Measuring Urinary Hydroxylated PAH Metabolites as Biomarkers of Exposure to Diesel Exhaust in Underground Miners

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Abstract

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Background

Many people throughout the world are exposed to diesel exhaust (DE) in both occupational and non-occupational environments. High DE exposures are associated with acute and chronic adverse health outcomes including cardiovascular and respiratory disease, asthma and lung cancer. In recent years, The International Agency for Research on Cancer (IARC) has categorized DE as a known Group 1 human carcinogen, reaffirming the risk that DE poses to human health and the necessity for continuing research on the topic and enhanced control measures to further reduce risk. Thus, urinary hydroxylated PAHs were explored as biomarkers of exposure to diesel exhaust in underground miners.

Methods

In this research project, specific job task information was collected, exposure questionnaires were administered and air and biological samples were collected from a cohort of 20 underground miners. Air samples were previously analyzed for elemental carbon (EC) and 1-nitropyrene (1-NP). A subset of the urine samples (n=170) were previously analyzed for metabolites of 1-NP - 8-OHNP, 6-OHNP and 1-hydroxypyrene (1-OHP). The remaining urine samples (n=354) were analyzed using high performance liquid chromatography with fluorescence detection for six different hydroxylated PAH metabolites associated with DE

exposure. All urine samples were specific gravity adjusted prior to data analysis. In this project, we estimated associations between (1) hydroxylated PAH urinary metabolites and air exposures of EC and 1-NP and (2) hydroxylated PAH urinary metabolites and 1-NP-8-OHNP and 6-OHNP urinary metabolites. Lastly, the temporal variation was examined for the hydroxylated PAH urinary metabolites to determine if they accumulate in the body over continued exposures. This was done by (1) comparing post-shift urine voids to pre-shift urine voids and (2) comparing post-shift urine voids throughout the week.

Results

Of the six metabolites examined, the urinary metabolite 1-OHP is positively associated with EC in air. For every doubling of elemental carbon air exposure, 1-OHP in urine increases by 37% ($p = 0.001$ and 95% CI: 14%,64%). Similarly, 1-OHP is also positively associated with 1-NP in air. For every doubling of 1-NP in air exposure, 1-OHP in urine increases by 15% ($p = 0.02$ and 95% CI: 2%,28%).

An association is observed between 1-OHP and 6-OHNP and 8-OHNP. For every doubling of 6-OHNP and 8-OHNP, 1-OHP increases by 46% (95% CI: 24%, 74%) and 51% (95% CI: 26%, 79%) respectively ($p < 0.001$ for both associations).

When examining temporal variation throughout the week, there were no statistically significant increases in hydroxylated PAH urinary metabolite concentrations. This means that these metabolites are cleared from the body within 24 hours and do not accumulate over weekly exposures. Unlike weekly variability, when examining daily cross shift increases in hydroxylated PAH urinary metabolites, for the metabolite 1-OHP, daily post shift samples were on average, 21% greater than pre-shift samples collected on the same day ($p=0.02$). No statistically significant daily increases were observed in the five other metabolites.

Conclusions

Of the six hydroxylated PAH urinary metabolites examined in this study, it appears that 1-OHP has the greatest potential to be established as a reliable surrogate for DE exposure. 1-OHP is associated with EC and EC is frequently used as a surrogate for DE and in combination with total carbon (TC), is used for the determination of compliance with the Mine Safety and Health Administration PEL. 1-OHP is also positively associated with 1-NP in air and OHNPs in urine,

which are diesel specific and less prone to interferences to other sources. Thus, in the absence of interfering combustion sources, 1-OHP may be reliable for estimating DE exposure. It can be concluded that occupational DE exposures contribute to elevated hydroxylated PAH levels because of the elevated 1-OHP concentrations seen in miner's post shift urine samples compared to their pre-shift samples collected on the same day.

The ability to estimate DE exposures from hydroxylated PAH urinary metabolites reduces the time and cost burden of collecting air samples or the economic burden of sending samples to a lab to be analyzed. Continuing research to establish hydroxylated PAH urinary metabolites as reliable biomarkers for DE exposure could have many benefits, such as workers not having to don cumbersome sampling equipment, thus increasing their work output overall. Unexplained variability in associations is observed and continued research needs to be completed to determine the source of that variability.

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CHAPTER ONE: BACKGROUND AND SIGNIFICANCE

Diesel Exhaust Exposure and Health Effects

Many people throughout the world are exposed to diesel exhaust (DE) in both occupational and non-occupational environments. Non-occupational exposure to DE is associated with living in an urban setting or living near industrial areas, roads or freeways. Depending on the city and the nature of industry in the city's vicinity, the general public's exposure to DE can vary greatly (Bian et al. 2016). Occupational exposure to DE is associated with working in industries such as mining, bridge and tunnel building, trucking, farming, construction, maritime, automobile and truck maintenance and working on railroads, where diesel vehicles and equipment are used (OSHA, 2013). Occupational exposures can vary depending on the specific industry of a worker and their specific job/work tasks (OSHA, 2013; HEI, 2002).

Since the introduction of the first diesel engine, diesel engine technology has developed and continues to change. One of the many reasons for this is the attempt to reduce engine emissions by the implementation of more stringent emission regulations. Diesel oxidation catalysts and diesel exhaust particulate filters were introduced to aid in the reduction of emissions (IRSG, 2012). Fuel technology has also evolved with the reduction of sulfur content in fuels to meet particulate matter regulations (IRSG, 2012). Although diesel engines and fuels have become more efficient, diesel vehicle miles traveled continues to grow, especially in mining (HEI, 2012). Since diesel powered machines, equipment and vehicles are widespread, many workers are at risk for exposure to DE and developing associated adverse health outcomes (OSHA, 2013).

Due to the nature of diesel exhaust, high exposures are associated with both acute and chronic adverse health outcomes. Acute adverse health outcomes include: dizziness, headache and/or irritation of the throat, nose or eyes (EPA, 2002). Chronic exposure to diesel exhaust can result

in cardiovascular and respiratory disease, asthma and lung cancer (EPA, 2002). In recent years, the International Agency for Research on Cancer (IARC) has categorized DE as a Group 1 human carcinogen, thus reaffirming the risk that DE poses to human health and the necessity for continuing research on the topic (IARC, 2013).

DE is a complex mixture of gases, vapors and particulate matter. Some of the components by themselves are Group 1 human carcinogens and approximately half of the components are Group 2 probable human carcinogens (IARC, 2006). The majority of workplace exposures are via inhalation, but additional exposures from dermal contact or ingestion through contamination on skin is possible (ACGIH, 2005). Previous attempts have been made to establish a correlation between DE and other surrogate measures in air samples. However, misclassification of worker exposures to DE can occur due to interferences from other combustion sources, thus leading to poor correlation between DE and its surrogates, and compromising the ability to determine associations with adverse health effects (Noll et al, 2007).

Measuring Exposure via Total Carbon and Elemental Carbon

DE is a complex mixture with many components capable of being measured. Because of this, one, or a few of these components, can be selected as a surrogate to measure exposure. With so many components in diesel exhaust, it is difficult for regulatory agencies to come to a consensus on a specific standard for DE. The Mine Safety and Health Administration (MSHA) is the only agency to have a set standard for DE exposure. MSHA uses diesel particulate matter (DPM), more specifically total carbon (TC), as the regulatory surrogate for diesel exhaust (MSHA, 2005). DPM typically consists of greater than 80% carbon components, a core of elemental carbon (EC) with organic carbon (OC), including aromatic hydrocarbons, attached to the surface (Noll et al., 2007). Adding the measured EC mass to the measured OC mass results in TC mass,

the MSHA regulatory surrogate for DE. In an underground metal mine, MSHA states that an 8-hour time weighted average may not exceed 160 $\mu g/m^3$ of TC exposure (MSHA, 2005).

Although having a permissible exposure limit (PEL) for DPM is anticipated to reduce worker exposure, using TC as a regulatory surrogate nevertheless has two important limitations. (1) TC is measured in air and thus, does not account for additional routes of worker exposure, nor the modifying effect of varying worker inhalation rates on dose. (2) TC is also prone to interferences from additional combustion sources (MSHA, 2005). Because of these issues, the exploration for different markers of exposure to DE could aid in determining a better predictor of true exposure and dose, and hence, a better understanding of the aforementioned adverse health outcomes associated with DE exposure.

Measuring Exposure via 1-Nitropyrene

One of the many polycyclic aromatic hydrocarbons (PAHs) formed from the combustion of diesel fuel is 1-nitropyrene (1-NP), a nitro-PAH. A unique feature of 1-NP is that, compared to the carbon components, it is a more specific marker for DE, and not found in high concentrations in other combustion sources (IARC, 2013). Because of this, 1-NP is a highly useful marker of exposure to DE when there is a potential for interferences from other emission sources, such as interferences found in the mining environment (Toriba et al., 2007).

