE and purified air

Descriptive statistics for measured airborne analytes are presented in Table 2.2. Estimated mean exposure concentrations of measured analytes in DE are arranged in order of increasing magnitude, as shown by the black bars (from left to right), in Figure 2.1. Air levels were lowest for particle-bound PAHs, while volatile & semivolatile PAHs had intermediate values, and volatile aromatic compounds had the highest concentrations. Air concentrations of the particle-bound PAHs ranged from 0.068 ng/m^3 for

dibenz[a,h]anthracene (DaA) to 0.346 ng/m^3 for Chr; air concentrations for the volatile & semivolatile PAHs ranged from 17.1 ng/m^3 for fluoranthene (Fla) to 2,600 ng/m^3 for Nap; and air concentrations for the volatile aromatic compounds ranged from 3,160 ng/m^3 for ethylbenzene (Etb) to 62,100 ng/m^3 for 1,2,4-trimethylbenzene (Tmb*).

For most analytes, measured concentrations in purified air were similar to field blank values (Table 2.2.). Under the standard assumptions satisfying least squares linear regression of our calibration data (particularly linearity and homogeneity of variance), we calculated these low concentrations using comparisons to batch-wise analyses of control standards processed exactly as if they were regular samples at nominal "zero" and "span" levels. Ratios were calculated of the mean analyte air concentration in DE to the mean analyte concentration in purified air. The calculation of the ratio parameter is used as one of two discriminators to differentiate a good from a poor potential DE surrogate. Although a relatively high ratio may not be proof of a useful surrogate, a comparatively low ratio suggests discarding the compound, as it is difficult to differentiate even under the best of conditions. The estimated values of these exposure ratios are shown in Figure 2.1. by the crosshatched bars. The largest exposure ratios were observed for the particle-bound PAHs, which ranged from 27.2 for DaA to 288 for Chr, and the smallest exposure ratios were observed for the volatile aromatic compounds, which ranged from 0.776 for styrene (Sty) to 58.5 for Ben. In selecting chemical surrogates for DE exposure, it is desirable to select candidate compounds that are both abundant, to maximize detection, and which have high exposure ratios, suggesting that they are highly differentiated with respect to purified air. Our results indicate that although the volatile aromatic compounds were the most abundant, they had low exposure ratios and were, therefore, not highly differentiated from purified air.

Particle-bound PAHs, on the other hand, had large exposure ratios but had very low abundance, and would be difficult to detect in studies of health effects. The compounds having the best combination of large abundance and large exposure ratios were the volatile & semivolatile PAHs, notably Nap and Phe.

Table 2.2. Mean levels (± SD) and ranges of analyes measured in chamber air.

		Diesel exhaust (ng/m^3)			Purified air (ng/m^3)		
analyte	**phase**[†]	**mean ± SD**	**min**	**max**	**mean ± SD**	**min**	**max**
Ben	gas	3330 ± 790	217	5130	56.9 ± 33.0	24.3	117
Tol	gas	3670 ± 1090	1970	5320	604 ± 376	177	1290
Etb	gas	3160 ± 929	1660	4180	76.6 ± 62.8	24.0	195
mp-xyl	gas	11800 ± 4660	4720	19600	624 ± 610	101	1830
Sty	gas	4910 ± 1410	3140	7210	6330 ± 3280	2290	13800
o-xyl	gas	4470 ± 1550	2080	6890	306 ± 379	33.4	1310
Etol	gas	6520 ± 3120	2190	12700	381 ± 571	39.6	1940
Tmb	gas	10500 ± 4060	4440	18100	717 ± 847	114	2970
Tmb*	gas	62100 ± 41000	17900	164000	3210 ± 5280	393	18000
Nap	gas + particle	2600 ± 658	1690	3630	10.3 ± 4.94	5.14	21.0
Anap	gas + particle	173 ± 48.5	69.8	232	2.21 ± 1.55	0.354	5.01
Ace	gas + particle	118 ± 19.6	96.7	150	3.40 ± 2.60	0.521	6.92
Flu	gas + particle	158 ± 50.8	72.9	236	5.14 ± 5.20	0.613	14.4
Phe	gas + particle	765 ± 181	507	1070	8.28 ± 4.60	1.32	17.7
Ant	gas + particle	78.6 ± 20.6	53.2	114	4.78 ± 4.37	0.392	10.6
Fla	gas + particle	17.1 ± 6.0	8.17	29.3	3.52 ± 2.75	0.291	7.69
Pyr	gas + particle	89.9 ± 35.8	34.0	145	6.86 ± 6.55	1.31	22.2
BaA	particle	0.239 ± 0.076	0.160	0.345	0.0010 ± 0.0018	0.00006	0.0056
Chr	particle	0.346 ± 0.100	0.220	0.525	0.0012 ± 0.0018	0.00021	0.0056
BbF	particle	0.307 ± 0.066	0.177	0.390	0.0037 ± 0.0062	0.00068	0.0190
BkF	particle	0.153 ± 0.037	0.0874	0.214	0.0019 ± 0.0033	0.00011	0.0100
BeP	particle	0.264 ± 0.036	0.195	0.309	0.0028 ± 0.0043	0.00015	0.0133
BaP	particle	0.341 ± 0.072	0.169	0.410	0.0036 ± 0.0053	0.00051	0.0163
Ind	particle	0.205 ± 0.066	0.103	0.320	0.0022 ± 0.0033	0.00092	0.0104
BgP	particle	0.233 ± 0.076	0.132	0.330	0.0023 ± 0.0026	0.00070	0.0084
DaA	particle	0.068 ± 0.029	0.0348	0.121	0.0025 ± 0.0048	0.00001	0.0139

[†] Gas- and gas + particle-phase compounds collected using adsorbent cartridges and analyzed using TD GC-MS; Particle-phase compounds were collected using active filter sampling and analyzed using LE GC-MS.

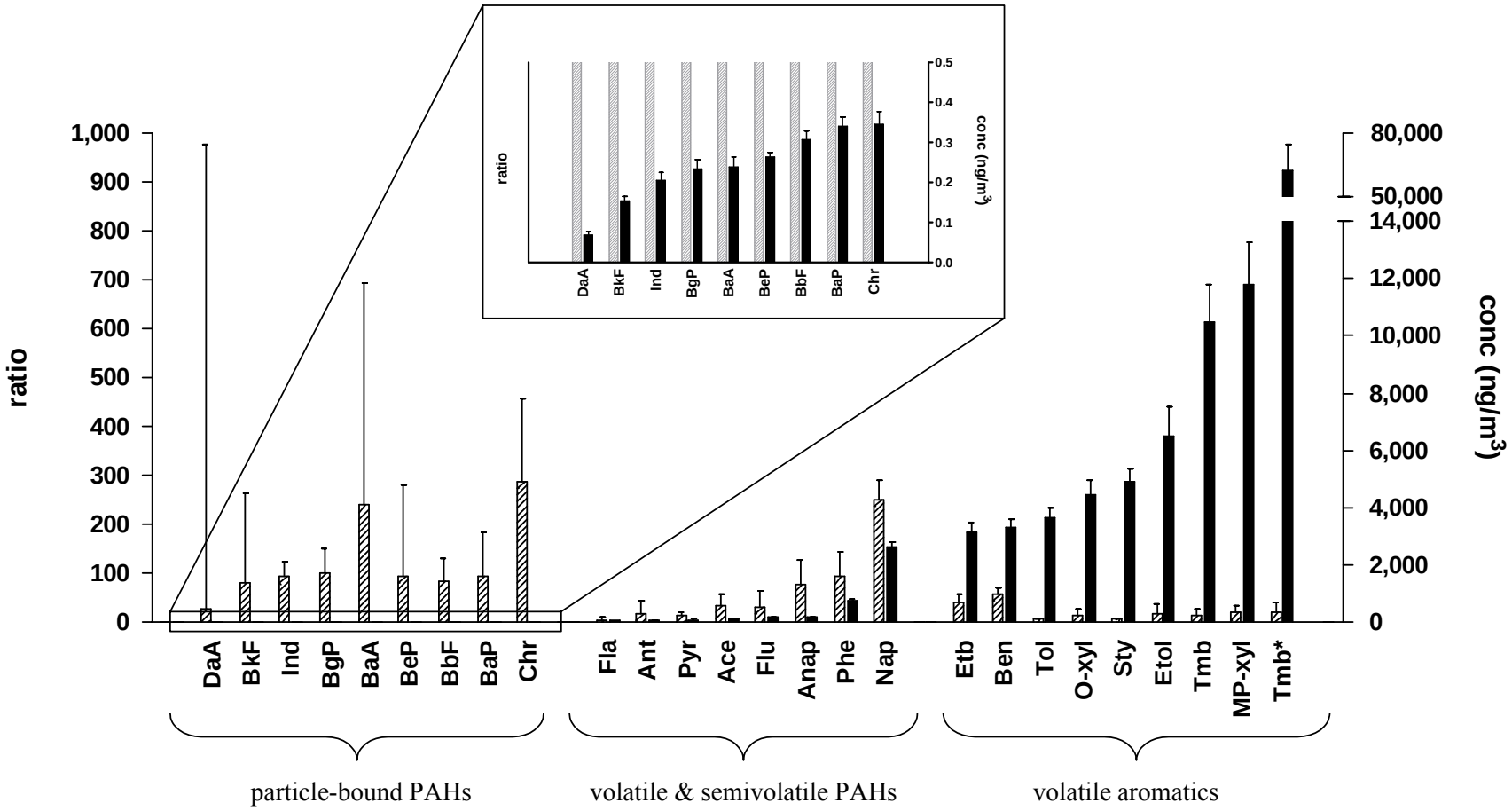

Figure 2.1. Air concentrations of volatile aromatics and PAHs in diesel exhaust, and corresponding exposure ratios. Data are arranged in order of increasing air concentration. Black bars represent estimated mean diesel exhaust exposure concentrations (right *y*-axis); crosshatched bars represent estimated exposure ratios [(mean diesel exhaust air concentration: mean purified air concentration); left *y*-axis]. Error bars represent estimated SE.

2.4.2. Nap and Phe as surrogates for DE exposure

Nap and Phe clearly stand out among volatile & semivolatile PAHs (see Figure 2.1.) due to their relatively high abundance (mean DE conc. = 2600 and 765 ng/m^3, respectively) and large exposure ratios (252 and 92.4, respectively). This finding supports results from previous observational studies in which air levels of Nap and Phe were relatively large among populations occupationally exposed to DE [i.e., truck drivers, maintenance workers and bus garage workers, and toll booth operators (Kuusimaki et al. 2002; Kuusimaki et al. 2003; Tsai et al. 2004)]. Table 2.3. compares the levels of Nap and Phe measured in our chamber experiments with the air concentrations from these earlier investigations. Levels of Nap and Phe in chamber purified air were lower than those measured among literature reported control subjects (228 and 37 ng/m^3, respectively). Levels of Nap and Phe in chamber DE were very similar to those measured among bus garage workers (1023 and 568 ng/m^3, respectively), and were higher than those measured among truck drivers (455 and 154 ng/m^3, respectively) and maintenance workers (162 and 53 ng/m^3, respectively). Whereas Nap levels in chamber DE were lower than those measured among toll booth attendants (8850 ng/m^3), Phe levels were higher in chamber DE compared to toll booth attendant exposure levels (264 ng/m^3). We note that Nap and Phe measurements among toll booth attendants reflect exposures from diesel and non-diesel sources (i.e., gasoline-powered engines). As few studies have published individual Nap and Phe measurements from strictly diesel sources, the data are included here for a general comparison with our controlled exposure levels.

Nap and Phe possess physiochemical properties that make them potentially desirable surrogates for DE. Nap is generally the most abundant PAH measured from a given source (Rappaport et al. 2004). Furthermore, because Nap exposure occurs almost entirely in the

vapor phase, it can be measured using passive adsorbent techniques (Egeghy et al. 2003). Such techniques eliminate the need for cumbersome and expensive sampling equipment, thereby increasing the ability to obtain more air measurements at a given cost for studies of health effects (Rappaport 1991). Nap also offers several potentially useful biomarkers of PAH exposure, including unmetabolized Nap in urine (Waidyanatha et al. 2003; Campo et al. 2006a), several urinary metabolites (Serdar et al. 2003b; Serdar et al. 2004; Wu et al. 2005), and protein adducts of the naphthoquinones (metabolites of Nap) in human serum albumin (Waidyanatha et al. 2004). Finally, since Nap is classified as a possible human carcinogen (IARC 2002), it is useful to characterize human exposures to Nap *per se*. Phenanthrene is also abundant in PAH exposure scenarios, both in vapor and particle-bound phases. Vapor-phase Phe can presumably be measured in air using passive adsorbent techniques. While Phe is not classified as a human carcinogen (IARC 1983; IARC 1987), its metabolism follows closely to that of carcinogenic PAHs (Shou et al. 1994; Carmella et al. 2004); numerous Phe metabolites can be measured in human urine as biomarkers of PAH exposure (Hecht et al. 2003; Carmella et al. 2004; Kim et al. 2005).

Table 2.3. Comparison of Nap and Phe levels in studies involving diesel exhaust exposure.

Study	Exposure Group	Air Nap (ng/m^3)		Air Phe (ng/m^3)	
Kuusimaki et al. 2002	Truck drivers [a,b]	455	(175 - 2500)	154	(20 -1135)
	Maintenance workers [a,b]	162	(50 - 476)	53	(< 20 - 395)
	Controls [a,b]	228	(100 - 510)	37	(< 20 - 215)
Kuusimaki et al. 2003	Bus garage workers [a,b]	1023	(204 - 3362)	568	(43 - 2664)
	Controls [a,b]	228	(100 - 510)	37	(< 23 - 215)
Tsai et al. 2004	Toll booth attendants [c]	8850	(5040 - 12300)	264	(111 - 422)
This Study	Diesel exhaust exposure	2600	(1690 - 3630)	765	(507 - 1070)
	Purified air exposure	< 27		< 20	

Mean exposure levels and ranges reported
[a] Mean values of combined winter and summer data
[b] Gas-phase measurements only
[c] Values reflect vehicle engine emissions including those from diesel sources

2.4.3. Variability assessment for Nap and Phe

The mean levels of Nap and Phe across ten DE exposure days were 2600 ng/m^3 and 765 ng/m^3, and the standard deviations were 658 ng/m^3 and 181 ng/m^3, respectively. These standard deviations reflect the dispersion of ten individually averaged values specific to each exposure day, and serve as a measure of the between-day exposure variability. For volatile & semivolatile PAHs including Nap and Phe, three measurements were made on each exposure day, allowing the simultaneous assessment of within-day and between-day variability. Estimates of within-day variability are assumed to represent assay variability across three replicate samples; between-day estimates represent variability in daily DE exposure levels. Calculation of the intraclass correlation coefficient [ICC $= \hat{\sigma}_b^2 / (\hat{\sigma}_w^2 + \hat{\sigma}_b^2)$; where $\hat{\sigma}_b^2$ represents the estimated between-day variance component and $\hat{\sigma}_w^2$ represents the estimated within-day variance component of the total estimated variance (i.e. $\hat{\sigma}_w^2 + \hat{\sigma}_b^2 = \hat{\sigma}^2$)] allows

assessment of the relative variability between exposure days to total measured variation. For Nap in DE, the majority of total measured variation came between exposure days (ICC = 0.91). In fact, subjects exposed to DE on days 5, 6, 9, and 10 received Nap exposures significantly different than the overall group mean (Figure 2.2.A). For Phe, about one third of the total measured variation came between exposure days (ICC = 0.36), and no subjects were identified as receiving Phe exposures significantly different than the group mean (Figure 2.2.B). Within-day variability estimates were nearly identical for Nap and Phe (Nap: $\hat{\sigma}_w^2$ = 39200; Phe: $\hat{\sigma}_w^2$ = 37400) suggesting uniform assay variability for these analytes. All measurements of Nap and Phe made during purified air exposures were determined to be indistinguishable from field-blank values, and were therefore not considered in this assessment of sources of variability.

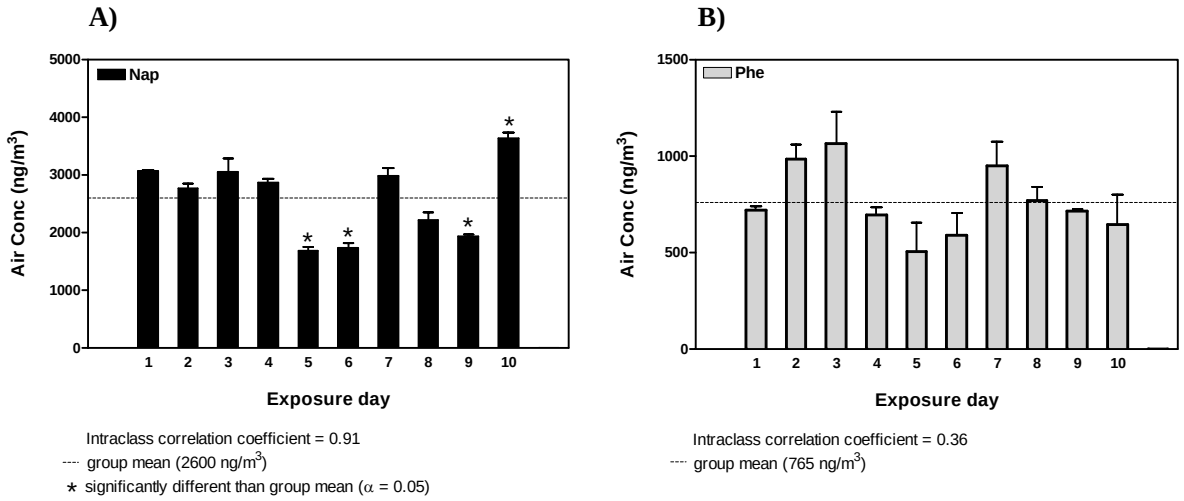

Figure 2.2. Chamber measurements of Nap (A) and Phe (B) across ten diesel exhaust exposure days. Each bar represents the day-specific mean exposure level. Error bars (SE) represent variability across three measurements per day.