In addition to measuring the parent compound 1-NP in air, metabolic products from 1-NP exposure can also be measured in urine as hydroxylated nitropyrenes (OHNPs). Because of the parent compound's specificity to DE, the associated urinary metabolite also has similar specificity to DE and thus, is proposed to be a reliable surrogate to estimate total exposure (Miller-Schulze et al., 2013).

Although having a surrogate marker of DE exposure that is more specific than the carbon components is extremely useful for determining worker exposure, measuring the parent compound 1-NP in air and its associated metabolites in urine is time consuming and expensive. This is due to the need for time intensive sample preparation procedures, and sophisticated analytical instrumentation required for these measurements.

Although 1-NP measured in air has the aforementioned benefits, similar to measuring for TC, this measurement does not account for additional routes of exposure, nor the modifying effect of worker specific ventilation rates on dose. Although inhalation is the major exposure pathway for DE, the use of biomarkers is beneficial in better understanding the relationship between total exposure and dose.

Other PAHs as Biomarkers for Exposure to Diesel Exhaust

Because of the limitations noted above for the measurement of the parent compound 1-NP and its urinary metabolites, we sought to evaluate a less expensive marker of DE exposure and dose: that is the measurement of hydroxylated metabolites of PAHs (OHPAHs) in urine (SOP for PAH urinary metabolites in Appendix III).

It is known that PAHs are highly enriched in diesel exhaust (Nelson, 1989). Thus, workers exposed to DE are exposed to elevated concentrations of the parent PAH compounds (ATSDR, 2009). In this study, the parent PAH compounds of interest are fluorene, phenanthrene, naphthalene and pyrene. The parent PAH compounds can enter the human body through various routes including, inhalation, ingestion and dermally and their associated metabolites can subsequently be measured in the urine. Because of this, it is advantageous to use biological monitoring instead of environmental monitoring to estimate internal dose (Lauwerys and Hoet 1993). To estimate PAH exposure, 1-hydroxypyrene (1-OHP), the major metabolite of pyrene,

one of the many components in a PAH mixture, can be used as a surrogate measure (Jongeneelen, 2001). The current study also examined the use of the urinary metabolites 2-naphthol and 1-naphthol (parent compound naphthalene), 2+9 hydroxyfluorene (parent compound fluorene), 2-hydroxyphenanthrene and 3-hydroxyphenanthrene (parent compound phenanthrene) as other potential exposure surrogates (NHANES, 2017).

OHPAH metabolites are eliminated in urine and have been previously studied as surrogates to estimate DE exposures (Hansen et al., 2008). Once the parent compound is inhaled, a substantial fraction is metabolized, mediated by the enzyme cytochrome P-450. Once oxidized and hydrolyzed by epoxide hydrolase, the PAHs are excreted as hydroxylated metabolites. These products can be eliminated either via the urine or via the feces (ACGIH, 2005). A significant fraction (~25%) will be delivered to the kidney for filtration of the cardiac output and then eliminated via urine (Van Rooij et al, 1994).

More specifically, 1-OHP is one of the most studied biomarkers of exposure to combustion emissions, with at least 100 reports of its use as a biomarker (Hansen et al., 2008). The popularity of 1-OHP as a biomarker is because pyrene is found at relatively high concentrations in combustion emissions, its metabolic pathways are relatively simple, and 1-OHP can be detected at low concentrations in urine when using validated laboratory methods. Additionally, pyrene has similar physical properties as carcinogenic PAHs and therefore is a good indicator of their exposure (ACGIH, 2005). Understanding the relationship between DE exposure, the resulting OHPAH metabolites and adverse health effects is important for the determination of mitigation efforts.

Underground Miners as a Study Cohort

Underground miners are one of the occupations who historically have experienced high levels of exposure to DE. This is due in part to both their close working vicinity to diesel powered vehicles for long periods of time and the confining spaces that they work in (Coble et al., 2010). Because of this, underground miners were chosen as the study subjects for this research project.

In mining, many vehicles run on diesel fuel including transportation vehicles, locomotives, tractors, graders, dozers, forklifts, bolters and front-end loaders (Coble et al., 2010). Although many mines use diesel powered vehicles and equipment, DE exposures are highly variable. Exposure and dose depend on many factors such as worker inhalation rate, mine ventilation rate and exposure mitigation efforts in the mine, such as vehicle exhaust filters and the use of respirators (Pronk et al., 2009). With so many variables potentially affecting exposure and dose, determining how and which of them contribute and/or change DE exposure concentrations, could help in controlling elevated exposures and mitigating the associated adverse health outcomes.

Specific Aims

The specific objective of this study is to take personal measurements of diesel exhaust exposure (measured as EC and 1-NP) in the breathing zone of underground mine workers and previously analyzed OHNP urinary biomarker concentrations and then compare those air and urine concentrations with worker's urinary metabolite concentrations of OHPAH metabolites. Establishing a relationship between diesel exhaust exposure, OHNP biomarker concentrations and the OHPAH metabolite concentrations in worker's urine would be beneficial in making it possible to mitigate the known negative health outcomes associated with DE exposure. A worker's health can be protected and/or improved if their exposures can be estimated and proper precautions taken to reduce exposures before they occur. The three specific aims of this project are:

(1) Compare urine biomarker concentrations of OHPAH metabolites to known air exposure concentrations of EC and 1-NP.

(2) Compare urine biomarker concentrations of OHPAH metabolites to known urinary biomarker concentrations of OHNP metabolites.

(3) Identify the temporal variation in exposures throughout the workweek by examining changes in urine biomarker concentrations between pre-shift and post-shift and changes in post-shift urinary concentration by day of the week. This will help identify potential accumulation of OHPAHs in the body.

These aims were completed using air and biological samples previously collected in an underground metal mine as a part of a 2014 study. The outcomes of the aforementioned aims will help to establish associations between air exposures and urinary biomarkers. Establishing those associations could reduce the time and economic burden of collecting and analyzing personal air samples in the future. Through identifying the most appropriate time for collection of spot urine samples, and quantifying the likely misclassification of exposure associated with specific spot urine samples, adverse health outcomes associated with DE exposure will be better understood and beneficial mitigation controls can be put into place to reduce worker exposure.

CHAPTER TWO: METHODS

Study Design

This research project is a part of a National Institute of Safety and Health (NIOSH) funded project entitled "Diesel Exposures in Mines: Biomarkers in Urine and Realtime Air Monitoring". The NIOSH project was an observational cohort study undertaken collaboratively by researchers at the University of Washington, Boise State University and Montana Tech of the University of Montana.

Research Location, Study Subjects and Data Collection

All samples were collected in an underground metal mine during 2014. The mine is one of the largest underground metal mines in the US, and it extracts, refines and smelts metal ore on site. The mine employs approximately 1,300 workers and is capable of processing more than 2,000 tones ore/day. This specific mine exclusively uses diesel or diesel electric engines underground, and it was anticipated that there would be minimal confounding from additional (non-diesel) exhaust sources in this setting. The diesel fuel used in all engines underground is a B70 blend biodiesel.

A 20-member cohort was enrolled in this study. This cohort consisted of 18 men and 2 women with a mean age of 41.4 years and an age range between 27 and 59 years. Subjects were not included in the study if they had physician diagnosed kidney, liver or bladder disease. These diseases could potentially interfere with biomarker concentrations in the urine samples.

Subjects had a variety of jobs and worked in varying locations throughout the mine. Each cohort member was given a cohort ID number of 1-20. Because two subjects (#5 and #16) switched to the night shift early in the study, data is reported for only the eighteen workers who remained on the day shift.

Mine safety and health staff categorized workers *a priori* into high, medium and low exposure groups based on job title and job location (table 1). These different exposure categories were selected in attempt to ensure a wide range of DE exposure levels across the cohort.

There were four sample collection campaigns, corresponding with the months of March, June, August and October. These months were chosen in attempt to control for seasonal variability in exposures throughout the mine. Each sampling campaign's duration was four days, the equivalent of a full workweek for these workers. Although the mine operates day and night shifts, to simplify study logistics all samples were collected during the day shifts. Work crews rotate shifts between night shift and day shift with four consecutive days off in-between shifts.

Both air and urine samples were collected for all cohort members. Personal air samples were collected in the workers' breathing zone using, in tandem, a polystyrene cassette/Teflon filter downstream from an SKC GS-3 cyclone and an MSHA-compliant sub-micron DPM SKC impactor with quartz fiber filter downstream from an SKC GS-1 cyclone. SKC AirChek and PXCR personal sampling pumps were used to draw air through the filters. Flow rate was 2.75 L/minute for the Teflon filter samples and 1.7 L/minutes for the DPM samples. Flow rate was calibrated prior to sample collection using a defender Drycal primary gas flow calibrator, and flow was verified after sample collection with the Drycal. The flow rate prior to sample collection and the flow rate after sample collection were then averaged to determine average flow. If those values varied by greater than 10%, the sample was not included in data analysis. The Teflon filters were analyzed by gravimetry, and the quartz filter samples were analyzed for EC/OC/TC and 1-NP. Because of the limited number of sampling devices available, personal air samples could not be collected on all workers simultaneously. Therefore, on days 1 and 3, air

samples were collected for cohort members 1-10 and on days 2 and 4, air samples were collected for cohort members 11-20.

Urine samples were collected on all of the four days for all cohort members both pre and post shift. Total void urine volume and urine specific gravity (measured via refractometer) were measured in the field. Up to 110 mL of each urine void was kept and any additional void was discarded. Duplicate specimens were retained for approximately 10% of samples. Samples were stored on dry ice until they could be shipped to the University of Washington for long term freezer storage (typically within four days of collection).

Additionally, daily post shift questionnaires focusing on job task and daily activities were administered to all cohort members (see Appendix II).

Table 1. Worker task and worker location characteristics

Study ID	Job Title	Job Location	Exposure Category			Personal Air Sampling Days	
			High	Medium	Low	Days 1 & 3	Days 2 & 4
01	Muck Hauler	Area 2		X		X	
02	Sand plant Operator	49 Sand plant		X		X	
03	Operator	2000 level to 7800 level	X			X	
04	Miner I	2300 West FWL	X			X	
05	Miner III	Area 3, 3800 East 9900			X	X	
06	Miner I	2000 West Stope Block	X			X	
07	Geologist	All Locations		X		X	
08	Miner I	2600 East	X			X	
09	Mechanic	2900 shop			X	X	
10	Diamond Driller	2300 West & 5000 West			X	X	
11	Electrician	All Locations		X			X
12	Miner I	5000 West	X				X
13	Miner I	4100 West FWL	X				X
14	Stationary Mechanic	All Locations			X		X
15	Operator	Lower Off Shaft		X			X
16	Raise Bore I - Driller	5200 East 5900		X			X
17	Geologist	All Locations			X		X
18	Beat Mechanic	3200 level to 4800 level	X				X
19	Surface Mill Operator	Surface Mill			X		X
20	Miner 1	38w Block Area 2	X				X

Sample Analysis

Air Samples

Air samples were analyzed for EC, OC and TC by ALS Environmental in Salt Lake City, Utah using NIOSH method 5040 (NIOSH, 1999). After being analyzed for EC/OC/TC, the filters were sent to the University of Washington to be analyzed for 1-NP using liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) (Carpenter, 2014).

Urine samples

Over all campaigns, 535 urine samples and 51 duplicates were collected. On average, each cohort member gave approximately 32 samples (range: 8 – 39). 170 of these samples had previously been analyzed for 1-NP metabolites and 1-OHP via HPLC/MS/MS (Ramsay, 2014).

Urinary Hydroxy PAHs Analyses

All remaining urinary samples were previously frozen and then thawed for analysis. They were randomized and distributed into batches prior to being analyzed. Urine samples were analyzed for six OHPAH metabolites (2-naphthol, 1-naphthol, 2+9 hydroxyfluorene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene and 1-hydroxypyrene) using HPLC with fluorescence detection. The Simpson Laboratory has developed an extensive SOP for this analysis method (found in Appendix III) (Chetiyanukornkul et al., 2006; ACGIH, 2005). Quality control samples were included for every batch of samples analyzed as follows:

1. an internal spike standard: 10 mL water spiked with a known mass (20 µL) of d^9-1-hydroxypyrene glucuronide
2. an internal standard and metabolite spike: 10 mL water spiked with a known mass (20 µL) of d^9-1-hydroxypyrene glucuronide and (25 µL) OHPAH protonated spike
3. a blank: 10 mL water, with no spike solutions added

4. a benchmark: extracted from a large pooled urine batch to monitor the assay reproducibility
5. 100% control: 120 µL water and 55 µL methanol spiked with known mass (25 µL) protonated OHPAH spike

The pH was stabilized to a pH of 5.5 using a 1 M sodium acetate buffer. Metabolites were deconjugated in the urine using the enzyme β-glucuronidase and incubated in a 37°C shaking water bath for 3 hours. The time that samples spent in the water bath was changed partway through the sample analysis due to changes of activity with the specific batch of β-glucuronidase used in the assay. Samples sets analyzed in the fall quarter of 2016 were in the hot water bath overnight.

The deconjugated metabolites were extracted using solid phase extraction (SPE) columns (Supelco, Supelclean 6 mL SPE tubes). They were eluted from the SPE columns using 5 mL of an 80:20 methanol: water mixture. Once eluted, the extracts were placed in a TurboVap to be evaporated. The samples were evaporated to approximately 2 mL and then ~4.5 mL of acetonitrile was added to aid in the evaporation process. This was step was repeated a second time. Once the samples were brought to approximately 1 mL, 20 µL of DMSO was added and samples were vortexed for 1 minute. The samples were then brought to near dryness in the TurboVap. Metabolites were reconstituted in 200 µL of 60:40 water: methanol mixture and lastly, the samples were filtered (Fisherbrand, 13mm syringe filter) into HPLC vials for analysis. The complete urinary analysis SOP for the PAH metabolites can be found in Appendix III. Duplicate extraction and analyses were performed on approximately 6.5% of the urine samples to determine precision of the assay.

Data Analysis

To complete the data analysis, all relevant data and information was imported into Stata 13. Data was matched using the subject specific and date specific identifiers from four different excel spreadsheets:

1. *Consolidated urinary PAH data* (OHPAH urinary biomarker data)
2. *urine full dataset JMR 5_22_15* (OHNP urinary biomarker data)
3. *survey data table_03may2015* (questionnaire data)
4. *compiled air data_2july2015* (air data)

The data traceability document found in Appendix IV outlines the original sources, locations and any previous manipulation completed on this data. Data was combined into one .xlsx Stata file (*PAH_1NPUrine_questionairre_airdata*) to complete Aim 1-3.

Aim 1

A linear mixed model was used to evaluate the relationship between OHPAHs biomarker levels and air exposure:

$$\ln(\text{OHPAH})_{n,t} = \beta_0 + \beta_1 \, x \, \ln(\text{air EC})_{n,t} + b_n + \varepsilon_{n,t}$$

$$\ln(\text{OHPAH})_{n,t} = \beta_0 + \beta_1 \, x \, \ln(\text{air 1-NP})_{n,t} + b_n + \varepsilon_{n,t}$$

In this model, β_0 represents the background OHPAH urinary concentration in the absence of occupational exposure to EC or 1-NP. $2^{\beta 1}$ represent the percent change in OHPAH urinary concentration for every doubling of air EC or air 1-NP concentration. b_n represents the subject-specific random effect and $\varepsilon_{n,t}$ is the residual error term for cohort subject *n* at shift *t*. Descriptive statistics were generated using the statistical software STATA.

Aim 2

A linear mixed model was used to evaluate the relationship between OHPAH biomarker levels and previously analyzed OHNP urinary metabolite concentrations:

$$\ln(\text{OHPAH})_{n,t} = \beta_0 + \beta_1 \, x \, \ln(\text{urinary OHNP})_{n,t} + b_n + \varepsilon_{n,}$$

In this model, β_0 represents the average OHPAH urinary concentration in the absence of OHNP. $2^{\beta 1}$ represent the percent change in OHPAH urinary concentration for every doubling of urinary OHNP concentration. b_n represents the subject-specific random effect and $\varepsilon_{n,}$is the residual error term for cohort subject *n*. Descriptive statistics were generated using the statistical software STATA.

Aim 3

Each cohort member's urine concentrations was examined throughout the week to establish the variation in the metabolite concentrations. This was done two ways. The relationship between pre-shift vs. post shift samples was first completed by using the mixed model equation:

$$\ln(\text{OHPAH post/pre-shift concentration})_{n,t} = \beta_0 + b_n + \varepsilon_{n,}$$

In this model β_0 = average daily cross shift variation b_n represents the subject-specific random effect and $\varepsilon_{n,}$ is the residual error term for cohort subject *n* . If workers' daily exposure contributes to higher PAH metabolite levels, then the ratio of post-shift urinary concentrations/pre-shift urinary concentrations will be > 1. Because this test was completed on the natural log scale, if workers' daily exposure contributes to higher PAH metabolite levels, the relationship of ln (post-shift urinary concentrations/pre-shift urinary concentrations) will be > 0. Thus, $e^{\beta 0}$ is equivalent to the GM cross shift ratio (post-shift/pre-shift) which is a measure of the magnitude of difference between post shift urine and pre shift urine concentrations.

Another model was used to determine if there is a significant cross-week change in biomarker levels among cohort members. This was completed using the mixed model:

$$\ln(\text{OHPAH post-shift concentration})_n = \beta_0 + \beta_1 x + \beta_2 x + \beta_3 x + b_n + \varepsilon_{n,}$$

In this equation, β_0 = average day 1 post-shift urinary concentration. β_1 = average increase/decrease of day 2 post-shift urinary concentration in respect to day 1. β_2 = average increase/decrease of day 3 post-shift urinary concentration in respect to day 1. β_3 = average increase/decrease of day 4 post-shift urinary concentration in respect to day 1. b_n represents the subject-specific random effect and $\varepsilon_{n,}$ is the residual error term for cohort subject *n*. If worker daily exposures contribute to higher PAH metabolite levels and accumulate over the week then it would be assumed β_1, β_2 and β_3 would be significantly higher than β_0.