2.4.4. Correlations among surrogate measures of DE exposure

Although chamber DE exposures were highly regulated based on real-time particulate measurements, individual analytes associated with DE displayed variability. While this variability was very modest compared to that observed in environmental and occupation settings (Lin et al. 2005), significant daily variations in individual analyte levels were observed. In considering Nap and Phe as potential surrogates for DE exposure, it is particularly useful to compare the air concentrations of Nap and Phe with those of accepted measures of particulate exposure, namely, OC, EC, and PM2.5, as well as with the combined air concentrations of PAHs in the particle-bound phase [containing the most carcinogenic compounds (IARC 1983; IARC 1987)]. Figure 2.3. shows a correlation matrix, for the estimated mean air levels of Nap, Phe, OC, EC, PM2.5, total volatile aromatics, total volatile+semivolatile PAHs (minus Nap and Phe), and total particle-bound PAHs, for the ten subjects exposed to DE in our study (only measurements made during actual DE exposures were included in this correlation analysis). Air concentrations of Nap were significantly correlated with OC (r = 0.85; p = 0.0016), EC (r = -0.71; p = 0.022) and particle-bound PAHs (r = 0.67; p = 0.033). The negative correlation between Nap and EC mirrors that which was observed between OC and EC [r = -0.64; p = 0.048 (not shown in Figure 2.3.)]. As DE is composed of an elemental carbon core with adsorbed organics, it stands to reason that when the total mass is held constant (as was the case during our controlled exposures), the EC and OC components of total mass should be negatively correlated. While EC is a useful DE exposure surrogate in occupational studies (Zaebst et al. 1991; Davis et al. 2006; Smith et al. 2006; Davis et al. 2007), the positive correlations between Nap and OC, and between Nap and total particle-bound PAHs, suggests that Nap may be a particularly good

surrogate for the organic constituents of DE. This finding is even more interesting when considering that air levels of Nap have previously been shown to be highly correlated with those of total PAHs in studies of workers in the steel and aluminum industries where PAH levels are particularly high (Rappaport et al. 2004). In addition to the observed Nap correlations, a highly significant pairwise correlation was observed between concentrations of Phe and total volatile+semivolatile PAHs ($r = 0.96$; $p < 0.0001$), suggesting that Phe may also be a useful surrogate for selected organic DE constituents.

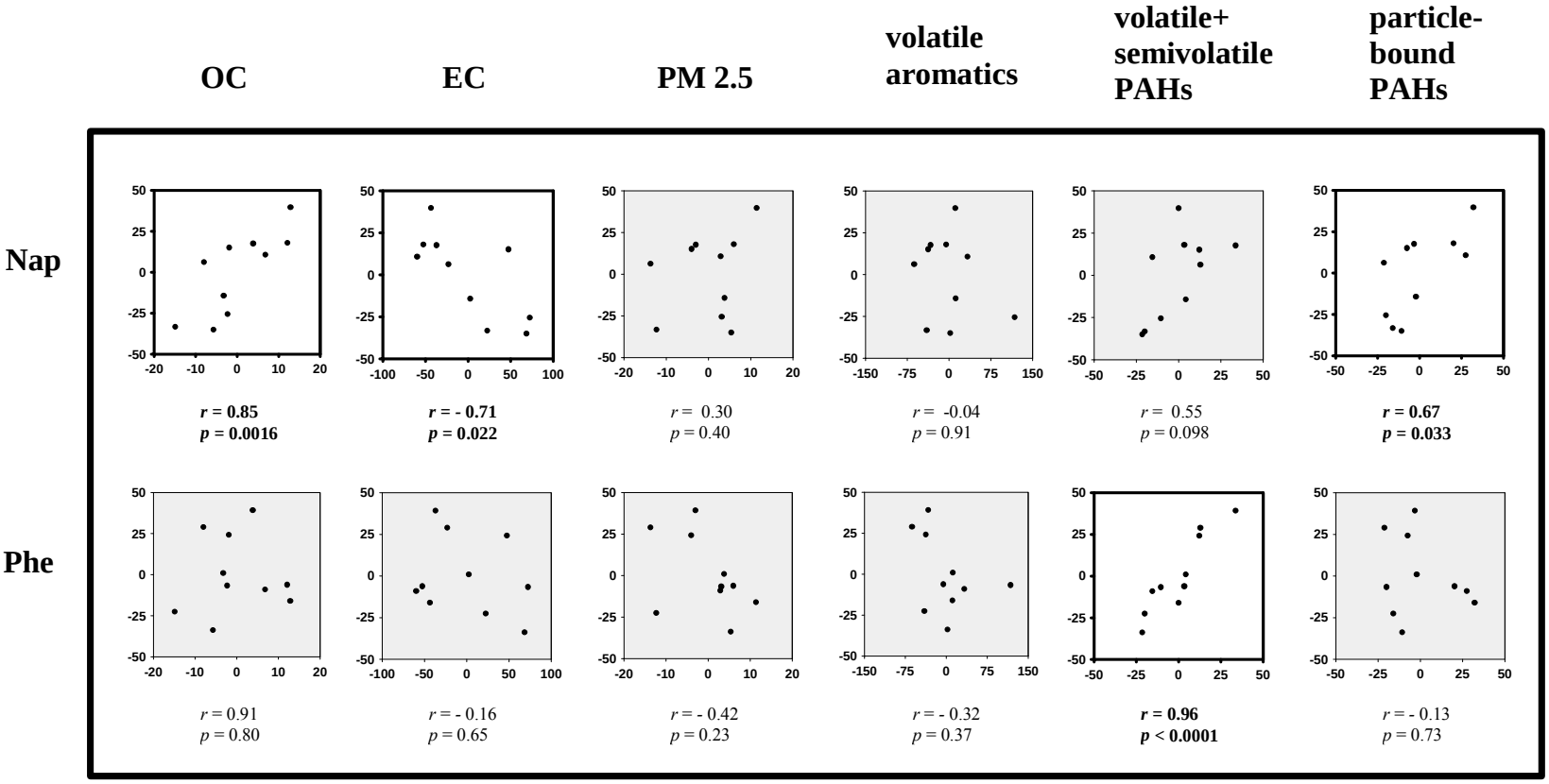

Figure 2.3. Correlations among constituents of diesel exhaust; total DE mass was feedback regulated to achieve 100 µg/m^3.

Nap = naphthalene; Phe = phenanthrene; OC = organic carbon; EC = elemental carbon; PM2.5 = fine particulate matter
x- and y-axes represent % deviation from mean exposure level (n =10)
r = Spearman's *rho*; p = p -value

2.4.5. Concluding remarks

Nap and Phe are promising surrogate markers for the broad spectrum of airborne constituents resulting from DE sources. They are both abundant in DE and highly differentiated in trace-level measurements with respect to purified ambient air. In addition, Nap and Phe are easily measured in air and can also be assayed as biomarkers of exposure. Although the chamber concentrations were highly regulated based on real-time particulate measurements, individual analytes associated with DE displayed variability. We attribute this to small differences in organic enrichment of the particles, stemming from subtle changes in truck engine performance and weather conditions. Despite this observed variability, significant positive correlations between concentrations of Nap and other DE markers (i.e., OC and total particle-bound PAHs), and between Phe and total volatile+semivolatile PAHs, suggest that Nap and Phe could serve as quantitative markers for DE concentrations in future health effects studies and epidemiological risk assessments. We caution that all of the common DE markers may have other environmental sources as well, and that other meta-data will be important for assessing their relative contributions. However, we conclude from this work that Nap and Phe are optimal surrogates if a direct comparison between biomarker and external source is sought. We are currently assessing a series of metabolic biomarkers and response parameters in human biological specimens collected during this study and will discuss those results in ensuing articles.

3. Paper II. Urinary Naphthalene and Phenanthrene as Biomarkers of Occupational Exposure to Polycyclic Aromatic Hydrocarbons [Sobus, J.R., Waidyanatha, S., McClean, M.D., Herrick, R.F., Smith, T.J., Garshick, E., Laden, F., Hart, J.E., Zheng, Y., Rappaport, S.M., submitted to Occupational and Environmental Medicine; provisional acceptance, June 2008.]

3.1. Abstract

Objectives: We investigated the utility of unmetabolized naphthalene (Nap) and phenanthrene (Phe) in urine as surrogates for exposures to mixtures of polycyclic aromatic hydrocarbons (PAHs).

Methods: Our study included workers exposed to diesel exhausts (low PAH exposure level, $n = 39$) as well as those exposed to emissions from asphalt (medium PAH exposure level, $n = 26$) and coke ovens (high PAH exposure level, $n = 28$). Levels of Nap and Phe were measured in urine from each subject using head space-solid phase microextraction and gas chromatography-mass spectrometry. Published levels of airborne Nap, Phe, and other PAHs in the coke-producing and aluminum industries were also investigated.

Results: In post-shift urine, the highest estimated geometric mean concentrations of Nap and Phe were observed in coke-oven workers (Nap: 2,490 ng/l; Phe: 975 ng/l), followed by asphalt workers (Nap: 71.5 ng/l; Phe: 54.3 ng/l), and by diesel-exposed workers (Nap: 17.7 ng/l; Phe: 3.60 ng/l). After subtracting logged background levels of Nap and Phe from the logged post-shift levels of these PAHs in urine, the resulting values [referred to as ln(adjNap) and ln(adjPhe), respectively] were significantly correlated in each group of workers ($0.71 \leq$ Pearson $r \leq 0.89$), suggesting a common exposure source in each case. Surprisingly, multiple linear regression analysis of ln(adjNap) on ln(adjPhe) showed no significant effect of the

source of exposure (coke ovens, asphalt, and diesel exhaust) and further suggested that the ratio of urinary Nap/Phe (in natural scale) decreased with increasing exposure levels. These results were corroborated with published data for airborne Nap and Phe in the coke-producing and aluminum industries. The published air measurements also indicated that Nap and Phe levels were proportional to the levels of all combined PAHs in those industries.

Conclusion: Levels of Nap and Phe in urine reflect airborne exposures to these compounds and are promising surrogates for occupational exposures to PAH mixtures.

3.2. Introduction

Polycyclic aromatic hydrocarbons (PAHs) comprise a class of chemicals composed of two or more fused aromatic rings. Since PAHs are produced by the incomplete combustion of organic matter, including petroleum, coal, and other carbonaceous materials (e.g. wood, tobacco products, food products), they are ubiquitous contaminants of the human environment. Humans are exposed to PAHs via inhalation, ingestion, and dermal contact. Moderate to high-level PAH exposures stemming from coke ovens, aluminum production, and asphalt use have been associated with cancers of the lung, skin, and bladder (Boffetta et al. 1997). Exposure to diesel exhausts, containing lower levels of PAHs, has been associated with increased lung cancer risks (Bhatia et al. 1998; Lipsett and Campleman 1999).

The quantitative assessment of PAH exposure has been complicated by the large number of individual compounds in a given mixture and by the presence of PAHs in both the gas phase (2-ring and 3-ring compounds) and the particulate phase (4-ring to 6-ring

compounds). While certain particulate-phase PAHs (notably benzo(*a*)pyrene) have been classified as known or probable human carcinogens (IARC 1983; IARC 1987), air concentrations of these 4-6 ring PAHs tend to be very low and difficult to measure. Recently, attention has focused upon the more abundant gas-phase PAHs, notably naphthalene (Nap, two rings) and phenanthrene (Phe, three rings), as possible surrogates for PAH exposure (Jacob and Seidel 2002; Rappaport et al. 2004). Naphthalene is typically the most abundant PAH measured from a given source (Rappaport et al. 2004), and air levels of Nap tend to be highly correlated with the sum of all measured PAH levels (hereafter 'total PAHs') in workplaces (Rappaport et al. 2004). Since Nap is a known carcinogen of the lung in rodents (Abdo 1992; NTP 1992; NTP 2000; Abdo et al. 2001), it is important to characterize human exposure to Nap *per se*. Phenanthrene is also present at high concentrations in PAH emissions and, while not classified as a carcinogen, is the smallest PAH to contain a bay region, a feature closely associated with carcinogenicity (Shou et al. 1994; Carmella et al. 2004).

Small amounts of Nap and Phe are eliminated unchanged in the urine (Waidyanatha et al. 2003). Here we report levels of unmetabolized Nap and Phe in urine from workers exposed to three sources of PAHs that had been classified *a priori* as having low, medium, and high levels of PAHs, namely, diesel-exhausts, asphalt emissions, and coke-oven emissions, respectively (Brandt and Watson 2003). We show that urinary levels of Nap and Phe in these workers followed the expected low, medium, and high designations of the sources, and that they were highly correlated, suggesting a common source of exposure to Nap and Phe in each group. We further compare results from measurements of urinary Nap

and Phe with published data representing air concentrations of Nap, Phe, and total PAHs in the coke-producing and aluminum-producing industries (Bjorseth et al. 1978a; Bjorseth et al. 1978b; Petry et al. 1996; Strunk et al. 2002).

3.3. Methods

3.3.1. Chemicals and supplies

Naphthalene (99+%), phenanthrene (99.5%), ($^2H_8$)naphthalene (98+%), ($^2H_{10}$)phenanthrene (98+%), and methanol (purge and trap grade) were obtained from Aldrich Chemical Company (Milwaukee, WI). Sodium chloride was obtained from Fisher Scientific (Pittsburgh, PA). Head space-solid phase microextraction (HS-SPME) supplies were obtained from Supelco (Bellefonte, PA) and MicroLiter Analytical Supplies, Inc. (Suwanee, GA).

3.3.2. Sources of urine samples and published air measurements

Post-shift urine samples were obtained from 28 coke-oven workers (15 top workers and 13 side and bottom workers) and 22 control workers (office and hospital workers) from a single steel-producing complex in Northern China. Since levels of Nap and Phe had previously been determined in urine from these subjects (Serdar et al. 2003b; Waidyanatha et al. 2003), here we compare the original data with those obtained from other groups of workers in the current investigation. Urine samples were also obtained from 26 asphalt workers in road paving crews in the Northeastern U.S. (McClean et al. 2004a; McClean et al. 2004b). This group included 20 paving workers who applied hot mix asphalt to roads, and six milling workers who removed old asphalt from roads. Urine samples were collected from

asphalt workers on either two (milling workers) or three consecutive days (paving workers) both before and after work shifts, starting at the beginning of the work week. Finally, urine samples were obtained from 39 diesel-exposed workers who performed various tasks in trucking terminals throughout the U.S. (Davis et al. 2006; Smith et al. 2006). (While workers in trucking terminals may have experienced PAH exposures from ambient air, diesel exhaust is believed to be their primary source of PAH exposure, and we refer to this group as 'diesel-exposed workers' for simplicity). This group included 27 loading-dock workers who drove propane forklifts and loaded trailers, 8 truck-repair-shop workers involved with truck maintenance and refueling activities, and 4 office workers who had only background exposure to diesel exhausts. Pairs of urine samples were collected from each diesel-exposed worker before and after a work shift. The smoking status of all workers was obtained by questionnaire.

Air levels of Nap, Phe, and total PAHs were derived from published data reporting exposures in the coke-producing and aluminum industries. The data were previously summarized by Rappaport et al. (Rappaport et al. 2004), who focused upon Nap exposures. For this analysis, we consider air levels of both Nap and Phe that were reported along with various other PAHs in several workplaces. The data include 32 measurements (area, breathing-zone, and personal samples) for up to 39 PAHs in a coke production plant (Bjorseth et al. 1978b), three measurements (grouped personal samples from 24 individuals) for 16 PAHs in a coke-production plant (Strunk et al. 2002), 28 measurements (including area and personal samples) for up to 36 PAHs in an aluminum reduction plant (Bjorseth et al. 1978a), and six measurements (midrange of five personal samples from 6 workers) for 26 PAHs in a carbon anode plant (Petry et al. 1996).

All subjects included in this study provided informed consent to participate according to protocols approved by ethics committees at the Harvard University School of Public Health (Boston, Massachusetts, U.S.A.) and the Institute for Occupational Medicine (Beijing, China).

3.3.3. Analysis of urinary naphthalene and phenanthrene

Urine samples from asphalt and diesel-exposed workers were analyzed for Nap and Phe as previously described, with minor modifications (Waidyanatha et al. 2003). Samples were stored at either -20°C or -80°C prior to analysis. After thawing, 0.7-ml portions were transferred into 2-ml crimp top vials containing 0.3 g of NaCl. Urine samples were spiked with 1.0 µl of an internal standard mixture containing ($^2H_8$)Nap and ($^2H_{10}$)Phe in methanol, to give a final concentration of 0.5 µg/l of urine. Samples were immediately capped and stored at -20°C for up to 24 h prior to analysis. Prior to use, vials, caps, and NaCl were conditioned at 160°C to remove background Nap and Phe.

Headspace solid-phase microextraction (HS-SPME) was performed to extract Nap and Phe from urine using a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland). Prior to analysis, samples were brought to room temperature, and were incubated at 55°C for three minutes. Analytes were sampled from urine headspace using a PDMS fiber (10 mm, 100-µm film thickness). Adsorption and desorption times were 30 and 20 min, respectively. Levels of Nap and Phe were measured with a model 6890N gas chromatograph (GC) coupled to a model 5973N mass spectrometer (MS) (Agilent, Palo Alto, CA). The MS was operated with electron impact ionization at an ionization voltage of 70eV. The MS transfer line was maintained at 280°C, the source temperature at 200°C, and the quadrupole at 100°C. A DB-

1 (J&W Scientific Inc., Folsom, CA) fused silica capillary column (60 m, 0.25-mm i.d., 0.25-μm film thickness) was used with He as the carrier gas. A 0.75-mm i.d. SPME injection sleeve was used in the injector port, with the temperature maintained at 250°C. The GC oven was held at 75°C for 8 min, and then ramped at 5°C/min to 260°C, where it was held for ten min. Ions selected for analysis included *m/z* 128 (Nap), *m/z* 136 [($^2H_8$)Nap], *m/z* 178 (Phe), and *m/z* 188 [($^2H_{10}$)Phe]. Quantitation was based on response ratios of the analytes to the corresponding internal standards [($^2H_8$)Nap or ($^2H_{10}$)Phe]. Standard curves were prepared with pooled urine from human volunteers, which had been spiked with Nap and Phe at concentrations of 0.40, 2.0, 10, 25, 50, 75, and 100 ng/l and the same levels of internal standards used for experimental samples. The estimated coefficients of variation for Nap and Phe were 0.25 and 0.26, respectively, and the estimated limit of detection (LOD) was 0.40 ng/l for each analyte. Two observations, with analyte levels below the LOD, were assigned levels of LOD/$\sqrt{2}$ for statistical analyses.

3.3.4. Statistical analyses

Statistical analyses were performed after (natural) logarithmic transformation of urinary levels, to remove heteroscedasticity and satisfy normality assumptions, using SAS statistical software (v. 9.1, SAS Institute, Cary, NC). A p-value < 0.05 was considered significant (two-tailed test). For asphalt workers, subject-specific means of logged pre-shift and post-shift Nap and Phe levels (from urine samples collected on two or three consecutive days) were used for statistical analyses. General linear models were used with dummy variables to test for effects on analyte levels of the source of exposure (diesel exhausts, asphalt emissions, and coke-oven emissions), the job category (coke-top, coke-side, coke-

control, asphalt-paver, asphalt-miller, diesel-office, diesel-shop, and diesel-dock workers), smoking status, and interaction terms for job category and smoking status (Proc GLM). Correlation and regression analyses of subject-specific urinary analyte levels were based upon background-adjusted values as follows: Since asphalt and diesel-exposed workers had paired pre-shift and post-shift urine samples, background adjustment was performed by subtracting logged pre-shift analyte levels from logged post-shift analyte levels. Background adjustment for coke-oven workers, who had only post-shift urine samples, was performed by subtracting the mean logged level estimated in factory control workers from the logged level observed for each coke-oven worker. Since background adjustment was based upon subtraction of logged pre-shift or control values of Nap and Phe from logged post-shift values, the adjusted values, designated adjNap and adjPhe, in natural scale, represent the ratios of post-shift analyte levels to pre-shift or control levels. Pairwise correlations between ln(adjNap) and ln(adjPhe) were estimated using Pearson correlation coefficients (Proc CORR). Least squares multiple linear regression was used to investigate the relationship between ln(adjNap) and ln(adjPhe) using dummy variables to test for effects of worker group and job category (Proc REG). Individual observations were investigated as possible outliers based upon influence as measured by leverage, Studentized residuals, Cook's distance, and change in adjusted R^2. Considering these criteria, one observation (out of a total of 93 observations) was rejected from the final model (removing this outlier increased the adjusted R^2 of the final model by 7.7%). Least squares multiple linear regression was also used to investigate the relationship between logged air concentrations of Nap and Phe using dummy variables for sources of exposure (coke- and aluminum-producing industries) (Proc REG). Three observations of air data (out of a total of 85 observations) were not included in the

analysis, because they were reported as containing little or no Nap and/or Phe in the gas phase, a physical impossibility for the environments in question.

3.4. Results and discussion

3.4.1. Effects of job category on urinary analyte levels

Summary statistics [geometric means (GMs), geometric standard deviations (GSDs), and numbers of subjects] of Nap and Phe levels in pre-shift urine samples (asphalt and diesel-exposed workers only) and post-shift urine samples are presented in Table 3.1., for workers classified by source of PAHs and job category. Results from general linear models showed a significant effect of job category on Nap and Phe levels in both pre-shift ($p < 0.0005$) and post-shift urine ($p < 0.0001$). After adjustment by job category, smoking status did not significantly affect Nap and Phe levels in either pre-shift or post-shift urine ($p > 0.05$).

Table 3.1. Urinary naphthalene and phenanthrene levels [a] for workers grouped by source of PAHs and job category.

Source of PAHs	Job Category	No. Subjects	Naphthalene Level (ng/l)		Phenanthrene Level (ng/l)	
			Pre-shift	Post-shift	Pre-shift	Post-shift
Diesel exhausts	Dock workers	27	21.2 (1.69)	17.1 (1.71)	3.39 (2.02)	2.67 (1.87)
	Office workers	4	14.9 (1.51)	18.0 (1.42)	4.05 (2.20)	5.18 (1.40)
	Shop workers	8	13.0 (1.55)	19.6 (2.28)	4.40 (2.16)	8.20 (2.31)
Asphalt emissions	Asphalt milling workers	6	33.3 (1.30)	34.4 (2.01)	10.1 (2.01)	23.8 (2.87)
	Asphalt paving workers	20	32.3 (1.90)	89.1 (1.90)	11.0 (2.61)	69.6 (2.01)
Coke-oven emissions	Office and hospital workers (factory controls)	22	*NA*	765 (2.31)	*NA*	58.2 (3.27)
	Coke-oven workers (side and bottom)	13	*NA*	1710 (3.39)	*NA*	735 (4.56)
	Coke-oven workers (top)	15	*NA*	3450 (5.14)	*NA*	1250 (7.65)

[a]Geometric mean (geometric standard deviation) levels are displayed.
Legend: *NA* = not available.

Significant differences in pre-shift urine levels suggest job-specific variations in background exposures stemming from variability in ambient air exposures, diet, lifestyle factors, and/or geographic location. The post-shift data point to an approximate 200-fold range of Nap levels and a 470-fold range of Phe levels across the different job categories, with the highest levels observed in the top coke-oven workers and the lowest levels observed in the diesel-exposed workers. The ranks of observed Nap and Phe levels are consistent with *a priori* reports of PAH exposures (Brandt and Watson 2003), where coke-oven workers had the highest concentrations (top > side and bottom > steel-factory controls) (Serdar et al. 2003b; Waidyanatha et al. 2003), followed by asphalt workers (paving > milling) (McClean et al. 2004a; McClean et al. 2004b), followed by diesel-exposed workers. We note that the asphalt workers' Nap and Phe levels in urine are very similar to median levels measured by

Campo et al. in road paving workers and road construction workers (Campo et al. 2006a; Campo et al. 2007). We also note that the control workers from the Chinese steel-making complex had higher levels of Nap and Phe in their urine than even the asphalt workers, indicating a significant source of background exposure to PAHs in the air of that factory.

3.4.2. Background-adjusted levels of urinary naphthalene and phenanthrene

Summary statistics for Nap and Phe levels are shown in Table 3.2. by source of PAHs, after adjustment for background concentrations of Nap and Phe. Because (in natural scale) the adjusted values represent ratios of post-shift levels to background levels, values of adjNap and adjPhe of approximately one for diesel-exposed workers imply little difference between post-shift and background levels for a typical subject. Adjusted post-shift levels for asphalt workers indicate an approximate 2-fold and 5-fold increase over background levels for Nap and Phe, respectively and results for coke-oven workers suggest 3-fold and 17-fold increases over background levels for Nap and Phe, respectively. While background-adjusted values follow the same rankings as the unadjusted levels of Nap and Phe (coke-oven workers > asphalt workers > diesel-exposed workers), the ranges are considerably smaller, i.e., about 3-fold for adjNap and 17-fold for adjPhe. This reflects the large range of background levels of Nap and Phe observed for each worker group. (We recognize that our use of the large median values of urinary Nap and Phe measured in factory controls to adjust the corresponding urinary levels for the Chinese coke-oven workers could have introduced uncertainty into this analysis). Since Nap is typically the most abundant PAH measured from a given source (Rappaport et al. 2004), the larger adjPhe values suggest greater urinary excretion of Phe compared to Nap and/or lower relative background concentrations of Phe

compared to Nap. This result is consistent with previous results estimating the percentages of excreted Nap and Phe in the coke oven-workers to be 4 and 13%, respectively, of the urinary levels of the metabolites of these PAHs (Waidyanatha et al. 2003).

Table 3.2. Background-adjusted levels of naphthalene and phenanthrene in the urine of groups of workers exposed to PAHs from three different sources. [These background-adjusted values represent the ratios of levels of naphthalene and phenanthrene in post-shift urine to either pre-shift urine levels (diesel-exhaust and asphalt sources) or factory-control urine levels (coke-oven source)].

Source of PAHs	No. Subjects	Adjusted Naphthalene Level (ratio of post-shift level to background level)			Adjusted Phenanthrene Level (ratio of post-shift level to background level)		
		GM (GSD)	Min.	Max.	GM (GSD)	Min.	Max.
Diesel exhaust	39	0.959 (2.00)	0.159	7.61	0.989 (2.74)	0.228	17.5
Asphalt	26	2.20 (2.19)	0.651	10.4	5.04 (3.35)	1.21	219
Coke ovens	28	3.26 (4.38)	0.144	32.8	16.7 (6.06)	0.228	330

Legend: GM = geometric mean; GSD = geometric standard deviation.

3.4.3. Relationships between urinary levels of naphthalene and phenanthrene

Significant correlations between ln(adjNap) and ln(adjPhe) levels were observed in all three groups of workers ($p < 0.0001$), suggesting common sources of exposure to Nap and Phe in each case. Pearson correlation coefficients for ln(adjNap) and ln(adjPhe) increased from $r = 0.71$ for diesel-exposed workers, to $r = 0.82$ for asphalt workers, to $r = 0.89$ for coke-oven workers (one outlier excluded).

Multiple linear regression analyses of ln(adjNap) on ln(adjPhe) showed no significant effect of the source of PAHs (coke ovens, asphalt, diesel exhaust) or the job category ($p >$

0.05). Since the sources of PAH were dramatically different, this finding was unexpected. However, it is clear from Figure 3.1. that the overall relationship between ln(adjNap) and ln(adjPhe) was essentially the same for each of the three groups of workers. Figure 3.1. also shows that the intra-group variability of the data pairs [ln(adjNap), ln(adjPhe)] was very large for each of the three sources of PAHs. The final regression model (after removal of one outlier) is given as: ln(adjNap) = -0.121 + 0.551×ln(adjPhe) (n = 92), with an adjusted R^2 value of 0.751. The log-scale regression coefficient of 0.551, with 95% confidence limits of [0.485, 0.617], was significantly less than one. This suggests that, in natural scale, the ratio of Nap/Phe diminished with increasing levels of Phe.

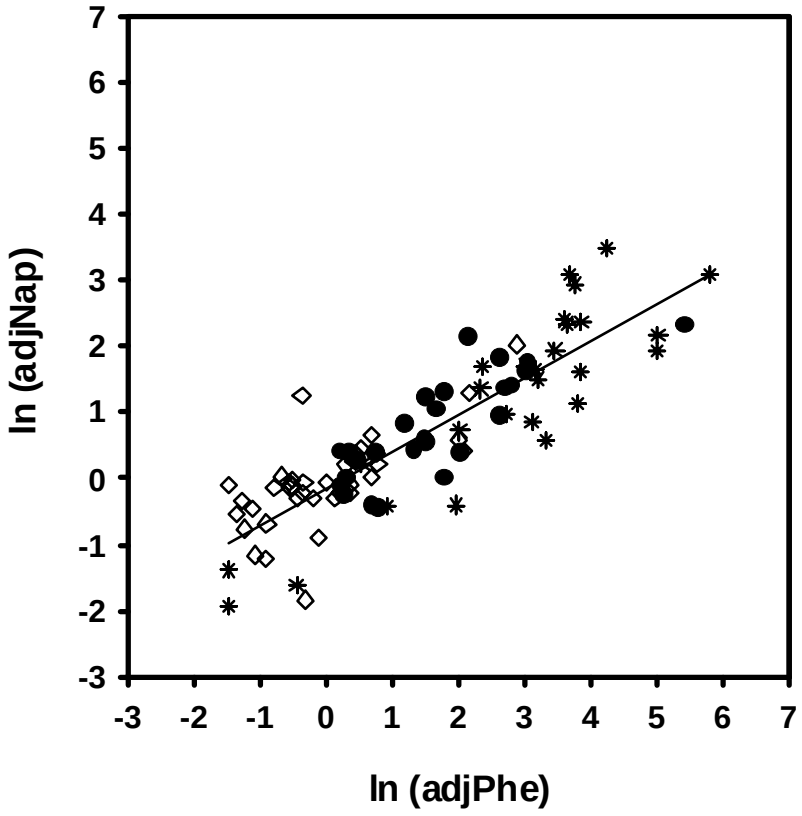

Figure 3.1. Logged background-adjusted naphthalene level in urine [ln(adjNap)] regressed on the corresponding logged background-adjusted phenanthrene level [ln(adjPhe)]. Diamonds represent diesel-exposed workers; circles represent asphalt workers; asterisks represent coke-oven workers. Overall regression equation: ln(adjNap) = -0.121 + 0.551[ln(adjPhe)]; adjusted R^2 = 0.751.

3.4.4. Relationships between air levels of naphthalene, phenanthrene, and total PAHs

In selecting Nap and Phe as possible surrogates for PAH exposure, it is important that urinary levels accurately reflect air concentrations of total PAHs at the time of urine collection. Rappaport et al. (Rappaport et al. 2004) reported high correlations between logged levels of airborne Nap and logged levels of total airborne PAH in published data from several industries, namely, creosote impregnation (Pearson $r = 0.815$), coke production ($r = 0.917$), an iron foundry ($r = 0.854$), and aluminum production ($r = 0.933$). Furthermore, the estimated slopes of the log-scale relationships between ln(Nap) and ln(total PAHs) ranged from 0.824 to 1.19, indicating that air concentrations of Nap were roughly proportional to those of total measured PAHs in each case.

Using the same data previously analyzed by Rappaport et al. (Rappaport et al. 2004) we investigated the straight-line relationships between logged levels of airborne Phe and logged levels of total PAHs. Datasets reporting air Phe levels were only available in studies of the aluminum-producing and coke-producing industries (Bjorseth et al. 1978a; Bjorseth et al. 1978b; Petry et al. 1996; Strunk et al. 2002). Across four datasets, 60 out of 66 observations of total PAH levels included measurements of Phe. We note that, for studies from Bjorseth et al. (Bjorseth et al. 1978a; Bjorseth et al. 1978b) some observations specified both gaseous and particulate air levels, while others specified only particulate levels. We distinguished between these types of observations in Figure 3.2., where we examined the log-log relationships of Phe and total PAHs. As shown in Figure 3.2., the estimated slopes of the log-log relationships were 1.09 and 1.15 for aluminum-producing and coke-producing industries, respectively, indicating that levels of airborne Phe were roughly proportional to those of total PAHs in these industries.

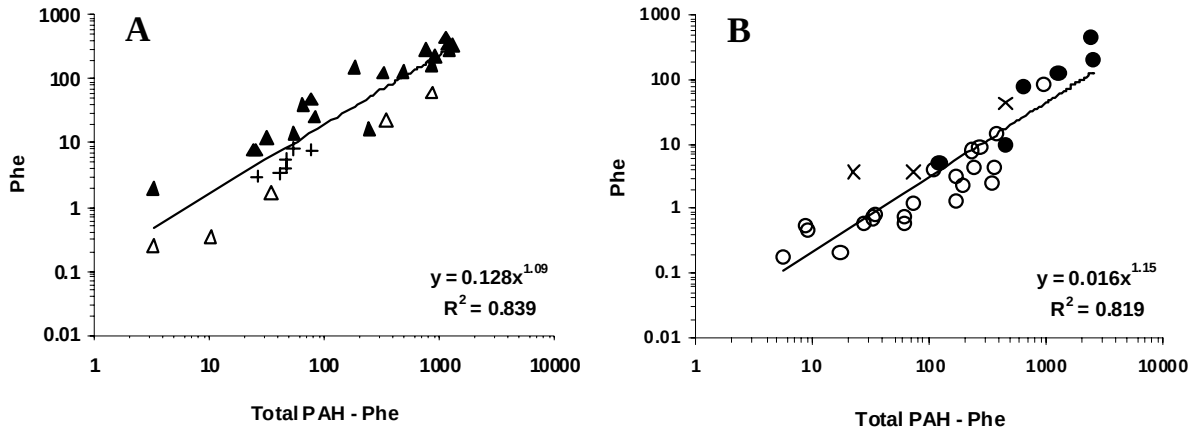

Figure 3.2. Air phenanthrene concentration (μg/m^3) vs. total PAH concentration (μg/m^3) (minus phenanthrene concentration) in aluminum-production facilities (A) and coke-production facilities (B). Closed triangles: data from (Bjorseth et al. 1978a) (gas+particulate measurements); open triangles: data from (Bjorseth et al. 1978a) (particulate measurements only); plus symbols: data from (Petry et al. 1996); closed circles: data from (Bjorseth et al. 1978b) (gas+particulate measurements); open circles: data from (Bjorseth et al. 1978b) (particulate measurements only); ×: data from (Strunk et al. 2002).

We used these same data (Bjorseth et al. 1978a; Bjorseth et al. 1978b; Petry et al. 1996; Strunk et al. 2002) to investigate the relationship between air concentrations of Nap and Phe in the aluminum-producing and coke-producing industries. The final analysis included six observations from (Bjorseth et al. 1978b), 15 observations from (Bjorseth et al. 1978a), three observations from (Strunk et al. 2002), and six observations from (Petry et al. 1996). As shown in Figure 3.3., the relationship between air Nap and air Phe is given by the regression model: ln[Nap (μg/m^3)] = 0.723 + 0.769×ln[Phe (μg/m^3)] + 1.50[source]; where coke-production source = 1 and aluminum-production source = 0. A significant effect of the source of exposure indicates that the ratio of airborne Nap/Phe was greater in coke-producing industries when compared to aluminum-producing industries. Since the overall regression coefficient for ln(Nap) on ln(Phe) was significantly less than one, with an estimated value of

0.769 and 95% confidence interval [0.607, 0.931], we infer that ratio of airborne Nap/Phe (in natural scale) decreased with increasing air levels of Phe, consistent with results based upon the urinary data (see Figure 3.1.). This finding lends indirect support to the conjecture that urinary levels of Nap and Phe reflect the corresponding air levels of these compounds in occupational settings at the time of urine collection. Our results support those from a recent study by Campo et al. (Campo et al. 2007), which showed that measurements of urinary Nap and Phe in highway construction workers and asphalt workers were highly correlated with personal airborne exposures to these compounds.