CHAPTER 3: RESULTS

Sample Corrections

All urinary concentrations were adjusted for specific gravity (SG) prior to data analysis using the equation below.

SG adjusted urine concentration = urine concentration * ((1.02 – 1)/(urine concentration – 1))

Metabolites were not corrected for differences in internal standard recovery with the exception of 1-OHP. It was determined to correct for the internal standard recovery in 1-OHP because this correction reduced the variability in duplicate samples, but did not consistently do so for the other metabolites.

Additionally, all metabolites were log normally distributed (see Appendix I) and were natural log transformed for figures and statistical tests.

Flagging Data

Following sample analysis, data files were reviewed for potential errors before any statistical testing began. Flags were assigned to data values depending on the associated error. Explanation of flagging variables can be found in table 1 of Appendix I. According to the type of flag assigned to a value, an associated action was taken in attempt to control for those errors.

Table 2. Summary of the number of data flags and percentage of total censored data

	Number of Flagged Data Points	Number of Censored Data Points	Percent of Total Samples Censored
2-Naphthol	19	19	5.4 %
1-Naphthol	148	135	38.2%
2+9 Hydroxy-Fluorene	25	25	7.0%
2-Hydroxy-Phenanthrene	85	77	22.0%
3-Hydroxy-Phenanthrene	88	77	21.9%
1-Hydroxypyrene (fluorescence detection)	16	16	4.5%
1-Hydroxypyrene (mass spectrometry detection)	48	48	28.2%

The metabolite 1-naphthol had over 38% of its final data censored. The majority of those flags were associated with interferences from other sources in the HPLC chromatograms. Approximately 28% of 1-OHP analyzed by HPLC/MS/MS was also censored due to spike recoveries < 10%. In addition, 2-hydroxy phenanthrene and 3-hydroxy phenanthrene were censored for approximately 22% of the total samples. This was largely due in part to a particular batch where the HPLC/fluorescence system could not detect those peaks due to instrument malfunction. This resulted in 29 samples being censored.

Samples Under the Limit of Detection

One of the flags indicated samples that were under the limit of instrument detection. For statistical significance testing, samples less than the LOD were substituted with a pre-determined substitution value. The LOD was determined to be the lowest concentration of standard detected that was used for the instrument calibration curve. The substitution for samples less than the LOD was the LOD/$\sqrt{2}$. This substitution only applied to values where a peak was not detected. If a peak was detected at the appropriate retention time, but the extract concentration was less than

the LOD, the extract concentration from the chromatogram was used and a substitution was not made.

Table 3. LOD values of extract concentration and number of substitutions/percent of total substitutions by metabolite

	LOD (pg/mL)	Number of Substitutions	Percent of Total Samples
2-Naphthol	13.2	0	0%
1-Naphthol	1320	13	6.0%
2+9 Hydroxy-Fluorene	0.99	0	0%
2-Hydroxy-Phenanthrene	0.825	8	2.9%
3-Hydroxy-Phenanthrene	0.825	11	4.0%
1-Hydroxypyrene (fluorescence detection)	0.66	0	0%
1-Hydroxypyrene (mass spectrometry)	LOD varied by batch	48	28.2%

Duplicates and Quality Control Samples

Twenty three duplicate samples were run (6.5% of total samples). Of the 23 sample duplicates analyzed, no duplicate sample pairs were analyzed in the same batch and thus, the within batch variability for duplicates cannot be determined. However, the between batch variability can be determined for sample duplicates, seen in table 5.

Each batch contained duplicate benchmark urine samples, duplicate D+H spike samples and duplicate 100% control samples in order to determine within batch and between batch variability. Tables 4 and 5 display the within and between batch variability respectively.

Table 4. Within batch variability

	2-Naphthol	1-Naphthol	2+9 Hydroxy-Fluorene	2-Hydroxy-Phenanthrene	3-Hydroxy-Phenanthrene	1-Hydroxypyrene
CV Benchmark Urine	9%	27%	6%	12%	12%	8%
CV D+H Spike Samples	9%	10%	8%	8%	5%	7%
CV 100% Controls	3%	3%	3%	3%	3%	8%

Table 5. Between batch variability

	2-Naphthol	1-Naphthol	2+9 Hydroxy-Fluorene	2-Hydroxy-Phenanthrene	3-Hydroxy-Phenanthrene	1-Hydroxypyrene
CV Urine Duplicates	32%	66%	28%	31%	44%	25%
CV Benchmark Urine	20%	100%	54%	38%	35%	19%
CV D+H Spike Samples	21%	21%	19%	28%	13%	17%
CV 100% Controls	12%	13%	11%	14%	14%	51%

Variability seen in the 100% control samples displays instrument variability. Variability seen in benchmark data, D + H spiked samples and duplicates displays overall assay reproducibility, taking into account instrument and assay variability.

There is less variability within batches than there is between batches, on average approximately 10% instrument and assay variability within batches. Because of this, it can be assumed that for samples analyzed within the same batch, the variability due to the instrument is approximately 10% and the variability due to the assay is approximately 10%. The compound 1-naphthol displayed the greatest within batch assay variability at approximately 19%.

The variability between batches was greater than the variability within batches. The between batch instrument variability was different depending on the compound, but on average was approximately 19%. The compound 1-naphthol displayed the greatest between batch assay variability at approximately 62%. The compound 1-hydroxypyrene displayed the lowest between batch assay variability at approximately 20%. An attempt was made to control for batch to batch variability using benchmark urine concentrations to make adjustments, but this did not reduce overall sample variability in the duplicates and thus, it was determined not to adjust for differences in batches for statistical analysis and testing.

Combining 1-OHP Data for Statistical Testing

Statistical testing for aim 1 and aim 2 utilize combined 1-OHP data from HPLC/fluorescence detection and 1-OHP data from HPLC/MS/MS. 1-OHP data from solely HPLC/fluorescence detection matched to EC air data resulted in $n < 33$, matched 1-NP air data resulted in $n < 15$ and matched to OHNP urinary data resulted in $n < 13$. More data was available for statistical testing after combining 1-OHP data from HPLC/MS/MS to the 1-OHP data from HPLC/ fluorescence detection (matched to EC air data resulted in $n = 100$, matched 1-NP air data resulted in $n = 81$ and matched to OHNP urinary data resulted in $n = 104$).

It was determined that 1-OHP data from HPLC/fluorescence detection and 1-OHP data from HPLC/MS/MS were comparable and could be reasonably combined for statistical analysis. Seven of the same samples were analyzed for 1-OHP via HPLC/fluorescence and also via HPLC/MS/MS. Those 7 data points were compared to determine if an association existed, seen in figure 1.

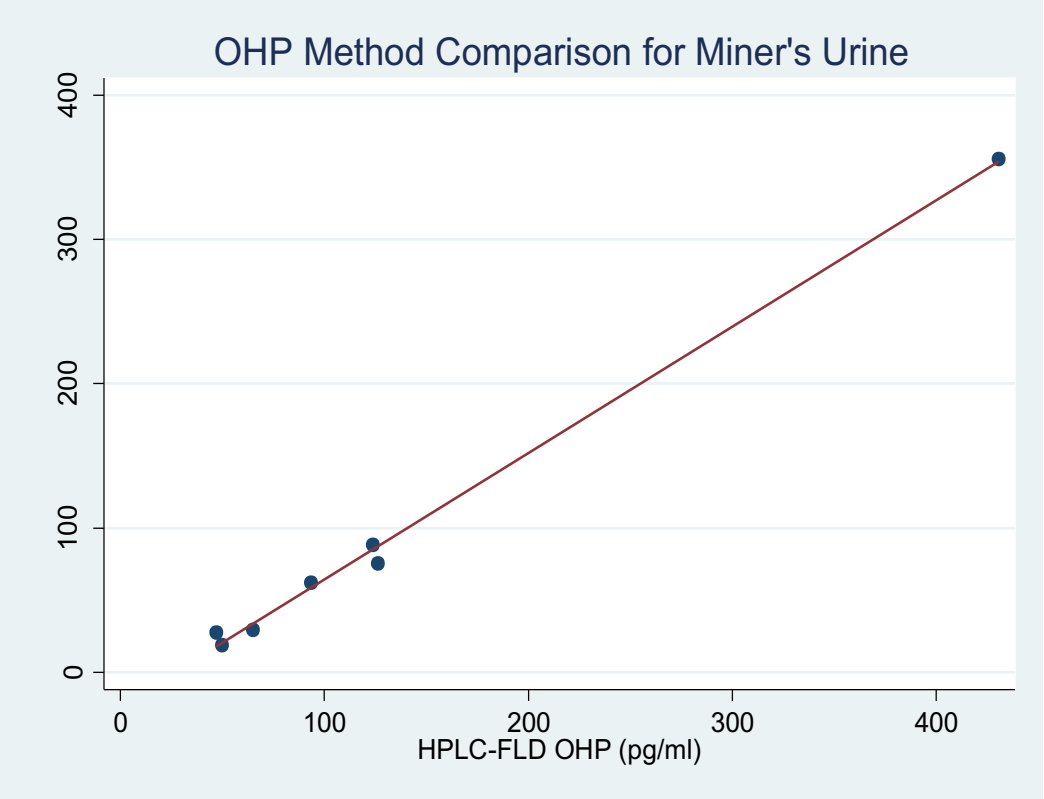


Figure 1. 1-OHP urine concentration measured via HPLC/fluorescence vs 1-OHP urine concentration measured via HPLC/MS/MS

An $R^2 = 0.98$ was observed in this data set. The 1-OHP data from HPLC/MS/MS were adjusted for, using the slope (0.8566), so that data could be combined with 1-OHP data from HPLC/fluorescence detection. For the 7 points where data existed for both 1-OHP from HPLC/MS/MS and 1-OHP from HPLC/fluorescence detection, the 2 urine concentration values were averaged for statistical analysis.