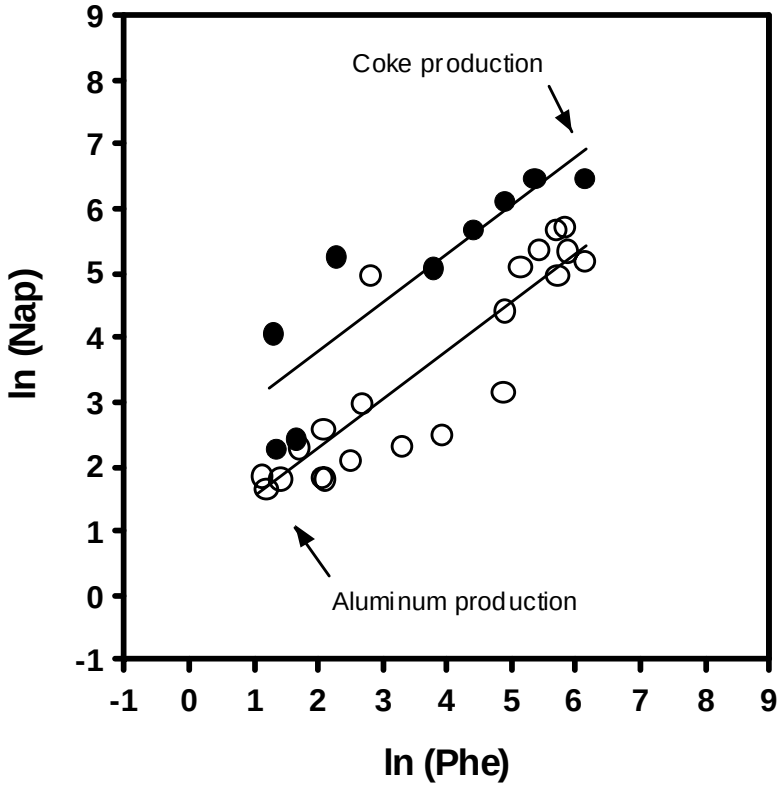

Figure 3.3. Log-transformed air concentration (μg/m^3) of naphthalene [ln(Nap)] regressed on the corresponding logged air concentration (μg/m^3) of phenanthrene [ln(Phe)] in the coke-producing and aluminum-producing industries. Closed circles: data from (Bjorseth et al. 1978b) and (Strunk et al. 2002); open circles: data from (Bjorseth et al. 1978a) and (Petry et al. 1996). Overall regression equation: ln(Nap) = 0.723 + 0.769[ln(Phe)] + 1.50[source]; where coke-production source = 1 and aluminum-production source = 0; adjusted R^2 = 0.796.

3.5. Conclusions

We conclude that levels of Nap and Phe in urine from diesel-exposed workers, asphalt workers, and coke-oven workers followed the expected low, medium, and high exposure designations, and were highly correlated with each other, suggesting common sources of the two PAHs. Regression analyses of ln(adjNap) values on the corresponding ln(adjPhe) values showed no significant effect of the source of PAH in these three cases. Furthermore, these analyses indicated that the ratio of urinary Nap/Phe decreased with increasing levels of urinary Phe. Since the ratio of airborne Nap/Phe was also found to decrease with increasing airborne Phe, in independent sets of data from the coke-producing and aluminum-producing industries, we conclude that urinary levels of Nap and Phe very likely reflect the corresponding levels of airborne Nap and Phe at the time of urine collection. Also, we observed that air concentrations of Nap and Phe were both proportional to total PAHs in the coke-producing and aluminum-producing industries. Taken together, these findings, plus the ease and sensitivity of measuring Nap and Phe in urine, lead us to conclude that urinary levels of Nap and Phe are promising surrogates for occupational exposures to total PAHs. Of the two urinary analytes, Phe may be a more useful surrogate than Nap, given the much larger fold increases above background values observed for Phe (one to 17-fold) compared to Nap (one to 3-fold) across the three sources of PAHs in our study (see Table 3.2.).

4. Paper III. Analysis of Urinary Biomarkers of Asphalt Exposure Using Mixed Models [Sobus, J.R., McClean, M.D., Herrick, R.F., Waidyanatha, S., Onyemauwa, F., Kupper, L.L., Rappaport, S.M., to be submitted to Annals of Occupational Hygiene]

4.1. Abstract

Hot-mix asphalt used in the road-paving industry is known to contain polycyclic aromatic hydrocarbons (PAHs). Individual PAH biomarkers have been used to represent internal dose through inhalation and dermal routes of exposure. We investigated urinary biomarkers of naphthalene [urinary naphthalene (U-Nap) and total hydroxynaphthalene (OH-Nap)], phenanthrene [urinary phenanthrene (U-Phe) and total hydroxyphenanthrene (OH-Phe)], and pyrene [1-hydroxypyrene (OH-Pyr)] in a group of 26 highway construction workers; 20 workers were involved with the application of hot-mix asphalt (pavers), and 6 were involved in road removal processes (millers). Our analysis included baseline urine samples, as well as postshift, bedtime, and morning samples collected over three consecutive days. Overall, significant pairwise correlations between all biomarkers suggest common exposure sources. In baseline samples, no significant differences in biomarker levels were observed between pavers and millers, suggesting similar background exposures. For millers, postshift, bedtime, and morning biomarker levels were not significantly elevated over baseline levels. For pavers, all biomarkers were significantly elevated in postshift samples, indicating rapid uptake and elimination following occupational asphalt exposure. Biological half-times were estimated to be 8 h for U-Phe, 10 h for U-Nap, 13 h for OH-Phe and OH-Pyr, and 26 h for OH-Nap. Results from linear mixed-effects models showed significant effects

of work tasks on levels of U-Nap, U-Phe, OH-Phe, and OH-Pyr. While no task-related effects were observed for OH-Nap, cigarette-smoking status significantly affected OH-Nap levels. 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 asphalt.

4.2. Introduction

Asphalt, a byproduct of petroleum refining, has been widely used in the road-paving industry. When hot asphalt is applied to roads, workers are exposed to gaseous and particulate emissions via inhalation and dermal contact. These emissions include polycyclic aromatic hydrocarbons (PAHs), some of which are known or suspected carcinogens (IARC 1987). Epidemiological evidence suggests an increased risk of cancer among asphalt-exposed workers (Partanen and Boffetta 1994; Hooiveld et al. 2002; Burstyn et al. 2003; Randem et al. 2004; Boffetta et al. 1997). However, the relationship between occupational exposures and cancer risk is not well defined in this population (Chiazze et al. 1991; NIOSH 2000). The inability to characterize associations between asphalt-derived PAH exposures and cancer stems, in part, from a lack of quantitative exposure data and the presence of confounding variables that influence exposures from airborne and dermal routes (Chiazze et al. 1991; NIOSH 2000).

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, which represent internal doses of PAHs through multiple exposure routes. Such biomarkers may be good indicators of total PAH exposure for use in epidemiologic studies. Indeed, a urinary metabolite of pyrene (Pyr) [namely 1-hydroxypyrene (OH-Pyr)] has been shown to

reflect asphalt exposure (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 exposure. More recently, naphthalene (Nap) and phenanthrene (Phe) have been identified as potential PAH-exposure surrogates. Urinary levels of unmetabolized Nap (U-Nap) and Phe (U-Phe) (Campo et al. 2006a; Campo et al. 2007), and of hydroxylated Nap and Phe metabolites (Vaananen et al. 2003; Vaananen et al. 2006; Buratti et al. 2007), have been shown to reflect occupational exposures to asphalt emissions. (Hereafter, the sum of 1- and 2-hydroxynaphthalene will be referred to as 'OH-Nap' and the sum of 2-, 3-, 4-, and 9-hydroxyphenanthrene will be referred to as 'OH-Phe'). However, the rates of uptake and elimination of these biomarkers are uncertain and the influence of covariates on urinary analyte levels has not been thoroughly examined.

In a previous analysis of 26 highway construction workers, the relationship between asphalt exposure and urinary OH-Pyr was evaluated using distributed lag models (McClean et al. 2004b). Results showed that OH-Pyr concentrations in paving workers were significantly affected by inhalation and dermal Pyr exposure. Here, we extend the analysis to include selected biomarkers of Nap and Phe. Our objectives are to: 1) characterize urinary levels of Nap, Phe, and Pyr biomarkers in asphalt-exposed workers; 2) assess correlations between measured analytes; 3) evaluate the influence of sample collection time on urinary analyte levels; 4) estimate the rate of elimination of each analyte from the body; and 5) evaluate the influence of covariates [creatinine, time, workday, work task, smoking, age, and body mass index (BMI)] on urinary analyte levels.

4.3. Methods

4.3.1. Study population and design

Specific details of this study population and design have been published (McClean et al. 2004a; McClean et al. 2004b). Here we considered 26 male highway construction workers residing in the Greater Boston area of the U.S. Workers were recruited with informed consent according to a protocol reviewed for the protection of human subjects at Harvard University. Twenty workers were involved with the application of hot-mix asphalt on road surfaces (hereafter 'pavers') and 6 workers were involved in road removal processes (hereafter 'millers'). Since the millers did not work with hot-mix asphalt, they were included in this analysis as a reference group. The pavers consisted of workers from four task categories, namely paver operators, screedmen, rakers, and roller operators. 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 a three-day period of evaluation, starting at the beginning of the workweek, urine samples were collected from all workers, before work (morning), after work (postshift), and at bedtime of each day. Morning samples collected on the first day of the workweek (after a work-free weekend) were treated as baseline samples. Overall, seven urine samples were collected from each milling worker (two sampling days), and ten urine samples were collected from each paving worker (three sampling days). All urine samples were collected in sterilized polypropylene containers and stored at -20˚C for up to seven years prior to analysis. Covariate information (e.g., age, height, weight, smoking status) was obtained by questionnaire. Creatinine levels

were determined using a colorimetric procedure (Procedure No. 555, Sigma Diagnostics, Dorset, UK) (Sigma Chemical Co. 1984).

4.3.2. Analysis of urinary naphthalene and phenanthrene

A detailed description of this procedure is described elsewhere (see Chapter 3, section *3.3.3.*). Briefly, Nap and Phe were extracted from urine using solid-phase microextraction and analyzed using gas chromatography-mass spectrometry. Standard curves were prepared by spiking Nap, Phe, and an internal standard mixture [($^2H_8$)Naphthalene and ($^2H_{10}$)Phenanthrene] into pooled urine collected from anonymous nonsmoking unexposed volunteers. Quantitation of U-Nap and U-Phe in the samples was based on response ratios with respect to the corresponding internal standards.

4.3.3. Analysis of urinary PAH metabolites

A detailed description of this procedure is in preparation. Briefly, hydroxy-PAHs (OH-PAHs) were enzymatically digested using β-glucuronidase/arylsulfatase (Roche Diagnostics, Indianapolis, IN), extracted from urine using solid-phase extraction (EnvirElute PAH cartridges, Varian, Palo Alto, CA), and analyzed via liquid chromatography-tandem mass spectrometry using a Surveyor LC system (Thermo Fisher Scientific, San Jose, CA) and a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA). A Thermoelectron Gold 3μm C18 column (Thermo Fisher Scientific, San Jose, CA, 150 X 2.1 mm) was used for analyte separation with a flow rate of 250 μl/min. Methanol and water were used as the mobile phase with a gradient of 55% to 81.8% methanol over 25 min. After holding the column for 10 min at 97% methanol, it was

equilibrated to 55% methanol/45% water for 10 min. The following product ion masses were observed: 115.09 *m/z* for 1- and 2- hydroxynaphthalene (1- and 2-OHNap, respectively), 165.08 *m/z* for 2-, 3-, 4-, and 9-hydroxyphenanthrene (2-, 3-, 4-, and 9-OHPhe, respectively), and 189.08 *m/z* for OH-Pyr. Calibration curves were prepared by adding standards and a mixture of isotopically labeled internal standards into pooled urine collected from anonymous nonsmoking unexposed volunteers. Quantitation of 1- and 2-OHNap, 2-, 3-, 4-, and 9-OHPhe, and OH-Pyr were based on response ratios with respect to the corresponding internal standards [($^{2}H_{8}$)1-hydroxynaphthalene, ($^{13}C_{6}$)3-hydroxyphenanthrene, and ($^{2}H_{8}$)1-hydroxypyrene].

4.3.4. Statistical methods

For all statistical analyses, levels of 1- and 2-OHNap, and levels of 2-, 3-, 4-, and 9-OHPhe 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) after natural log-transformation to satisfy normality assumptions and to remove heteroscedasticity. 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. (Hamlett et al. 2003). To test the effects of work group and sample type 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 linear mixed-effects models (Proc MIXED). A p-value < 0.05 was considered significant (two-tailed test). Mixed models were used to assess covariate influence [creatinine (g/l), time (h), work task, workday, age (y), BMI (kg/m^2), and

smoking status (current smoker vs. nonsmoker)] on analyte levels. Rather than dividing analyte concentration by creatinine concentration, creatinine was included as an independent variable in the multivariable models to allow analyte levels and other covariates to be 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, bedtime range = 2.50 - 14.3 h; morning median = 15.3 h, morning range = 11.8 - 19.1 h). Elimination rate constants (k) and biological half-times ($T_{1/2} = -0.693/k$) 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 yielded by other tested matrices (homogeneous autoregressive, heterogeneous autoregressive).

4.4. Results

4.4.1. Descriptive statistics and correlation analysis for urinary analytes

Summary statistics [geometric means (GMs), geometric standard deviations (GSDs), and numbers of observations] for urinary analytes, categorized by work group and sample type, are displayed in Table 4.1.

Table 4.1. Descriptive statistics for urinary analytes (ng/L) measured in pavers and millers.

		Baseline			Postshift			Bedtime			Morning		
Analyte	Work Group	*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
*** significantly different from baseline level ($p < 0.0001$)
** significantly different from baseline level ($p < 0.05$)
* marginally different from baseline level ($p < 0.10$)

Overall, OH-Nap was the most abundant urinary analyte (median = 12,100 ng/l) followed by OH-Phe (median = 3,500 ng/l), OH-Pyr (median = 1,130 ng/l), U-Nap (median = 42.3 ng/l) and finally U-Phe (median = 29.7 ng/l). The geometric means of urinary analyte levels were greater for pavers than for millers in postshift, bedtime, and morning samples. Figure 4.1. shows a correlation matrix including pairwise comparisons of all analytes. Significant correlations between all pairs were observed, suggesting common exposure sources for all analytes. The strongest associations were observed between OH-Phe and OH-Pyr ($r = 0.90$), between U-Phe and OH-Phe ($r = 0.84$), and between U-Nap and U-Phe ($r = 0.80$). Although still statistically significant, weaker associations were observed when comparing OH-Nap to other analytes ($r \leq 0.53$). We note that levels of OH-Pyr measured here were highly correlated with the original OH-Pyr measurements from McClean et al. ($r = 0.89$, $p < 0.0001$) (McClean et al. 2004b), indicating good agreement between studies.

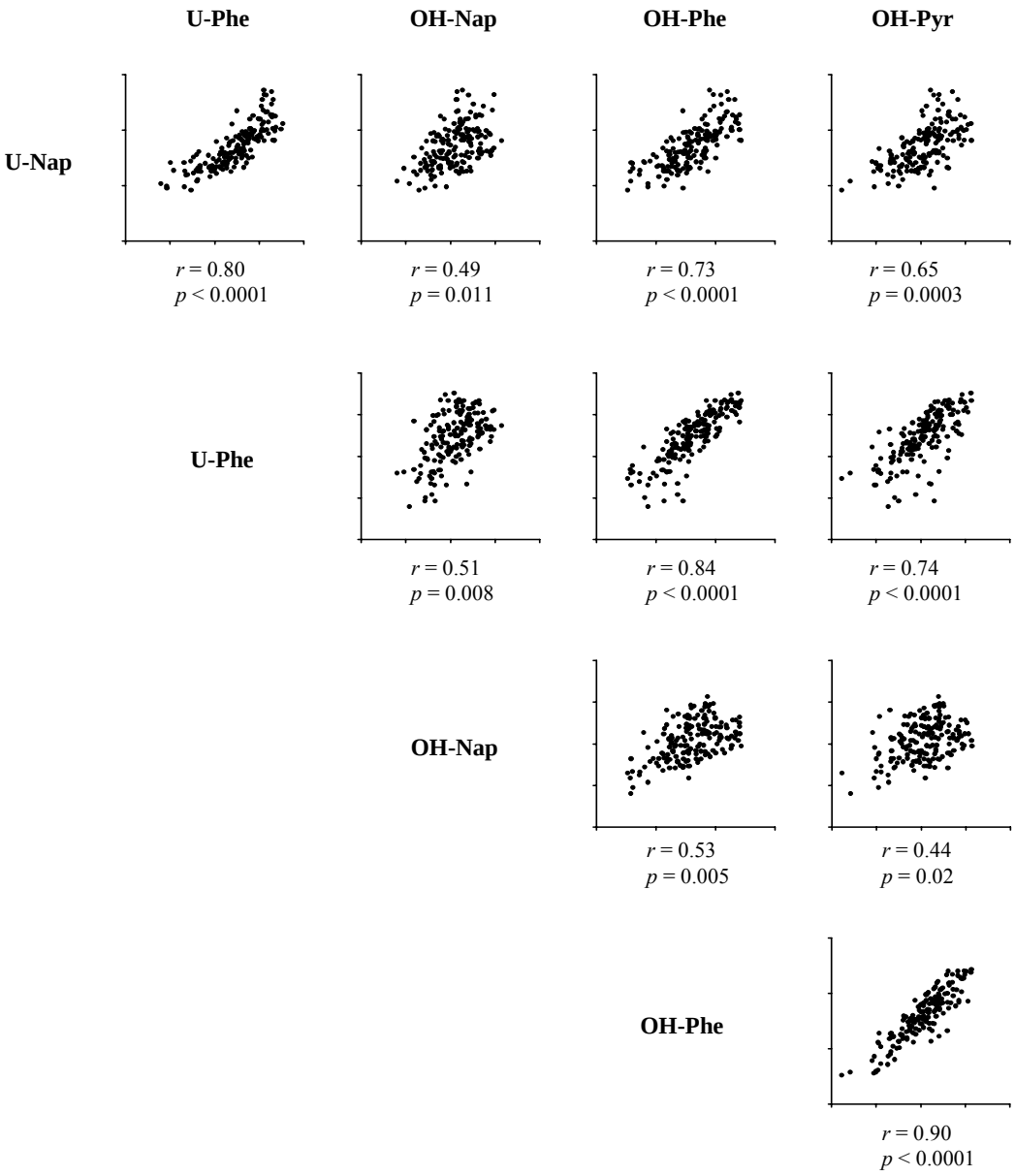

Figure 4.1. Pairwise correlations between urinary analytes.

4.4.2. Effects of work group and sample type on urinary analyte levels

In baseline urine samples, results from general linear models showed no significant effect of work group on urinary analyte levels (p-value ≥ 0.37). When we considered all other sample types (i.e., postshift, bedtime, and morning measurements), a significant work group effect was observed after adjusting for sample type (p-value < 0.05 for U-Nap, U-Phe, OH-Phe and OH-Pyr; p-value $= 0.07$ for OH-Nap). Table 4.1. displays group-specific geometric mean levels of urinary analytes for each sample type. For millers, neither postshift, bedtime, nor morning analyte levels were significantly elevated over baseline levels (p-value ≥ 0.09). For pavers, levels of OH-Phe and OH-Pyr were significantly elevated above baseline levels in postshift samples (p-value < 0.0001), bedtime samples (p-value < 0.0001), and morning samples (p-value ≤ 0.02) while 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 $= 0.07$).

4.4.3. Linear mixed-effects models for urinary analytes

Linear mixed-effects models were used to estimate elimination rate constants for each analyte, to analyze task-specific differences in analyte levels, and to assess the influence on analyte levels of urine dilution (as indicated by levels of urinary creatinine), workday, smoking status, age, and BMI. Observations from millers were not included in this analysis because preliminary results showed no effect of occupational exposure on analyte levels (see Table 4.1.). Additionally, as morning measurements of U-Nap, U-Phe, and OH-Nap from

pavers were not significantly elevated above baseline levels (see Table 4.1.), only postshift and bedtime measurements were included in the final models. Table 4.2. shows estimated parameters from linear mixed-effects models for each urinary analyte. In each model, a significant positive effect of urinary creatinine concentration was observed (p-value < 0.0001), indicating increased analyte levels with decreased urine flow. After adjusting for creatinine concentration, a significant negative effect of time was observed for each analyte (p-value $\leq$ 0.01), indicating a decrease in analye levels from the time of postshift urine collection to bedtime. Using estimated elimination rate constants, $T_{1/2}$ 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, a significant work task effect was observed for U-Nap, U-Phe, OH-Phe, and OH-Pyr. These results (shown in Table 4.2.) indicate that analyte levels were lower in roller operators compared to paver operators, screedmen, and rakers. When roller operators were removed from the multivariable models, no significant work task effect was observed (p-value $\geq$ 0.5).

Table 4.2. Results from the linear mixed-effects models evaluating urinary analytes in paving workers.

	U-Nap			U-Phe			OH-Nap			OH-Phe			OH-Pyr		
Parameters	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)	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
task			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)	
screedmen	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 (σ^2_B)	0.194			0.210			0.190			0.127			0.406		
within-subject (σ^2_W)	0.279			0.648			0.148			0.223			0.440		

Figure 4.2.A-E shows baseline analyte levels along with postshift and bedtime analyte levels over three consecutive days. As no work task effect was observed across paver operators, screedmen, and rakers, the mean values of these combined workers, along with the mean values of roller operators, are displayed at each time point. The task-based differences in analyte levels between roller operators and all other paving workers are apparent for all analytes except OH-Nap (Figure 4.2.C). Increases in analyte levels (p-value < 0.1) over each workday were also observed for U-Nap (Figure 4.2.A), OH-Phe (Figure 4.2.D), and OH-Pyr (Figure 4.2.E). While no work task or workday effect was observed for OH-Nap, a highly significant smoking effect was observed, with smokers having higher analyte levels than nonsmokers (Table 4.2.). No other analytes were significantly affected by smoking status. Neither body mass index (BMI) nor age were significantly associated with postshift and bedtime analyte levels.

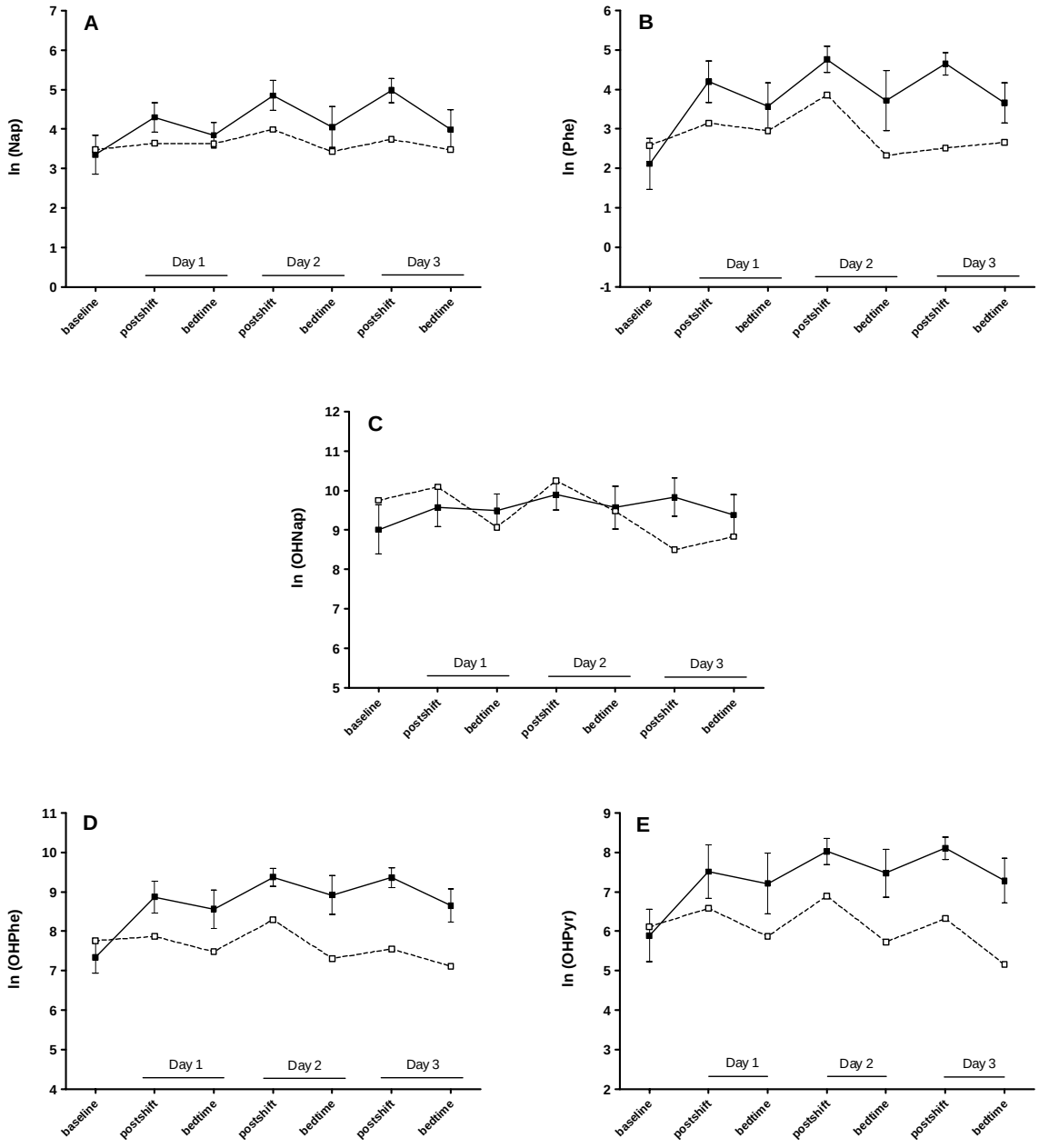

Figure 4.2. Estimated mean concentration (ng/l) and 95% confidence intervals of urinary analytes over time. Closed squares represent paver operators, screedmen, and rakers; open squares represent roller operators. Urinary analytes: (A) Naphthalene; (B) Phenanthrene; (C) OH-Naphthalene; (D) OH-Phenanthrene; (E) OH-Pyrene.

4.5. Discussion

Assessing occupational exposures to asphalt emissions has largely focused upon the content of carcinogenic PAHs, such as benzo(*a*)pyrene (IARC 1987). Although some relationships between airborne exposures to PAHs and urinary PAH biomarkers have been examined (Vaananen et al. 2003; McClean et al. 2004b; Campo et al. 2006a; Campo et al. 2006b; Vaananen et al. 2006; Buratti et al. 2007; Campo et al. 2007), questions remain regarding the rates of uptake and elimination for individual PAHs and influence of covariates, such as work groups and tasks on analyte levels. To address these questions, we analyzed five PAH biomarkers in the urine of 26 road construction workers using a repeated-measures sampling design (McClean et al. 2004a; McClean et al. 2004b). Linear mixed-effects models were used to estimate elimination rates for individual analytes, to evaluate work-group-related and task-related differences in analyte levels, and to determine the influence on analyte levels of urine dilution (measured by creatinine level), smoking status, BMI, and age.

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; Campo et al. 2006b; Buratti et al. 2007; Campo 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 approximately 3.5% of OH-Nap, and the concentration of U-Phe was 8.5% of OH-Phe (not including 1-hydroxyphenanthrene, which was not measured). These results agree with those observed in a previous study of coke-oven workers (Waidyanatha et al. 2003).

Overall, significant pairwise correlations between analyte levels suggest common exposure sources for Nap, Phe, and Pyr in asphalt workers. 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 similar background exposures, from the ambient environment and the diet, dominated PAH biomarkers in both of these groups of workers. Considering postshift, bedtime, and morning measurements in millers, no significant increases above baseline levels were observed for any analyte. This result was expected because millers did not work with hot asphalt and, generally, had low levels of exposure to PAHs (McClean et al. 2004a; McClean et al. 2004b). For pavers, the largest departures from baseline levels were observed in postshift samples (see Table 4.1.). A comparison of geometric mean levels showed that U-Phe was the most sensitive biomarker, with postshift levels approximately 7-fold greater than baseline levels. Postshift levels of OH-Phe and OH-Pyr were approximately 5-fold greater than baseline levels, and postshift levels of U-Nap and OH-Nap were approximately 3- and 2-fold greater than baseline levels, respectively.

Results from our final linear mixed-effects models showed that urinary creatinine was significantly associated with each urinary analyte. The positive regression coefficients suggest that as creatinine concentration increased analyte concentration also increased, indicating elevated analyte concentration with reduced urine output. This result was expected for OH-Nap, OH-Phe, and OH-Pyr, because these compounds are removed from the body via renal filtration. However, the significant positive effect of creatinine on U-Nap and U-Phe was unexpected. Unmetabolized volatile organic compounds are thought to be eliminated by passive diffusion (Ghittori et al. 1993; Waidyanatha et al. 2001; Serdar et al.

2003a). As the elimination rates of these compounds are directly proportional to urine flow rate, urinary analyte concentration should be less dependent on urine output (Boeniger et al. 1993; Barr et al. 2005). We note that final model results for each analyte were nearly identical whether or not creatinine was included as a covariate; the inclusion of creatinine reduced random sources of variation and had little effect on coefficient estimates for other covariates.

After adjusting for urinary creatinine, we were able to estimate elimination rate constants for each analyte, by including a fixed effect for time in our models. We first examined work-task-specific elimination rates by including 'time × task' 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 task. The estimated $T_{1/2}$ values were approximately 8 h for U-Phe, 10 h for U-Nap, 13 h for OH-Phe and OH-Pyr, and 26 hours for OH-Nap. Literature reported estimates of the first-order elimination half-time for OH-Pyr range from 4 - 48 h (Jacob and Seidel 2002; Brandt and Watson 2003; Hansen et al. 2008), thus, the estimated half-time of OH-Pyr in this study population is within the range of previous estimates. Additionally, we observed that among workers exposed to hot asphalt, the half-time of OH-Phe is very similar to that of OH-Pyr, and the respective half-times of U-Nap and U-Phe are shorter than that of OH-Pyr. We note that, while the model estimate for OH-Nap indicates a half-time of approximately 26 h, this value may be overestimated due to the highly significant smoking effect.

Considering the estimated half-times, we incorporated a fixed effect for 'workday' into our models to investigate bioaccumulation effects from previous workdays. No workday effects were observed for U-Phe, which had the shortest estimated biological half-time, 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 the estimated half-times and significant workday effects indicated a contribution to analyte levels from previous workdays, these contributions were relatively modest in relation to that of exposure on the current day (see Figure 4.2.).

After adjusting for time and workday effects, a significant work task effect was observed for U-Nap, U-Phe, OH-Phe, and OH-Pyr. Results from the linear mixed-effects models suggest that analyte levels in roller operators were significantly lower than in pavers performing other work tasks. In fact, when roller operators were removed from the models, no significant effects were observed across paver operators, screedmen, and rakers. Prior assessments of this study population showed that the rank order of PAH exposures across work tasks varied according to the exposure type (air measurement or dermal patch), and, in the case of dermal patches, the particular exposure surrogate (total dermal PAHs vs. dermal Pyr) (McClean et al. 2004a; McClean et al. 2004b). However, roller operators were shown to have experienced both the lowest air levels (measured by both total airborne PAHs and airborne Pyr) and lowest dermal-patch levels (measured by both total dermal PAHs 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-Nap, and OH-Pyr may all be good surrogates for occupational exposure to PAHs in jobs employing asphalt.

Results from the final model for OH-Nap indicate that smoking status had a highly significant effect on analyte levels. This smoking effect probably explains the lower

correlation coefficients for OH-Nap compared to the other analytes (see Figure 4.1.). Furthermore, after adjusting for the smoking effect, no significant effect of work task was observed for OH-Nap in contrast to the other urinary biomarkers in our study (i.e., U-Nap, U-Phe, OH-Phe, and OH-Pyr). This effect of smoking reduces the utility of OH-Nap as a measure of total-PAH exposure among asphalt workers compared to the other urinary biomarkers measured in our study.

5. Paper IV. Biomarkers of Naphthalene, Phenanthrene, and Pyrene are Predictors of Particulate Exposures to Polycyclic Aromatic Compounds in Asphalt-Exposed Workers [Sobus, J.R., McClean, M.D., Herrick, R.F., Waidyanatha, S., Nylander-French, L., Kupper, L.L., Rappaport, S.M., to be submitted to Annals of Occupational Hygiene]

5.1. Abstract

During road paving, workers are exposed to gaseous and particulate constituents of asphalt, the latter containing numerous carcinogenic polycyclic aromatic hydrocarbons (PAHs). Urinary PAH analytes have been independently validated as biomarkers of asphalt exposure in many studies. Specifically, levels of urinary naphthalene (U-Nap) and hydroxynaphthalene (OH-Nap), urinary phenanthrene (U-Phe) and hydroxyphenanthrene (OH-Phe), and hydroxypyrene (OH-Pyr) have been associated with asphalt exposures. Here, we compare these urinary analytes to determine the most suitable biomarker(s) of exposure to particulate asphalt emissions. Our study included 20 paving workers whose exposures were measured repeatedly over three consecutive workdays. Daily breathing-zone air and dermal patch measurements of 4-6 ring polycyclic aromatic compounds (PACs) were evaluated along with postshift, bedtime, and morning levels of U-Nap, U-Phe, OH-Nap, OH-Phe, and OH-Pyr. These data were used to model exposure-biomarker relationships while controlling for other covariates. Overall, OH-Phe was identified as the most promising biomarker of asphalt exposure because air and dermal patch measurements of PACs were significant determinants of OH-Phe in postshift, bedtime, and morning urine samples (the only measured biomarker to show these effects at bedtime and the following morning). For U-Nap, U-Phe, and OH-Pyr, both air and dermal patch

measurements of PACs were also significant determinants of the respective levels in postshift urine, and dermal patch measurements were significant predictors of the levels in bedtime urine (all-three analytes) and morning urine (U-Nap and OH-Pyr only). While a significant effect of creatinine concentration was observed for each of these analytes, modest effects of smoking status and BMI were also observed for U-Phe and OH-Pyr, respectively. Levels of OH-Nap were not associated with PAC measurements in air or dermal patch samples but were significantly affected by smoking status, age, day of sample collection, and urinary creatinine concentration. We conclude that OH-Phe, U-Nap, U-Phe, and OH-Pyr can be used as biomarkers of exposure to particulate asphalt emissions, with OH-Phe being the most promising biomarker. Indications that levels of U-Nap, U-Phe, and OH-Pyr were significantly associated with dermal patch measurements well into the evening after a given work shift, combined with the small ratios of within-person variance to between-person variance at bedtime, suggest that bedtime measurements may be useful for investigating dermal PAH exposures.