OHPAHs in Urine

In theory, with a cohort of 20 workers providing two urine samples/day for a 4-day period during 4 different sampling campaigns, there should be 640 urinary samples total. Due to the nature of research, 640 urinary samples were not available to be analyzed. Table 6 outlines the reason that specific urinary data is missing.

Table 6. Summary of missing urine samples

Urine Samples Analyzed	n	Percent of Total Samples
Total estimated samples	640	100%
Total actual samples analyzed	354	55%
Samples missing due to cohort dropout (2 dropouts)	64	10%
Samples unable to be located	18	3%
Samples previously analyzed and emptied (for urinary 1-NP and 1-OHP)	148	23%
Samples not collected (due to worker illness, vacation etc.)	56	9%

A large percentage of the samples (23%) were used and emptied for the analysis of OHNP and 1-OHP in urine and thus, could not be analyzed for the five additional urinary OHPAHs. Another large percentage of the samples (10%) were not available due to cohort dropout. The subset of 354 urine samples (55%) analyzed for urinary OHPAH represents all available remaining samples. Those 354 urine samples contained pre and post shift urine voids and samples for workers at all mine locations: face, shop and surface seen in table 7.

Table 7. Summary of urine samples analyzed for OHPAHs

Urine Samples Analyzed	N
Total samples	354
Pre-shift	227
Post-shift	127
Face	218
Shop	80
Surface	56

The 354 samples were analyzed for OHPAH urinary metabolites and summary statistics were generated. The geometric mean of post-shift OHPAH metabolites for all miners is compared to

the U.S. general population OHPAH metabolite concentrations, seen in table 8. The geometric mean of the post-shift OHPAH metabolites for miners was between the 75th percentile for non-smokers in the US and the 90th percentile for smokers in the US. These results suggest that miners have elevated urinary OHPAH metabolite levels when compared to the US general population. It is assumed that the observed elevated metabolite levels are due to occupational exposure to DE.

Table 8. 2017 NHANES PAH metabolite concentrations for the general public (NHANES, 2017) compared to the miner's post-shift geometric mean urine concentrations detected via fluorescence

Metabolite	50th percentile (non-smokers)	75th percentile (non-smokers)	90th percentile (non-smokers)	90th percentile (smokers)	Geometric Mean – Miners (post-shift)
2-naphthol (µg/L)	3.17	6.35	13.1	36.1	9.3
1-naphthol (µg/L)	1.09	2.31	5.4	34.6	80
2+9 hydroxyfluorene (ng/L)	352	677	1,315	5,940	741
2-hydroxyphenanthrene (ng/L)	52	93	170	334	222
3-hydroxyphenanthrene (ng/L)	47	88	165	517	101
1-hydroxypyrene (ng/L)	83	147	262	773	166

Although miners in this study had elevated OHPAH metabolite levels in comparison to the general public, they had lower OHPAH metabolite levels in comparison to several other occupationally exposed workers. Coke workers, asphalt pavers, aluminum plant workers, foundry workers, steel plant workers and other miners show more elevated urinary OHPAH metabolite levels.

Table 9. Various occupations' metabolite concentrations compared to the miners' geometric mean OHPAH urinary concentrations measured via HPLC/fluorescence detection (Strickland, 1996; Scheepers et al., 2002)

	n	1-OHP (µmol/mol creatinine)
Miners (pre-shift)	215	0.08
Miners (post-shift)	122	0.09
Miners at the face (post-shift)	72	0.11
Coke oven workers (post-shift)	44	2.5
Asphalt pavers (post-shift)	43	1.8
Aluminum plant workers (post-shift)	55	1.9 – 3.6
Foundry workers (exposed group)	16	0.11 (nonsmokers)
	20	0.42 (smokers)
Steel plant workers	40	0.02 – 0.89 (nonsmokers)
	44	0.15 – 3.57 (smokers)
Estonian miners		
Cigarettes/ day = 0	35	0.07
Cigarettes/ day = 1-19	39	0.11
Cigarettes/ day = 20+	26	0.16
Grilled Meat in the past day = yes	15	0.18
Grilled Meat in the past day = no	85	0.10
1 hour or longer in room with open fire in the last day = yes	13	0.21
1 hour or longer in room with open fire in the last day = no	87	0.10

The summary statistics for OHPAH urinary metabolites measured via HPLC with fluorescence detection and HPLC/MS/MS were stratified by worker location. For post-shift samples for the metabolites 2-naphthol, 1-naphthol, 2+9-hydroxyfluorene, 3-hydroxyphenanthrene and 1-hydroxypyrene, the geometric mean for the face locations displayed the largest urinary OHPAH concentrations overall and the surface locations displayed the lowest urinary OHPAH concentrations. The only metabolite where this relationship was not seen was in the metabolite 2-hydroxyphenanthrene.

The collected air samples show that workers in face locations experience the highest DE exposures and thus, these workers display the greatest OHPAH urinary concentrations. It is also known that workers in surface locations experience the lowest DE exposures and thus, surface workers display the lowest OHPAH urinary concentrations. The summary statistics displayed in table 10 reinforce these conclusions.

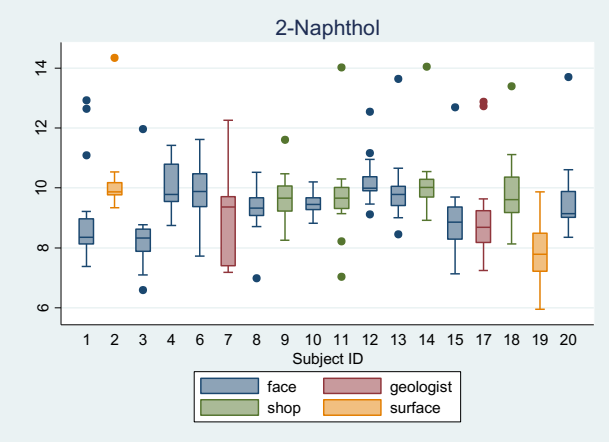
Table 10. Summary statistics for pre and post shift SG-adjusted OHPAH metabolite urinary concentrations by shift location

	All Locations	**Face**	**Shop**	**Surface**
	(n = 216)	(n =136)	(n =46)	(n =34)
2-Naphthol Pre-shift (pg/mL)				
Range	384 – 1,260,000	726 – 895,000	1,140 - 1,260,000	384 – 1,240,000
AM(ASD)	44,100 (156,000)	35,900 (118,000)	59,900 (204,000)	55,500 (212,000)
GM(GSD)	12,700 (3.4)	11,500 (3.1)	19,200 (2.9)	10,500 (4.7)
	(n =119)	(n =72)	(n =25)	(n =22)
2-Naphthol Post-shift (pg/mL)				
Range	937 – 1,710,000	1,260 – 413,000	3,420 – 110,000	937 – 1,710,000
AM(ASD)	40,700 (161,000)	33,200 (58,800)	21,400 (23,100)	87,500 (362,000)
GM(GSD)	13,500 (3.7)	15,200 (3.6)	14,700 (2.3)	8,170 (5.1)
	(n =135)	(n =88)	(n =33)	(n =14)
1-Naphthol Pre-shift (pg/mL)				
Range	61.6 – 2,810,000	61.6 – 2,810,000	21,600 – 963,000	1,820 – 1,320,000
AM(ASD)	243,000 (426,000)	248,000 (492,000)	263,000 (227,000)	162,000 (337,000)
GM(GSD)	89,200 (5.5)	71,200 (6.7)	189,000 (2.4)	62,7000 (4.1)
	(n =83)	(n =53)	(n = 16)	(n = 14)
1-Naphthol Post- shift (pg/mL)				
Range	308 – 18,700,000	631 - 18,700,000	13,100 – 1,040,000	308 – 403,000
AM(ASD)	746,000 (2,670,000)	1,100,000 (3,290,000)	143,000 (251,000)	87,500 (107,000)
GM(GSD)	110,000 (5.9)	174, 000 (5.8)	60,300 (3.7)	38,400 (5.7)