5.2. Introduction

Asphalt (also known as bitumen) is a highly viscous petroleum product that is commonly used as a binding agent in the road-paving industry. While the chemical composition of asphalt varies according to the source of petroleum and other ingredients used in the manufacturing process, constituents of asphalt include a complex assortment of hydrocarbons (NIOSH 2000). Of the chemicals identified in asphalt emissions, attention has focused primarily upon the polycyclic aromatic hydrocarbons (PAHs), a group of fused-ring aromatic compounds, which includes several carcinogenic forms (IARC 1987). During the

application of hot-mix asphalt, workers are exposed to PAHs in both the vapor phase (primarily 2-ring and 3-ring compounds) and the particulate phase (primarily 4-ring to 6-ring compounds). Asphalt contains varying amounts of PAHs, depending upon the source of raw materials and whether coal tar was used in its production (Burstyn and Kromhout 2000; Burstyn et al. 2000; Burstyn et al. 2002). Because the particulate emissions from asphalt contain the most carcinogenic PAHs, health investigators are particularly concerned about asphalt workers' exposures to particulate matter via inhalation and dermal contact.

Although epidemiological studies point to increased cancer risks among asphalt workers (Partanen and Boffetta 1994; Hooiveld et al. 2002; Burstyn et al. 2003; Randem et al. 2004; Boffetta et al. 1997) that are likely related to PAH exposures, the quantitative relationship between exposures to asphalt chemicals and cancer risk is uncertain (Chiazze et al. 1991; NIOSH 2000). This uncertainty stems largely from difficulties in assessing exposures to asphalt emissions, particularly the PAH content, via mixed routes (i.e., air and dermal contact, as well as ingestion) and physical forms (i.e., vapors and particulate matter).

Several previous investigations have quantified asphalt exposures in both air samples and dermal patches to gauge the amounts of PAHs potentially inhaled and deposited on the skin (Jongeneelen et al. 1988; McClean et al. 2004a; Vaananen et al. 2005). Analytes have included specific PAH compounds as well as several nonspecific measures of PAH exposure, including particulate matter (PM), benzene-soluble PM, and polycyclic aromatic compounds (PACs, a group of aromatic hydrocarbons containing primarily PAHs and heterocyclic compounds with 4 or more rings). Urinary biomarkers have also been used to assess exposures and internal doses of PAHs in asphalt-exposed workers. Urinary levels of unmetabolized PAHs in urine and of hydroxylated PAH metabolites, notably those of

naphthalene (Nap), phenanthrene (Phe), and pyrene (Pyr), have been associated with airborne and dermal exposures to PAHs in asphalt-exposed populations (Vaananen et al. 2003; McClean et al. 2004b; Campo et al. 2006a; Campo et al. 2006b; Vaananen et al. 2006; Buratti et al. 2007; Campo et al. 2007). However, only a few studies have been published which reported concentrations of multiple urinary PAH biomarkers and the corresponding levels of PAHs or PACs in air and on the skin (Vaananen et al. 2005; Vaananen et al. 2006).

In a study involving 20 road-paving workers who worked with hot-mix asphalt, PACs were measured in air samples and on dermal patches (McClean et al. 2004a). Levels of the PACs varied significantly according to the specific tasks performed by the pavers, and also differed between air measurements and dermal patch measurements, suggesting that exposures of PACs were differentially expressed regarding the air and skin (McClean et al. 2004a). Recently, urinary levels of Nap and Phe and hydroxylated metabolites of Nap, Phe, and Pyr were measured in archived urine from the same 20 road pavers (see Chapter 4). Results showed that urinary biomarker measurements significantly varied by task, as had been observed for PAC measurements in the same workers (see Chapter 4). The purpose of the current investigation was to statistically model the relationships between levels of urinary PAH analytes and the corresponding PAC measurements in air and dermal patch samples from these same 20 asphalt-exposed workers. This analysis further delineates the differences between air samples and dermal patch samples as measures of exposure to PAHs and points to particular urinary PAH analytes that may be useful biomarkers of exposure to PAHs among asphalt-exposed workers. These results should be useful in the design of future studies to assess exposures to PAHs in asphalt emissions for both hazard control and epidemiological investigations.

5.3. Methods

5.3.1. Subjects and air and urine measurements

Twenty male road-paving workers, all residing in the Greater Boston area of the U.S., were included in the study. Workers were recruited with informed consent according to a protocol reviewed for the protection of human subjects at Harvard University. Information about smoking habits and body size were obtained by questionnaire.

Starting at the beginning of the workweek, daily air measurements and dermal patch measurements were collected from each worker over three consecutive days. Personal air measurements of PACs were collected according to NIOSH Method 5506 (NIOSH 1998b), using a personal sampling pump operating at 2 l/min to draw air through a Teflon filter (for particulate-phase PACs, hereafter 'P-PACs') followed by a XAD-2 sorbent tube (for vapor-phase PACs). Levels of PACs on dermal patch samples (D-PACs) were measured using a method described by Jongeneelen et al. (Jongeneelen et al. 1988) and Van Rooij et al. (VanRooij et al. 1993), with minor modifications. A soft polypropylene filter was attached to an exposure pad to create a dermal patch with an effective surface area of 8.71 cm^2. Using an adhesive backing, the dermal patch was attached to the underside of each wrist (the average of the left and right wrist measurements were used in this study). Levels of PACs in air and dermal patch samples were analyzed using liquid extraction coupled with high-pressure liquid chromatography as described previously (McClean et al. 2004a; McClean et al. 2004b).

Urine samples were collected from all workers before work (morning), after work (postshift), and at bedtime of each workday. Morning samples collected on the first day of the workweek (after a work-free weekend) were treated as baseline samples for each subject.

Each morning sample, collected on a subsequent workday, was treated as the final observation from the previous workday. All urine samples were collected in sterilized polypropylene containers and stored at -20˚C for up to seven years prior to analysis. Levels of urinary Nap and Phe (U-Nap and U-Phe, respectively) were determined using head space-solid phase microextraction coupled with gas chromatography-mass spectrometry (see Chapter 3, section *3.3.3.*), and levels of OH-Nap, OH-Phe, and OH-Pyr were determined using solid-phase extraction coupled with liquid chromatography-tandem mass spectrometry as briefly summarized in the companion to this paper (see Chapter 4, section *4.3.3.*). We note that 'OH-Nap' is defined here as the sum of 1- and 2-hydroxynaphthalene, 'OH-Phe' is the sum of 2-, 3-, 4-, and 9-hydroxyphenanthrene, and 'OH-Pyr' is 1-hydroxypyrene. Descriptive statistics of the levels of U-Nap, U-Phe, OH-Nap, OH-Phe and OH-Pyr are described in a companion paper (see Chapter 4). Creatinine levels were determined using a colorimetric procedure (Procedure No. 555, Sigma Diagnostics, Dorset, U.K.) (Sigma Chemical Co. 1984).

5.3.2. Statistical analyses

In preliminary analyses, levels of PACs in particulate-phase and vapor-phase samples were found to be highly correlated. Thus, to remove problems related to collinearity in statistical models, and for ease of interpretation, we used only airborne particulate measurements (i.e., *P-PACs*) along with dermal PAC measurements (i.e., *D-PACs*) in statistical analyses. This approach also had the advantage of focusing upon the larger (4-ring to 6-ring) carcinogenic PAHs that were common to both air samples (*P-PACs*) and dermal patch samples (*D-PACs*). (Very similar results were obtained when vapor-phase PACs were

used instead of *P-PACs* in statistical models). The level of creatinine measured in each urine specimen (*CREATININE*) was included in the models as an independent variable to allow for adjustments on covariates as well as the dependent variables (Barr et al. 2005).

All statistical tests were performed using SAS statistical software (v. 9.1, SAS Institute, Cary, NC). All levels of air, dermal patch, and urinary analytes were evaluated after natural-log transformation to satisfy normality assumptions and to remove heteroscedasticity. Considering the repeated measures study design, data were evaluated using linear mixed-effects models (Proc MIXED). Correlation coefficients for exposure data were determined according to Hamlett et al. (Hamlett et al. 2003). Postshift, bedtime, and morning urinary analyte data were individually regressed on their respective workday exposures, along with covariates. Baseline urinary analyte data were not included in this statistical analysis, but were described in a companion paper (see Chapter 4). The following numbers of observations were available for the different sampling times: U-Nap and U-Phe models included 51 postshift, 50 bedtime, 48 morning measurements while OH-Nap, OH-Phe, and OH-Pyr models included 49 postshift, 48 bedtime, and 46 morning measurements.

For dermal patch samples, McClean et al. defined the limit of quantitation (LOQ) as three times the standard deviation of field-blank values (McClean et al. 2004a). Using this criterion, approximately 30% of dermal patch measurements used in this analysis were below the LOQ. However, because values were reported for these observations, we used the reported levels in our analyses and did not impute values below the LOQ.

The full mixed model for an exposure-biomarker relationship at the hth sampling time (i.e., postshift = 1, bedtime = 2, and morning = 3) is defined as:

$$Y_{hij} = \ln(X_{hij}) = \beta_{0h} + \beta_1 CREATININE_{hij} + \beta_2 P\text{-}PACs_{hij} + \beta_3 D\text{-}PACs_{hij} + \beta_4 DAY_{hij} + \beta_5 HOUR_{hij} + \beta_6 AGE_i + \beta_7 BMI_i + \beta_8 SMOKE_i + b_{hi} + \varepsilon_{hij} \quad (1)$$

for j = 1, 2, ..., n_{hi} measurements of the ith individual at the hth sampling time;

for i = 1, 2, ..., 20 individuals;

for h = 1, 2, or 3;

where X_{hij} represents the analyte concentration for the jth measurement of the ith individual and Y_{hij} is the natural logarithm of the individual measurement X_{hij}. The coefficient β_{0h} represents the intercept for the hth sampling time. The coefficients β_1 - β_8 represent the coefficients for fixed effects, which include $CREATININE_{hij}$ [creatinine concentration (g/l) for the jth measurement of the ith individual at the hth sampling time], $P\text{-}PACs_{hij}$ (ng/m^3) and $D\text{-}PACs_{hij}$ (ng/cm^2) which correspond to the jth air and dermal patch measurements, respectively, for the ith individual at the hth sampling time, DAY_{hij} and $HOUR_{\mathbf{hij}}$ (hour: postshift = 0 h; bedtime and morning values equal elapsed hours since postshift sample) for the jth measurement of the ith individual at the hth sampling time, and AGE_i, BMI_i, and $SMOKE_i$ which represent the ith subject's age (y), body mass index (kg/m^2), and smoking status (nonsmoker = 0), respectively.

In Model 1, b_{hi} represents the random effect for the ith individual at the hth sampling time and ε_{hij} represents the random error for the jth measurement of the ith individual at the hth sampling time. It is assumed that b_{hi} and ε_{hij} are normally distributed with means of zero and variances of $\sigma^2_{bY_h}$ and $\sigma^2_{wY_h}$, representing the between-person and within-person components of total variance (i.e., $\sigma^2_{bY_h} + \sigma^2_{wY_h} = \sigma^2_{Y_h}$), respectively, for sampling time h.

Linear mixed-effects models were constructed using restricted maximum likelihood estimates (REML) of variance components. Fixed effects were maintained in the models at a significance level of $\alpha = 0.10$, using manual backwards stepwise elimination. Bayesian Information Criterion (BIC) values were used to select between competing models. Compound symmetry covariance matrices were used; these generally yielded the lowest BIC diagnostic values compared to those yielded by other tested matrices (homogeneous autoregressive, heterogeneous autoregressive). Observations with missing values (i.e., air or dermal patch measurement, urinary analyte measurement, significant covariate) were not included in the final models.

Linear mixed-effects models including only β_{0h}, b_{hi}, and ε_{hij} were also constructed for each urinary analyte. The estimate of $\sigma^2_{Y_h}$ from each null model (no additional fixed effects), designated $\hat{\sigma}^2_{Y_h\text{null}}$, was used along with the estimate of $\sigma^2_{Y_h}$ from each full model (including significant fixed effects), designated $\hat{\sigma}^2_{Y_h\text{full}}$, to calculate the percent of the variance explained by fixed effects, where: $[(\hat{\sigma}^2_{Y_h\text{null}} - \hat{\sigma}^2_{Y_h\text{full}})/\hat{\sigma}^2_{Y_h\text{null}}] \times 100 = \%$ explained.

5.4. Results

5.4.1. Postshift urine samples

Table 5.1. shows the final models for analytes in postshift samples. Levels of *P-PACs*, *D-PACs*, and *CREATININE* were significant predictors of levels of U-Nap, U-Phe, OH-Phe, and OH-Pyr and *BMI* was a significant predictor OH-Pyr. The levels of *P-PACs, D-PACs,* and *CREATININE* collectively explained 66%, 54%, 42%, and 41% of the variability in levels of OH-Phe, U-Nap, U-Phe, and OH-Pyr, respectively. When *CREATININE* was removed from the models, *P-PACs* and *D-PACs* explained 53%, 47%,

37%, and 35% of the variability in levels of OH-Phe, U-Nap, U-Phe, and OH-Pyr, respectively. Conversely, when *P-PACs* and *D-PACs* were removed from the models, *CREATININE* explained 13%, 8%, 6%, and 5% of the variability in levels of OH-Phe, U-Nap, U-Phe, and OH-Pyr, respectively. This indicates much weaker effects of *CREATININE* on analyte levels than those of air and dermal patch measurements, i.e., *P-PACs* and *D-PACs*, respectively. After adjusting for *CREATININE*, *P-PACs*, and *D-PACs*, a significant effect of *BMI* was observed on levels of OH-Pyr only. The negative regression coefficient suggests that OH-Pyr levels were lower in individuals with higher *BMI*s. Interestingly, the model for OH-Nap levels was quite different from those for all the other analytes in postshift urine. For OH-Nap, *P-PACs* and *D-PACs* were not significant predictors and the final model included only *CREATININE*, *DAY*, *AGE*, and *SMOKE*. Together, these variables explained 74% of the postshift variability in OH-Nap levels.

Table 5.1. shows, for each analyte, the estimated between-person and within-person variance components, designated $\hat{\sigma}^2_{bY_h}$ and $\hat{\sigma}^2_{wY_h}$, respectively, along with corresponding values of $\hat{\lambda}$, representing the ratio of the within-person variance component to the between-person variance component (i.e., $\hat{\lambda} = \hat{\sigma}^2_{wY_h} / \hat{\sigma}^2_{bY_h}$). For U-Phe, $\hat{\sigma}^2_{bY_h} = 0$ indicating that all of the variation in this biomarker between persons was explained by *P-PACs, D-PACs,* and *CREATININE.* For U-Nap and OH-Pyr, after adjusting for significant fixed effects, there was a greater estimated within-person variance component ($\hat{\sigma}^2_{wY_h}$) than estimated between-person variance component ($\hat{\sigma}^2_{bY_h}$), as shown by $\hat{\lambda}$ values of 2.23 and 1.29, respectively. Values of $\hat{\sigma}^2_{bY_h}$ and $\hat{\sigma}^2_{wY_h}$ were similar for OH-Phe ($\hat{\lambda}$ = 0.881), whereas $\hat{\sigma}^2_{wY_h}$ was considerably smaller than $\hat{\sigma}^2_{bY_h}$ for OH-Nap ($\hat{\lambda}$ = 0.483).

5.4.2. Bedtime urine samples

Table 5.2. shows the final models for analytes in bedtime samples. In these samples, OH-Phe was the only analyte to be significantly affected by the exposure variables, *P-PACs* and *D-PACs*, as well as *CREATININE*. These combined effects explained 56% of OH-Phe variability. When *CREATININE* was considered as the only fixed effect, it explained 22% of the variability in OH-Phe levels. When *P-PACs* and *D-PACs* were considered as predictors of OH-Phe without *CREATININE*, they explained 23% of OH-Phe variability. Thus, in bedtime samples, the exposure variables and *CREATININE* contributed equally to the levels of OH-Phe. No other covariates were significant determinants of OH-Phe levels.

As with OH-Nap levels in postshift samples, OH-Nap levels in bedtime samples were highly affected by *CREATININE* and *SMOKE* (explaining 65% of the variability in OH-Nap) but were not affected by exposure levels (i.e., *P-PACs* and *D-PACs*). For OH-Pyr, U-Nap, and U-Phe, significant effects of *CREATININE* and *D-PACs* on analyte levels were observed, together explaining 50%, 30%, and 30% of the respective analyte variability. When included in the models without other fixed effects, *CREATININE* explained 25%, 15%, and 14% of the variability in OH-Pyr, U-Phe, and U-Nap levels, respectively. When *CREATININE* was removed from each model, *D-PACs* explained 15%, 12%, and 11% of the variability in OH-Pyr, U-Nap, and U-Phe levels, respectively. After adjusting for *D-PACs* and *CREATININE*, a moderately significant effect of *SMOKE* on U-Phe was observed. When included in the model, the combined effects of *CREATININE*, *D-PACs*, and *SMOKE* explained 36% of variability in U-Phe levels.

Results from the final models showed that all analytes except U-Phe ($\hat{\lambda}$ = 1.13) had $\hat{\lambda}$ values less than one (U-Nap = 0.528; OH-Nap = 0.698; OH-Phe = 0.604; OH-Pyr = 0.483)

indicating greater between-person variability compared to within-person variability at bedtime.

5.4.3. Morning urine samples

Model results for morning samples are shown in Table 5.3. In these samples, only the levels of OH-Phe were significantly affected by both air and dermal patch levels, i.e., *P-PACs*, and *D-PACs*, as well as *CREATININE*; together, these three variables explained 65% of the variability in morning OH-Phe levels. *CREATININE* alone explained 38% of the variability in OH-Phe levels while *P-PACs* and *D-PACs* jointly explained 25% of OH-Phe variability.