	(n =207)	(n =128)	(n =43)	(n =36)
2+9 Hydroxy-Fluorene Pre-shift (pg/mL)				
Range	48.4 – 14,000	48.4 – 14,000	115 – 6,580	90.6 – 6,680
AM(ASD)	1,740 (1,980)	1,590 (2,100)	1,990 (1,560)	1,980 (1,990)
GM(GSD)	1,080 (2.7)	981 (2.6)	1,300 (2.9)	1,200 (2.8)
	(n =125)	(n =75)	(n =25)	(n =25)
2+9 Hydroxy-Fluorene Post-shift (pg/mL)				
Range	22.7 – 15,800	22.7 – 15,800	39.6 – 14,800	103 – 4,390
AM(ASD)	1,920 (2,650)	1,900 (2,580)	2,790 (3,630)	1,120 (1,040)
GM(GSD)	1,050 (3.1)	1,120 (2.9)	1,110 (4.8)	801 (2.4)
	(n =178)	(n =109)	(n =40)	(n =29)
2-Hydroxy-Phenanthrene Pre-Shift (pg/mL)				
Range	9.87 – 5,010	9.87 – 2,190	28.5 – 5,010	15.1 – 3,590
AM(ASD)	640 (733)	438 (4433)	902 (906)	1,040 (1,050)
GM(GSD)	345 (3.4)	275 (2.8)	478 (3.9)	510 (4.1)
	(n =95)	(n =58)	(n =17)	(n =20)
2-Hydroxy-Phenanthrene Post-Shift (pg/mL)				
Range	25.7 – 8,930	38.2 – 8,930	25.7 – 7,490	49.9 – 2,130
AM(ASD)	680 (1,300)	579 (1,240)	1,220 (1,910)	510 (567)
GM(GSD)	318 (3.2)	288 (2.9)	423 (5.2)	331 (2.6)
	(n =179)	(n =112)	(n =39)	(n =28)
3-Hydroxy-Phenanthrene Pre-shift (pg/mL)				
Range	2.86 – 5,980	9.87 – 2,280	2.86 – 3,650	15.1 – 5,980
AM(ASD)	300 (718)	153 (230)	425 (660)	710 (1,510)
GM(GSD)	132 (3.2)	99.6 (2.5)	195 (3.9)	236 (4.0)
	(n =96)	(n =56)	(n =19)	(n =21)
3-Hydroxy-Phenanthrene Post-shift (pg/mL)				
Range	11.6 – 1,580	11.6 – 1,580	22.4 - 931	30.5 - 590
AM(ASD)	212 (217)	231 (239)	215 (220)	158 (134)
GM(GSD)	143 (2.5)	157 (2.5)	134 (2.9)	117 (2.2)

		(n =215)	(n =137)	(n =43)	(n =35)
1-Hydroxypyrene (Fluorescence Detection) Pre-shift (pg/mL)					
	Range	10.0 – 1,120	43.4 – 1,120	10.0 – 1,020	23.2 - 682
	AM(ASD)	277 (194)	265 (185)	338 (223)	252 (180)
	GM(GSD)	220 (2.1)	219 (1.8)	257 (2.4)	184 (2.4)
		(n =122)	(n =72)	(n =25)	(n = 25)
1-Hydroxypyrene Post-shift (Fluorescence Detection) (pg/mL)					
	Range	19.4 – 3,170	47.5 – 3,170	52.6 – 1,690	19.4 - 504
	AM(ASD)	387 (448)	480 (503)	358 (406)	152 (121)
	GM(GSD)	234 (2.8)	309 (2.7)	215 (2.8)	116 (2.2)
		(n =23)	(n =15)	(n = 5)	(n =3)
1-Hydroxypyrene (Mass Spectrometry) Pre-shift (pg/mL)					
	Range	12.2 - 283	37.2 - 283	20.9 - 194	12.2 - 202
	AM(ASD)	113 (72.7)	108 (72.4)	118 (71.0)	130 (103)
	GM(GSD)	88.3 (2.2)	89.8 (1.8)	92.3 (2.5)	75.6 (4.9)
		(n =99)	(n =60)	(n =24)	(n =15)
1-Hydroxypyrene (Mass Spectrometry) Post-shift (pg/mL)					
	Range	1.6 – 4,120	22.9 – 4,120	1.6 – 1,080	2.5 - 568
	AM(ASD)	392 (594)	491 (718)	311 (299)	127 (146)
	GM(GSD)	168 (4.2)	220 (3.7)	157 (4.6)	62.3 (4.2)