For U-Nap and OH-Pyr, *D-PACs* (but not *P-PACs*) and *CREATININE* were significantly associated with analyte levels, together explaining 36% and 35% of the variability in OH-Pyr and U-Nap levels, respectively. When *D-PACs* was removed from the models, *CREATININE* alone explained 20% and 28% of the variability in OH-Pyr and U-Nap levels, respectively. When *D-PACs* was solely considered, 7% and 1% of the variability in OH-Pyr and U-Nap levels was explained, respectively. After adjusting for *CREATININE* and *D-PACs*, a significant negative *BMI* effect was observed for OH-Pyr. In total, the effects of *CREATININE*, *D-PACs*, and *BMI* explained 46% of the variability in OH-Pyr levels. Air and dermal patch measurements from the previous workday (i.e., *P-PACs* and *D-PACs*) were not significant determinants for either OH-Nap or U-Phe. In fact, *CREATININE* and *DAY* were the only significant determinants for U-Phe, explaining 33% of the variability, and *CREATININE*, *DAY*, and *SMOKE* were the only significant determinants for OH-Nap, together explaining 61% of the variability.

In morning urine samples, more variability in OH-Pyr levels was observed between subjects than was observed within subjects ($\hat{\lambda}$ = 0.744). Estimates of λ were close to one for U-Nap and OH-Phe ($\hat{\lambda}$ = 1.06 and 0.961, respectively) and were greater than one for U-Phe and OH-Nap ($\hat{\lambda}$ = 3.31 and 2.60, respectively). This indicates comparatively larger within-person variability for U-Phe and OH-Nap in morning urine samples.

Table 5.1. Results from linear mixed-effects models evaluating postshift urinary analyte levels.

	U-Nap			U-Phe			OH-Nap			OH-Phe			OH-Pyr		
Parameters	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value
Fixed effects															
Intercept	0.969	(0.536)	0.09	0.472	(0.674)	0.5	10.4	(0.489)	< 0.0001	5.25	(0.418)	< 0.0001	6.09	(1.15)	< 0.0001
CREATININE [a]	0.446	(0.158)	0.009	0.476	(0.204)	0.03	0.519	(0.112)	< 0.0001	0.490	(0.121)	0.0004	0.348	(0.198)	0.09
P-PACs [b]	0.235	(0.067)	0.002	0.234	(0.085)	0.01			NS	0.245	(0.051)	< 0.0001	0.258	(0.082)	0.004
D-PACs [c]	0.202	(0.053)	0.0008	0.255	(0.066)	0.0007			NS	0.215	(0.041)	< 0.0001	0.223	(0.066)	0.002
DAY			NS			NS	0.159	(0.051)	0.005			NS			NS
BMI			NS			NS			NS			NS	-0.072	(0.029)	0.02
AGE			NS			NS	-0.016	(0.008)	0.05			NS			NS
SMOKE			NS			NS			< 0.0001			NS			NS
no							-1.59	(0.221)							
yes							0	(ref.)							
Random effects															
σ^2_B	0.090			0			0.143			0.101			0.197		
σ^2_w	0.201			0.514			0.069			0.089			0.254		
λ	2.23			NA			0.483			0.881			1.29		

[a] creatinine concentration (g/l)
[b] air concentration of particulate PAC [ln(ng/m^3)]
[c] dermal patch concentration of particulate PAC [ln(ng/cm^2)]
σ^2_B = between-subject variance component
σ^2_w = within-subject variance component
λ = variance ratio (σ^2_w/ σ^2_b)

Table 5.2. Results from linear mixed-effects models evaluating bedtime urinary analyte levels.

	U-Nap			U-Phe			OH-Nap			OH-Phe			OH-Pyr		
Parameters	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value
Fixed effects															
Intercept	2.23	(0.442)	< 0.0001	1.46	(0.658)	0.04	9.48	(0.355)	< 0.0001	5.13	(0.603)	< 0.0001	4.04	(0.568)	< 0.0001
CREATININE [a]	0.499	(0.161)	0.004	0.766	(0.234)	0.003	0.720	(0.148)	< 0.0001	0.795	(0.144)	< 0.0001	0.942	(0.205)	< 0.0001
P-PACs [b]			NS			NS			NS	0.141	(0.066)	0.04			NS
D-PACs [c]	0.203	(0.067)	0.005	0.320	(0.097)	0.003			NS	0.213	(0.062)	0.002	0.339	(0.085)	0.0005
DAY			NS			NS			NS			NS			NS
HOURS			NS			NS			NS			NS			NS
BMI			NS			NS			NS			NS			NS
AGE			NS			NS			NS			NS			NS
SMOKE			NS			0.07						NS			NS
no				-0.819	(0.432)		-1.57	(0.299)	< 0.0001						
yes				0	(ref.)		0	(ref.)							
Random effects															
σ^2_B	0.352			0.452			0.255			0.255			0.603		
σ^2_w	0.186			0.509			0.178			0.154			0.291		
λ	0.528			1.13			0.698			0.604			0.483		

[a] creatinine concentration (g/l)
[b] air concentration of particulate PAC [ln(ng/m^3)]
[c] dermal patch concentration of particulate PAC [ln(ng/cm^2)]
σ^2_B = between-subject variance component
σ^2_w = within-subject variance component
λ = variance ratio (σ^2_w/ σ^2_b)

Table 5.3. Results from linear mixed-effects models evaluating morning urinary analyte levels.

	U-Nap			U-Phe			OH-Nap			OH-Phe			OH-Pyr		
Parameters	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value	Estimate	(SE)	*p*- value
Fixed effects															
Intercept	2.03	(0.382)	< 0.0001	0.731	(0.480)	0.1	8.76	(0.421)	< 0.0001	4.92	(0.419)	< 0.0001	6.97	(1.41)	0.0001
CREATININE [a]	0.705	(0.142)	< 0.0001	0.917	(0.216)	0.0002	0.755	(0.151)	< 0.0001	0.647	(0.105)	< 0.0001	0.719	(0.209)	0.002
P-PACs [b]			NS			NS			NS	0.184	(0.052)	0.002			NS
D-PACs [c]	0.146	(0.059)	0.02			NS			NS	0.135	(0.044)	0.005	0.322	(0.078)	0.0004
DAY			NS	0.318	(0.167)	0.07	0.246	(0.112)	0.04			NS			NS
HOURS			NS			NS			NS			NS			NS
BMI			NS			NS			NS			NS	-0.094	(0.041)	0.03
AGE			NS			NS			NS			NS			NS
SMOKE			NS			NS	-1.24	(0.291)	0.0003			NS			NS
no							0	(ref.)							
yes															
Random effects															
σ^2_B	0.202			0.241			0.126			0.103			0.434		
σ^2_w	0.214			0.798			0.328			0.099			0.323		
λ [d]	1.06			3.31			2.60			0.961			0.744		

[a] creatinine concentration (g/l)
[b] air concentration of particulate PAC [ln(ng/m^3)]
[c] dermal patch concentration of particulate PAC [ln(ng/cm^2)]
σ^2_B = between-subject variance component
σ^2_w = within-subject variance component
λ = variance ratio (σ^2_w/ σ^2_b)

5.5. Discussion

In a previous investigation of 26 highway construction workers, 20 asphalt-exposed paving workers (the same 20 workers included in this study) were shown to have higher levels of urinary OH-Pyr in postshift, bedtime, and morning samples when compared to 6 milling workers who did not work with hot-mix asphalt (McClean et al. 2004b). In a companion paper (see Chapter 4), we reanalyzed archived urine specimens from the same 20 paving workers for several PAH biomarkers, namely, unmetabolized Nap and Phe, and the metabolites, OH-Nap, OH-Phe, as well as OH-Pyr [levels of OH-Pyr measured in the reanalyzed samples were highly correlated with the original OH-Pyr measurements ($r = 0.89$, $p < 0.0001$) indicating good agreement between studies] (McClean et al. 2004b). We used these data to assess correlations between analytes, to determine biological half-times, and to assess task-based differences in analyte levels, while controlling for covariates (see Chapter 4). In this study, our major goal was to determine the effect of airborne particulate and dermal patch measurements (i.e., *P-PACs* and *D-PACs*, respectively) on these urinary analytes and to identify the most promising biomarkers of exposure to particulate-phase PAHs for asphalt-exposed workers and the best timing of specimen collection (postshift, bedtime, or morning).

Figure 5.1. shows the percentages of urinary analyte variance explained by the sum of *P-PACs* and *D-PACs* in postshift, bedtime, and morning samples. The sum of *P-PACs* and *D-PACs* explained much more of the variability of biomarker levels in postshift urine samples (up to 53%), compared to bedtime (up to 23%) and morning (up to 25%) samples. In postshift samples, the strongest association with exposure measurements (*P-PACs* plus *D-PACs*) was observed for OH-Phe (53% of explained variance) followed by U-Nap (47%), U-

Phe (37%), and OH-Pyr (35%). It is important to note that both *P-PACs* and *D-PACs* were each strongly associated with these urinary PAH analytes (see Table 5.1.) and, because the levels of *P-PACs* and *D-PACs* were essentially uncorrelated ($r < 0.3$), that air and dermal patch measurements appear to have contributed independently to the levels of the biomarkers. The fact that these PAH biomarkers were more highly associated with exposure variables in postshift urine samples, compared to bedtime and morning samples, indicates that Nap, Phe, and Pyr were rapidly absorbed and eliminated from the body during and immediately following the work shift (Rappaport 2008). Indeed, we estimated the biological half-times of these PAH biomarkers to range between 8 and 26 h (see Chapter 4).

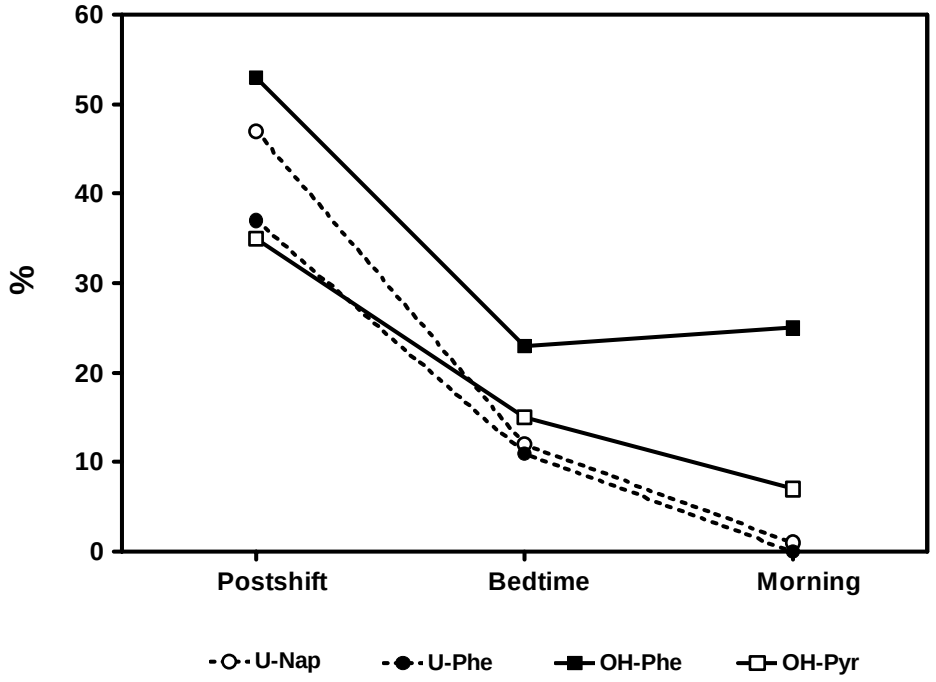

Figure 5.1. Percentage of urinary analyte variance explained by air and dermal patch concentrations of asphalt emissions.

In bedtime and morning urine samples, *P-PACs* and *D-PACs* were much weaker predictors of urinary biomarker levels than they were in the postshift urine samples. Nonetheless, levels of OH-Phe were associated with both *P-PACs* and *D-PACs* in bedtime and morning samples, while those of U-Nap and OH-Pyr were significantly associated with levels of *D-PACs* (but not *P-PACs*) in bedtime and morning samples, and those of U-Phe were associated with *D-PACs* in bedtime (but not morning) samples (Tables 5.2. and 5.3.). The consistent influence of *D-PACs* on levels of PAH biomarkers in bedtime-urine and morning-urine samples suggests that dermal contact with Nap, Phe, and Pyr persisted well into the evening after a given work shift. This finding is consistent with those of McClean et al. (McClean et al. 2004b) who used a distributed-lag model to investigate the influence of Pyr levels in air and dermal patches on levels of urinary OH-Pyr in these same 20 subjects. Based upon their modeling results, the authors concluded that the effect of the dermal patch Pyr levels on urinary OH-Pyr was approximately eight times that of the air Pyr levels. Moreover, the effect of inhalation exposure was determined to be more apparent immediately after the work shift (within 8 h), while the effect of dermal patch levels was more apparent during the period of 8 to 16 h after the work shift (McClean et al. 2004b). Results from our linear mixed-effects models are consistent with the earlier findings of McClean et al., and also suggest that levels of U-Nap, U-Phe, OH-Phe, as well as OH-Pyr are suitable biomarkers of both inhaled and dermal patch PAH levels emanating from asphalt.

Since *P-PACs*, *D-PACs*, and the urinary biomarkers were measured over three consecutive days, we included a 'workday' effect into our models (i.e., *WORKDAY*) to test the possibility that biomarker levels accumulated over the workweek. After adjusting for *P-PACs*, *D-PACs,* and other significant covariates, no significant workday effects were

observed for U-Nap, OH-Phe, or OH-Pyr. A significant positive workday effect was observed for U-Phe in morning samples only. While this indicates a linear increase in morning U-Phe levels, overall these results tend to rule out any strong effects towards accumulation of the measured biomarkers from day to day, and reinforce the notion that these urinary analytes reflect exposure primarily on the current day. A significant positive workday effect was also observed for OH-Nap in postshift samples and morning samples. As exposure effects (i.e., *P-PACs* and *D-PACs*) were not included in the final linear mixed-models due to a lack of significance (most likely due to the confounding effects of smoking), it is difficult to interpret the observed *WORKDAY* effect for OH-Nap.

In addition to assessing workday effects, we also added into the models a fixed effect of time in h postshift (*HOURS*) because bedtime and morning sample collection times varied from day to day and from person to person. The median collection time for bedtime samples was 6.00 h (range: 2.50 - 14.3 h) and the median collection time for morning samples was 15.3 h (range: 11.8 - 19.1 h) (see Chapter 4). Results from each model showed no significant effect of *HOURS* on analyte levels. This indicates that, after making adjustments for exposure levels and other covariates, subtle variations in sampling times did not affect urinary analyte levels.

Levels of urinary creatinine (*CREATININE*) were significant predictors of all urinary analytes. The positive regression coefficient in each model (Tables 5.1., 5.2., and 5.3.) indicates increased analyte levels with increased creatinine levels. Since the urinary concentration of creatinine increases with decreased urine volume, this finding indicates that urine volumes varied considerably within and between the road pavers under investigation.

Figure 5.2. shows the percent of each analyte's variance explained by urinary creatinine in urine collected postshift, at bedtime, and in the morning following exposure.

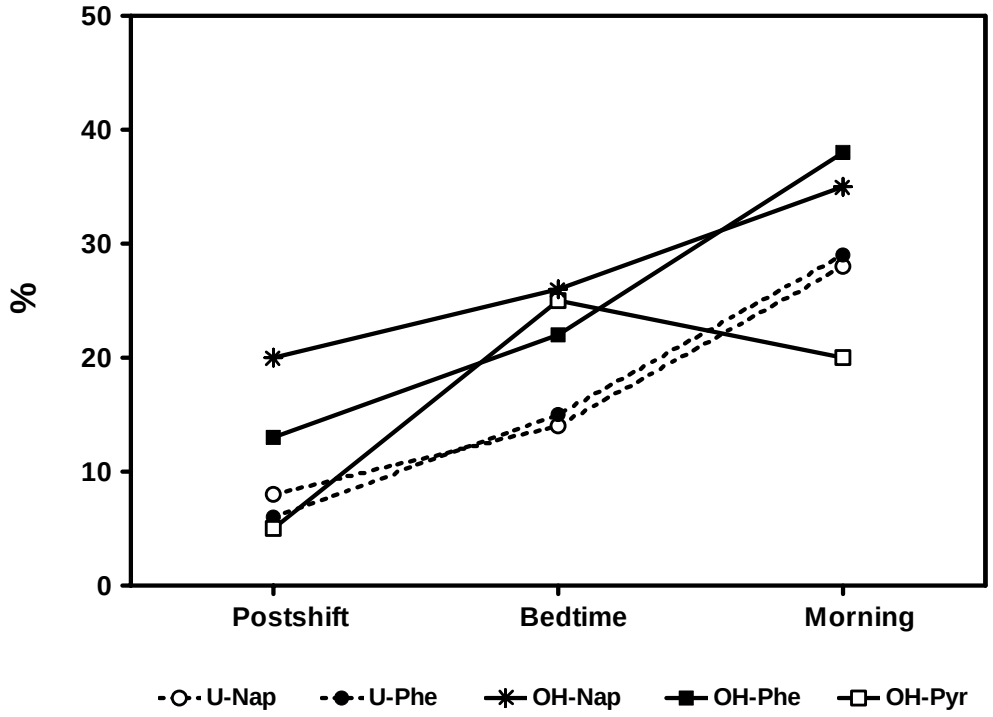

Figure 5.2. Percentage of urinary analyte variance explained by urinary creatinine concentration.

Overall, U-Nap and U-Phe were less affected by creatinine than OH-Nap and OH-Phe; this would be expected because unmetabolized organic compounds tend to enter urine via diffusion from blood rather than via glomerular filtration in the kidney, the latter mechanism being responsible for elimination of polar metabolites (Boeniger et al. 1993). To further investigate creatinine variability, we constructed a mixed model with *CREATININE* as the response variable and the period of sample collection (i.e., dummy variables for postshift, bedtime, or morning) as the predictor variables. Results showed a significant effect of the period of sample collection (p-value < 0.0001) on creatinine levels, with the highest levels

observed in postshift samples (mean = 1.93 g/l), followed by bedtime samples (mean = 1.55 g/l), and then morning samples (mean = 1.43 g/l). These changes in urinary creatinine levels may reflect a diurnal excretion pattern (Boeniger et al. 1993) as well as the dehydration of workers during hot work shifts. We note that the creatinine concentration was highest at the end of the workday as would be expected following outdoor work.

In postshift, bedtime, and morning samples, smoking status (i.e., *SMOKE*) was a highly significant determinant of OH-Nap levels. However, smoking status was not, at any time, a significant determinant of U-Nap levels. This result for U-Nap corroborates those from previous studies where smoking status had little effect on U-Nap levels, particularly in moderately-exposed to highly-exposed workers (Serdar et al. 2003a; Waidyanatha et al. 2003). The result for OH-Nap also supports those from previous studies where OH-Nap was shown to be significantly affected by smoking status (Serdar et al. 2003a; Serdar et al. 2004). Serdar et al. (Serdar et al. 2004) showed that, at low Nap exposure levels, smokers produced higher OH-Nap levels than nonsmokers and, at high Nap exposure levels, smokers produced lower OH-Nap levels than nonsmokers. The authors suggested metabolic induction by cigarette smoke at low Nap exposure levels. A similar mechanism may have influenced OH-Nap levels in this study. We also note a significant smoking effect on U-Phe levels in bedtime samples. Considering the lack of a smoking effect on U-Phe levels in postshift samples, this effect seems to be relatively modest compared to that of *P-PACs* and *D-PACs*.

As observed by McClean et al. (McClean et al. 2004b) in this population, we note a significant negative *BMI* effect on OH-Pyr levels. This observation may indicate increased storage of Pyr in adipose tissue with elevated exposures, thereby limiting the amount of Pyr

in the systemic circulation that is available for metabolism. *BMI* was not observed to significantly affect any other analyte after adjusting for other fixed effects.

Tables 5.1. - 5.3. list estimated values of the within-subject and between-subject variance components (i.e., $\hat{\sigma}^2_{wY_h}$ and $\hat{\sigma}^2_{bY_h}$, respectively) obtained from the mixed models of each urinary biomarker, as well as the corresponding estimated variance ratio $\hat{\lambda} = \hat{\sigma}^2_{wY_h} / \hat{\sigma}^2_{bY_h}$, in each case. Lin et al. (Lin et al. 2005) used values of $\hat{\lambda}$ to choose between air measurements and biomarker measurements in selecting the particular measure of exposure that should be the least biasing in estimating the slope of a log exposure-log disease linear relationship. In the current study, we can use $\hat{\lambda}$ values to choose among postshift, bedtime, and morning urine samples to find the minimally-biased biomarker of exposure for an epidemiological investigation. Interestingly, $\hat{\lambda}$ values for each of the biomarkers, save OH-Nap (which was affected primarily by smoking), were minimal in bedtime urine samples (see Tables 5.1. - 5.3.). Indeed, for U-Nap ($\hat{\lambda}$ = 0.53), OH-Phe ($\hat{\lambda}$ = 0.60), and OH-Pyr ($\hat{\lambda}$ = 0.48), the variance ratios were in the range of 0.5, which were smaller than those for the same analytes in postshift urine samples where values of $\hat{\lambda}$ = 2.2, 0.88, and 1.3, respectively. In light of the earlier discussion concerning the possibility that these biomarkers reflect continued effects of *D-PACs*, this finding opens the possibility that bedtime measurements of these biomarkers could be useful in investigating dermal PAH exposures. This conjecture will require further experimental confirmation.

Urinary OH-Pyr is a widely used PAH biomarker due to its reported associations with occupational exposures to PAHs (Jongeneelen 2001). In this study, we observed significant associations between particle-phase PACs (as measured by *P-PACs* and *D-PACs*) and OH-Pyr levels. This result was expected because Pyr, a 4-ring PAH, is often abundant in

measurements of PM from asphalt and combustion sources. Somewhat more surprising were the significant associations between Nap and Phe biomarkers (namely U-Nap, U-Phe, and OH-Phe) and particulate PAC measurements. Naphthalene, a 2-ring PAH, occurs almost exclusively in the gas phase, whereas Phe, a 3-ring PAH, is partitioned between the gas-phase and the particle-phase. Our results suggest that these abundant 2-ring and 3-ring PAHs may be very good surrogates for particulate exposures stemming from asphalt sources. Moreover, the results from our postshift models indicate stronger associations between levels of U-Nap, U-Phe, and OH-Phe and the particulate PAC levels in air and dermal patch samples than the corresponding associations for OH-Pyr. We do not recommend use of OH-Nap as a biomarker of exposure to particulate asphalt emissions, due to the apparent confounding by smoking.

6. Discussion and Conclusions

6.1. Summary and conclusions

The assessment of levels of PAH exposure has been challenged by the sheer number of individual PAH compounds, the distribution of species between gas- and particle-bound phases, and the presence of multiple routes of exposure. The purpose of this investigation was to evaluate the individual vapor-phase PAH constitutents, Nap and Phe, as possible surrogates for total PAH exposure, using measurements of air samples, dermal patch samples, and urine samples from groups of workers.

Diesel exhaust is a known source of occupational and ambient PAH exposure. In a controlled chamber study, where 10 human volunteers were exposure to 100 $\mu g/m^3$ of DE, we identified Nap and Phe as being both abundant in DE, and highly differentiated with respect to measurements of control samples (purified air). Furthermore, Nap and Phe were significantly correlated with accepted measures of particulate exposures emanating from diesel sources. That is, levels of Nap were highly correlated with levels of organic carbon and particle-bound PAHs, and levels of Phe were highly correlated with levels of total volatile+semivolatile PAHs. These findings offer promise that Nap and Phe can be useful surrogates for PAHs and organic particulate emissions in studies of DE exposure.

A previous study examined the relationship between air levels of Nap and total PAHs in several industries, namely creosote impregnation, coke production, aluminum production, and an iron foundry (Rappaport et al. 2004). Air levels of Nap and total PAHs were found to be highly correlated in each of these industries. Additionally, air concentrations of Nap were

found to be proportional to those of total PAHs. Using the available data from these same studies, we analyzed the relationship between air levels of Phe and total PAHs. Like Nap, air concentrations of Phe were found to be proportional to those of total PAHs. Thus, we have demonstrated that air concentrations of Nap and Phe track those of total PAHs, regardless of the source and exposure level, and conclude that Nap and Phe are promising surrogates of total PAH exposure through airborne routes. Interestingly, when we compared air concentrations of Nap and Phe, we found that the ratio of Nap/Phe decreased with increasing levels of airborne Phe, regardless of the source of PAH exposure, i.e., DE, asphalt emissions, or coke-oven emissions. This suggests that the particular chemical processes that generate PAHs tend to produce increasingly more Phe relative to Nap as the overall amount of PAH production increases.

Biomarkers offer a means to assess exposure to PAHs though multiple pathways, including inhalation, ingestion, and dermal contact. Since small amounts of unmetabolized PAHs are eliminated in the urine following passive diffusion from the blood to the urine, the urine concentrations of PAHs are thought to reflect blood levels. We measured levels of Nap and Phe in the urine of diesel-exposed workers, asphalt workers, and coke-oven workers, groups given *a priori* exposure designations of low, medium, and high, respectively. Levels of urinary Nap and Phe followed the *a priori* exposure designations, and were highly correlated, suggesting a common source of exposure to Nap and Phe in each case. Additionally, as observed with air levels of Nap and Phe, the ratio of urinary Nap/Phe decreased with increasing levels of urinary Phe. Therefore, we conclude that urinary levels of Nap and Phe likely reflect the corresponding airborne concentrations at the time of urine

collection. This suggests that urinary Nap and Phe are promising surrogates of occupational exposure to total PAHs.

In a group of asphalt-exposed workers, we measured urinary levels of unmetabolized Nap and Phe (U-Nap and U-Phe, respectively), along with phenolic metabolites of Nap, Phe, and Pyr [hydroxynapthalenes (OH-Nap), hydroxyphenanthrenes (OH-Phe), and hydroxypyrene (OH-Pyr), respectively]. We observed significant correlations between each of these analytes, suggesting a common exposure source of Nap, Phe, and Pyr. When we compared air and dermal patch measurements of particulate PAHs (or PACs) with urinary analyte levels, we observed significant associations between exposure levels (represented by air and dermal patch measurements) and biomarker levels. Specifically, U-Nap, U-Phe, OH-Phe, and OH-Pyr were strongly associated with both air and dermal patch measurements of particulate PAH emissions (OH-Nap was highly confounded by cigarette smoking). Therefore, we conclude that U-Nap, U-Phe, OH-Phe, and OH-Pyr can be useful surrogates of exposure to asphalt-derived PAHs via inhalation and dermal contact. Furthermore, we determined that measurements of U-Nap, U-Phe, OH-Phe, and OH-Pyr in urine specimens collected at bedtime were good predictors of PAH levels on dermal patches, and had lower ratios of within-person variability to between-person variability. This suggests that bedtime urine samples may be particularly useful for investigations of dermal PAH exposure in asphalt-exposed workers.

6.2. Significance of this study

The methods and results of this investigation should enhance our ability to assess PAH exposures, particularly those from derived from diesel and asphalt sources. As

previously mentioned, measures of particulate exposure, namely EC, OC, and PM2.5, have traditionally been used as surrogates for DE exposure, despite their inability to be linked with either the PAH content of diesel particulates or biomarkers of PAH exposure. This has made it difficult to determine whether the mechanism by which DE may cause lung cancer is related to the PAH content of DE or to the particles *per se*. Thus, by demonstrating that Nap and Phe are abundant in DE, are correlated with organic constituents of DE, and can be measured as biomarkers in human urine, we offer a means to link external exposure to internal PAH dose, a link that may help resolve this controversy.

The assessment of the carcinogenic effects of asphalt emissions is also fraught with uncertainty, due to limited quantitative exposure data from air and dermal measurements and possible confounding by smoking and other factors. Here, we have identified strong associations between biomarkers of Nap, Phe, and Pyr in asphalt workers and the corresponding air and dermal patch measurements of particulate PAHs. Our work indicates that particular urinary PAH biomarkers, namely, U-Nap, U-Phe, OH-Phe, and OH-Pyr are all promising surrogates for exposures to total particulate PAHs in asphalt workers. The fact that U-Nap, U-Phe, OH-Phe, and OH-Pyr were associated with dermal patch measurements of PAHs in bedtime urine points to the possibility that bedtime measurements have particular advantages in future epidemiological studies of asphalt-exposed persons.

Urinary PAH metabolites (i.e., OH-Nap, OH-Phe, and OH-Pyr) are the products of phase-I and phase-II metabolism that can vary between individuals (and within individuals over time) due to differences in levels of exposure and other factors that affect the uptake of the contaminant (e.g., inhalation rate, presence of dermal exposure, and genetic differences). Pyrene is a widely used surrogate for PAH exposure because it is a 4-ring compound, and

therefore, is representative of the particulate fraction of PAHs, containing the most carcinogenic compounds. However, because Pyr does not contain a bay region and is metabolized by a more simple scheme than BaP and the other carcinogenic 4-6 ring PAHs, it may not be particularly useful as a measure of PAH metabolism and the associated risk of cancer. To better understand PAH metabolism *in vivo*, we investigated the levels of urinary metabolites of the more abundant PAH, Phe, which does contain a bay region and undergoes essentially the same types of metabolism as the carcinogenic 4-6 ring PAHs. Thus, it is noteworthy that we detected stronger associations between particulate PAHs and U-Phe and OH-Phe, when compared to the association between particulate PAHs and OH-Pyr. This suggests that, in addition to being a better model for PAH metabolism, Phe is also at least as good a surrogate for particulate PAH exposure as Pyr.

6.3. Strengths and weaknesses of this study

The inability to discriminate DE from other exposure sources complicates the establishment of a causal association between DE exposure and lung cancer. In the chamber study (Chapter 2), air used in control exposures was purified with HEPA filters and activated charcoal. This reduced contamination through unknown sources of measured analytes. DE was introduced into the chamber after a dilution with purified air to achieve a highly regulated, feedback-controlled level. Considering these control measures, we were able to precisely quantify DE constituents without the influence of external sources, and were able to select candidate markers of exposure to DE particulates based on the differentiation of levels in DE with respect to purified air.

Results of the chamber study showed significant daily variations in specific analyte levels (e.g., Nap), most likely due to daily variations in truck-engine performance. While correlations were observed between selected analytes and other surrogate measures of DE, there existed very little dynamic range in exposure levels. Controlled DE exposures at varying concentrations (e.g., 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$) would allow for a better assessment of the correlations between DE constituents. Additionally, investigations of environmental and occupational DE exposure are warranted to further confirm that Nap and Phe are useful markers of DE exposure.

In Chapter 3, urinary measurements of Nap and Phe were proposed as biomarkers of occupational PAH exposure. A major strength of our analysis was the availability of a wide range of urinary analyte levels, i.e., 200-fold and 470-fold range of U-Nap and U-Phe levels, respectfully, due to our ability to use samples reflecting different types of occupational PAH exposures, namely diesel exhaust, asphalt emissions, and coke-oven emissions. For diesel-exposed workers and asphalt workers, pre-shift urine measurements were available, allowing each subject to act as his/her own control. However, our study was limited by the lack of available pre-shift measurements for coke-oven workers and we had to rely upon factory controls, who were also highly exposed. This introduced some uncertaintity into our analysis of adjusted Nap and Phe levels in our full models. Finally, while measurements of air and urine suggested a decrease in the ratio of Nap/Phe with increasing Phe levels, these results are based on a limited amount of data, and require further confirmation in future studies.

In the final study (Chapters 4 and 5), we were able to relate levels of urinary Nap, Phe, and Pyr analytes to air and dermal patch measurements of particle-bound PAHs. A major strength of this study was the repeated-measures sampling design, which allowed us to

assess biological half-times of urinary analytes, and the effect of repeated exposures over consecutive workdays. Additionally, covariate information allowed us to assess the effects on analyte levels of urine dilution (as measured by creatinine concentration), BMI, age, and smoking status. Although we had both air and dermal patch measurements for this analysis, little work has been done to validate patch sampling as a useful means of assessing PAH exposure through dermal routes. Therefore, dermal patch concentrations could only be included in our statistical models as measures of dermal deposition of asphalt emissions, and not necessarily true measures of dermal exposure. Also, the effect of particulate PAH exposure through ingestion was not considered in our models. Accounting for ingestion exposure may explain some of the unresolved variance in urinary analyte levels.

6.4. Suggestions for future research

Because air measurements of Nap and Phe were identified as promising surrogates for DE exposure in our chamber study, and because U-Nap and U-Phe were identified as promising biomarkers of PAH exposure, we will examine urinary levels of Nap and Phe in the 10 volunteer participants of the controlled chamber study. In addition, we will assess levels of Nap and Phe in samples of exhaled breath condensate and plasma, to better understand rates of uptake and elimination, and to validate additional Nap and Phe biomarkers. Finally, we will determine levels of OH-Nap, OH-Phe, and OH-Pyr in the urine samples from the 10 volunteer participants of the chamber study. We will pay particular attention to the effects of urine dilution on analyte concentrations given the low exposure levels of Nap, Phe, and Pyr in this study.

In the study of 26 road-construction workers, we measured U-Nap and U-Phe, as well as phenolic metabolites of Nap and Phe, including 1- and 2-OHNap, and 2-, 3-, 4-, and 9-OHPhe. However, our statistical analyses only considered the sum of phenolic Nap and Phe metabolites (i.e., total OH-Nap and total OH-Phe, respectively). In the future, we plan to develop linear mixed-effects models to assess the individual relationships between U-Nap and individual phenolic metabolites of Nap, and between U-Phe and individual phenolic metabolites of Phe. This work will highlight the influence of exposure and covariates on the production of individual phenolic isomers. Considering the observed effect of smoking status on OH-Nap levels, urinary cotinine concentration will be used to better explain the variability in OH-Nap levels. Ultimately, statistical models will be used to associate unmetabolized PAHs in urine (as a measure of internal PAH dose) with additional urinary metabolites (i.e., dihydrodiols and tetraols) and biomarkers of effect and susceptibility. This work will provide a further understanding of human PAH metabolism *in vivo*, and will help to inform dose-response relationships.

The relationship between low to moderate levels of PAH exposure (specifically those from diesel exhaust and asphalt sources) and cancer risk is uncertain due to a general lack of quantitative exposure data. In future occupational and environmental studies, air measurements of Nap and Phe should be considered as surrogates for total PAH exposure through airborne routes. Measurements of Nap and Phe in air can be obtained using inexpensive passive techniques, allowing for more measurements at a given cost. Additionally, levels of Nap and Phe in urine, whether unmetabolized or as phenolic metabolites, should be considered as measurements of internal dose through multiple exposure routes. These measurements of Nap and Phe in air and urine can be used to better

understand the cancer risk associated with low to moderate levels of PAH exposure. However, future studies should closely consider the effects of PAH exposure through dermal and ingestion routes. In our analysis, we considered dermal patch measurements of particulate PAHs. Little work has been done to validate this technique as being suitable for the assessment of dermal PAH exposure. Future work should evaluate the suitability of this method, specifically addressing the dermal absorption kinetics of particle-bound PAHs. Accurate data representing PAH exposure through inhalation, dermal, and ingestion routes are needed to better understand the contribution to total dose from each pathway. This information will ultimately aid in establishing links between exposure, internal dose, and health effects.

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