Each metabolite is examined by subject ID and worker location, seen in figure 1 below. A wide range of metabolite concentrations are observed, but each metabolite tends to follow a similar pattern where, when stratified by location, the face locations display the largest urine OHPAH concentrations overall and the surface locations displayed the lowest urine OHPAH concentrations.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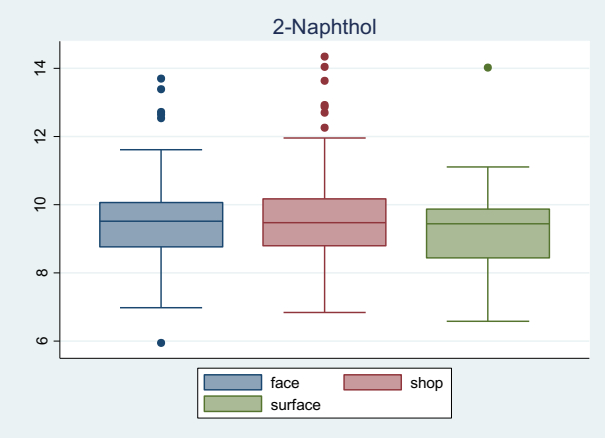
8
10
12
14
1 2 3 4 6 7 8 9 10 11 12 13 14 15 17 18 19 20
Subject ID
face
geologist
shop
surface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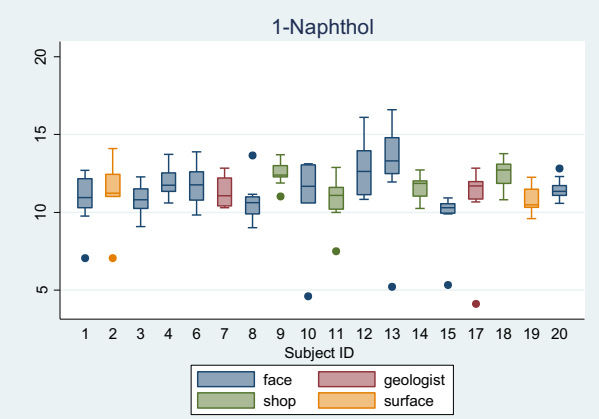
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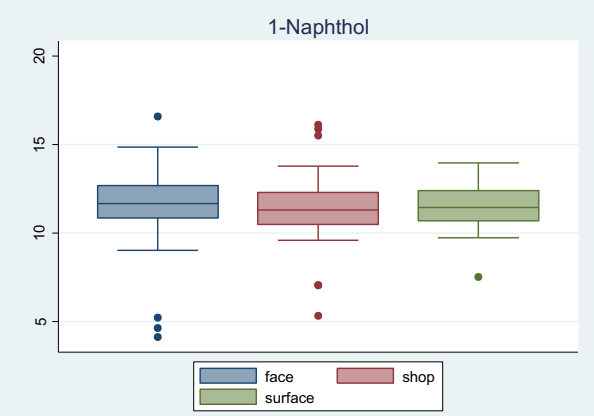
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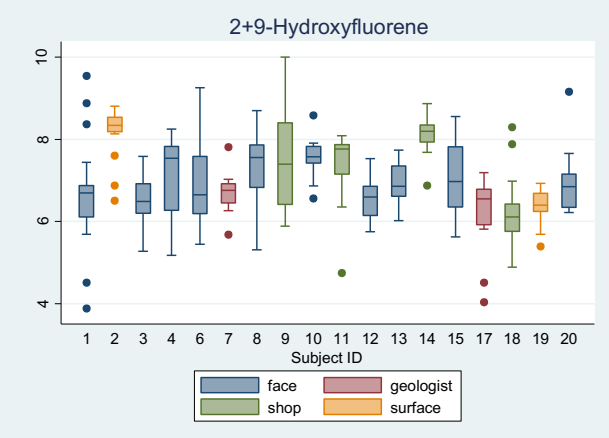
2+9-Hydroxyfluorene
4
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1 2 3 4 6 7 8 9 10 11 12 13 14 15 17 18 19 20
Subject ID
face
geologist
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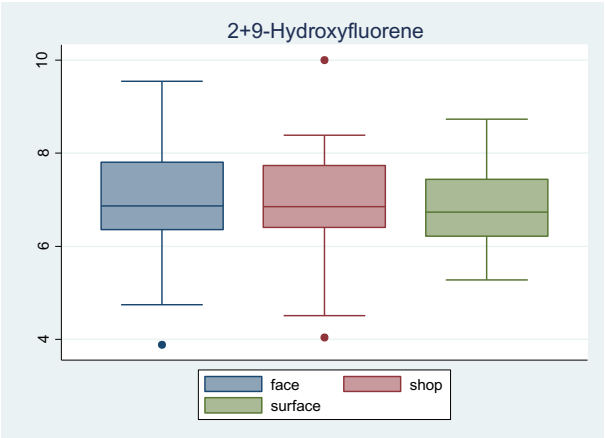
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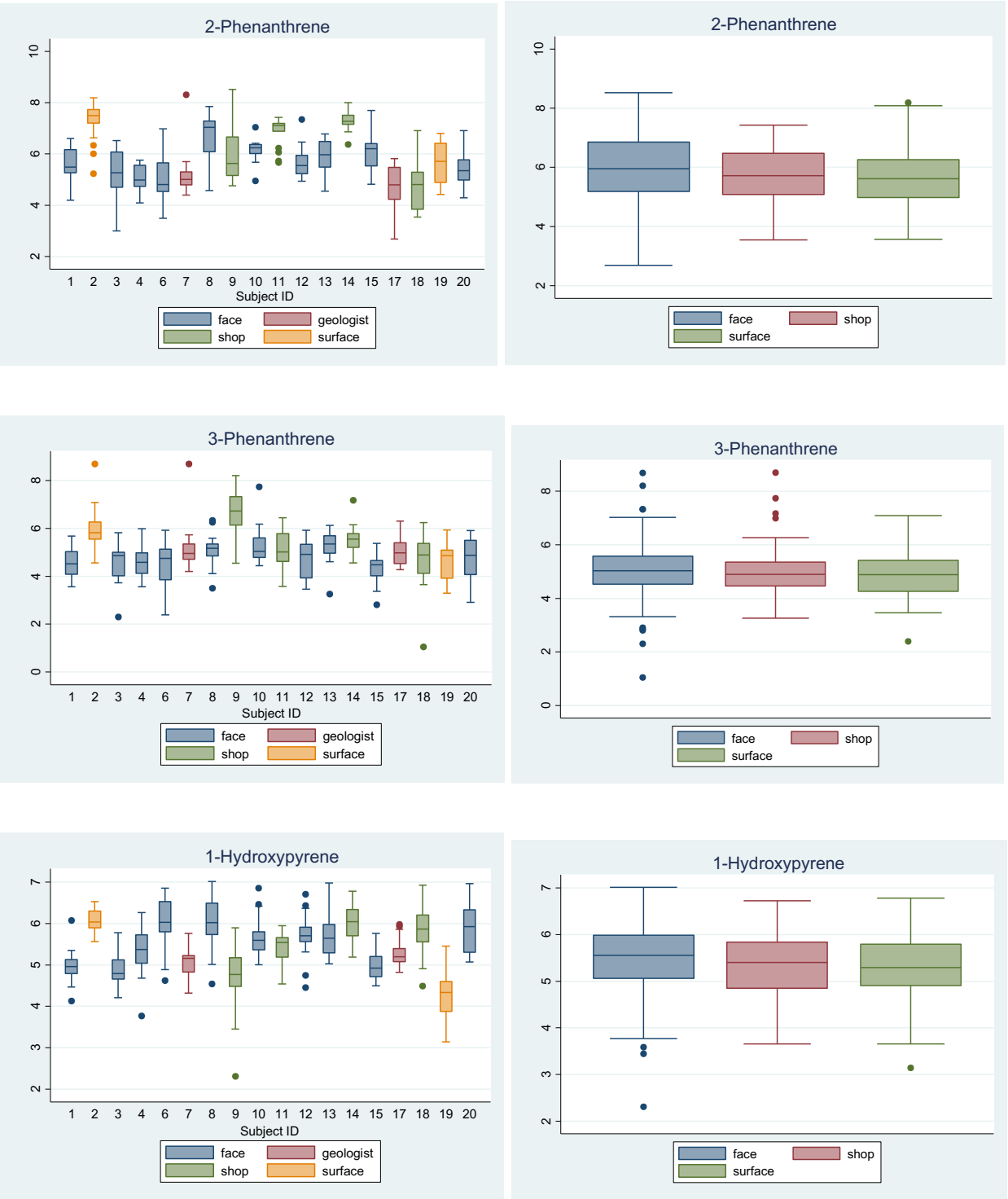

Figure 2. Distribution of natural log transformed SG adjusted OHPAH metabolites (measured via HPLC with fluorescence detection) by subject ID and job location

Air Data

Air concentrations for EC and 1-NP were log-normally distributed and thus, were natural log transformed for data analysis. Air data was obtained from two different sources.

1. EC data analyzed using NIOSH method 5040 by ALS Environmental laboratory in Salt Lake City, UT.
2. 1-NP extracted from quartz filters (the same filters that EC was captured on) and analyzed using HPLC/MS/MS (Carpenter, 2014).

Summary statistics for the subset of air samples with matching urinary OHPAH concentrations shows that workers in face locations had the highest exposures compared to surface workers who experience the lowest exposures for both 1-NP and EC, a similar pattern to the one seen in OHPAH urinary metabolites measured via HPLC with fluorescence detection.

Table 11. Summary statistics for the subset of 1-NP in air and EC in air samples by job location with matching urinary OHPAH concentrations

	All Locations	**Face**	**Shop**	**Surface**
	(n = 100)	(n = 59)	(n = 24)	(n = 17)
Elemental Carbon (µg/m^3)				
Range	1.04 – 101	2.4 - 101	1.3 – 25.1	1.04 – 44.9
AM(ASD)	11.4 (12.6)	14.9 (14.1)	6.3 (5.3)	6.8 (11.0)
GM(GSD)	7.5 (2.6)	11.5 (2.0)	4.8 (2.1)	3.3 (3.1)
	(n = 82)	(n = 49)	(n = 19)	(n = 14)
1-Nitropyrene (pg/m^3)				
Range	< 16.3 – 392	< 16.3 – 373	4.7 - 392	0.06 – 115
AM(ASD)	69.2 (80.6)	88.3 (81.9)	52.7 (86.9)	24.7 (36.2)
GM(GSD)	33.1 (5.2)	66.2 (2.2)	26.9 (3.1)	4.1 (12.7)

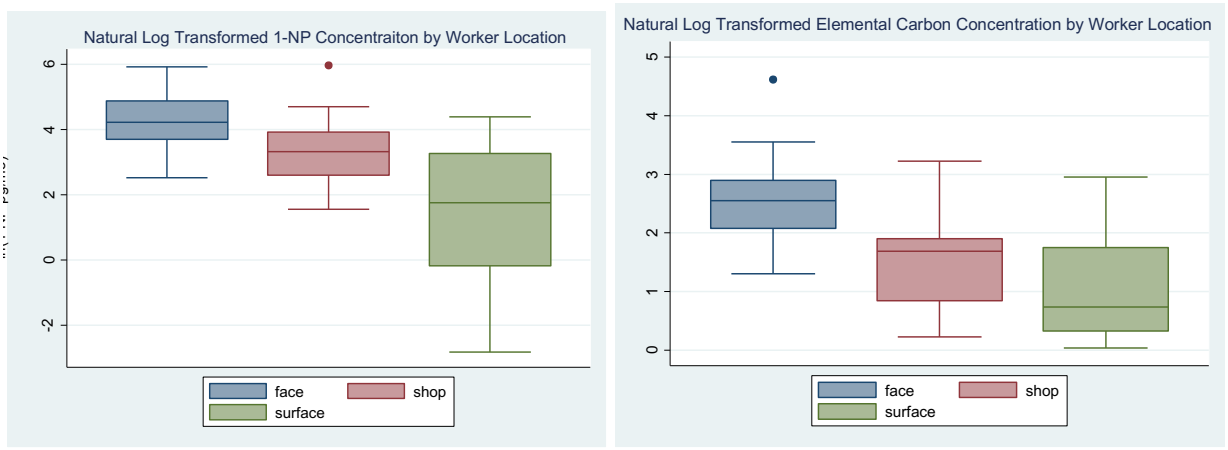

Figure 3. Distribution of natural log transformed 1-NP in air and EC in air by job location

Aim 1 – Air Data Compared to OHPAH Urinary Data

The association between post-shift OHPAH metabolites and exposure to EC and 1-NP air for samples collected on the same day was examined ($n < 33$ was observed for 5 of the 6 metabolites examined). 1-OHP had more samples than the other OHPAH metabolites because of combined data set from samples measured via HPLC with fluorescence and HPLC/MS/MS (n=100). The other metabolites only have data measured via HPLC with fluorescence detection. Table 12 displays the mixed model results comparing OHPAH urinary metabolite and EC. A positive relationship is observed between 1-OHP and ECOn average, as EC air concentration doubles, 1-OHP urinary metabolite concentration increases by 37%. This relationship is statistically significant at $p < 0.05$ level ($p = 0.001$). Additionally, a statistically significant association was observed between 2+ 9 hydroxyfluorene and EC, with a 33% increase in 2+ 9 hydroxyfluorene observed for every doubling of EC concentration. Although 5 of the 6 metabolites measured had relatively small n values, the results still display that on average as EC increases, all metabolite concentrations increase (even though only two metabolites displayed statistically significant increases). The results also suggests that occupational exposures to the parent compounds

naphthalene, pyrene and fluorene are greater than occupational exposure to phenanthrene because the derived urinary metabolites from phenanthrene increase a smaller magnitude as EC concentration increases.

Table 12. Results for the mixed effects model of association between natural log-transformed specific gravity adjusted OHPAH urinary metabolite concentrations (pg/mL) and natural log-transformed EC in air (µg/m^3)

	N	**Percent Increase in Concentration**	**p**	**95 % CI**	
Predicted from Doubling Elemental Carbon Air Concentrations					
1-Hydroxypyrene	100	37%	0.001	14%	64%
2-Naphthol	32	28%	0.11	-6%	75%
1-Naphthol	18	39%	0.36	-45%	182%
2+9-Hydroxy-Fluorene	32	33%	0.02	6%	66%
2-Hydroxy-Phenanthrene	24	12%	0.49	-22%	52%
3-Hydroxy-Phenanthrene	26	10%	0.35	-11%	37%

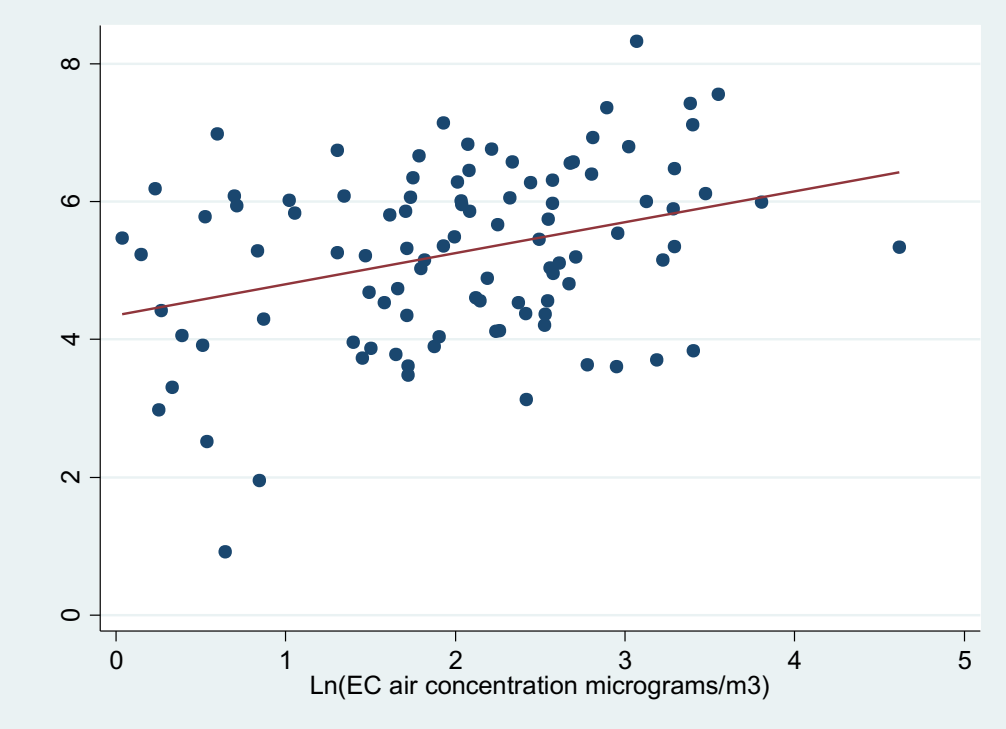

Figure 4. Scatter plot of Ln (EC in air concentration µg/m^3) vs. SG adjusted Ln (1-OHP urine concentration pg/mL)

Using the mixed model described previously, the relationship between post-shift OHPAH metabolite concentrations and exposure to 1-NP samples collected on the same day was

examined. Similar to the comparison made with EC, n < 15 was observed for 5 of the 6 metabolites examined, because only urine concentrations measured via HPLC with fluorescence detection were examined. . 1-OHP had more samples than the other OHPAH metabolites because of combined data set from samples measured via HPLC with fluorescence and HPLC/MS/MS (n=81). Table 13 displays the mixed model results comparing the OHPAH urinary metabolite and 1-NP in air. A positive relationship is observed between 1-OHP and 1-NP.On average, as 1-NP air concentration doubles, 1-OHP urinary metabolite concentrations increase by 15%. This relationship is statistically significant at $p < 0.05$ level ($p = 0.02$). Although 5 of the 6 metabolites measured had small n values (n<15), the results still display that on average as 1-NP increases, all urinary metabolite concentrations increase. These results also suggest that occupational exposures to the parent compounds naphthalene and pyrene, are greater than exposure to fluorene and phenanthrene because the urinary metabolites derived from fluorene and phenanthrene increase less as 1-NP concentration increases.

Table 13. Results for the mixed effects model of association between natural log-transformed specific gravity adjusted OHPAH urinary metabolite concentration (pg/mL) and natural log-transformed air 1-NP in air (pg/m^3)

	n	**Percent Increase in Concentration**	**p**	**95 % CI**	
Predicted from Doubling 1-NP Air Concentrations					
1-Hydroxypyrene	81	15%	0.02	2%	28%
2-Naphthol	14	16%	0.11	-4%	39%
1-Naphthol	7	13%	0.37	-15%	45%
2+9-Hydroxy-Fluorene	14	1%	0.90	-16%	18%
2-Hydroxy-Phenanthrene	10	<1%	0.93	-10%	11%
3-Hydroxy-Phenanthrene	12	<1%	0.93	-9%	10%

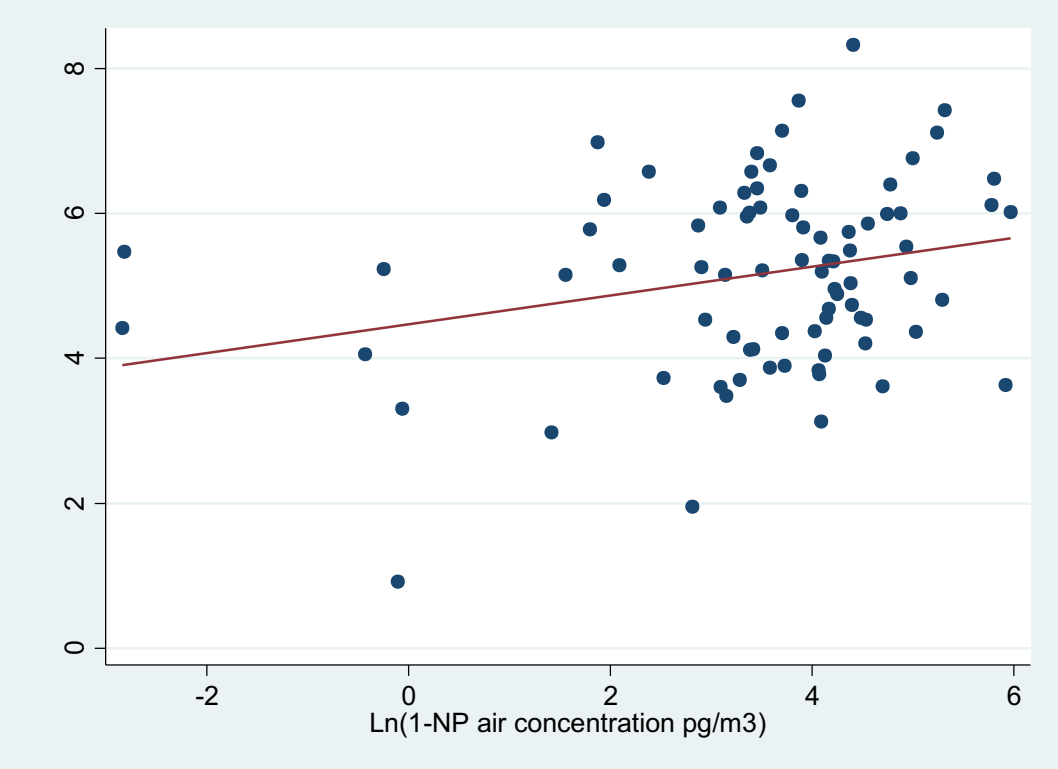

Figure 5. Scatter plot of Ln(1-NP in air concentration pg/m^3) vs. SG adjusted Ln (1-OHP urine concentration pg/mL)

Aim 2 - Comparison of Urinary PAHs to Urinary 1-NP metabolites

A subset of the miners' urine samples have previously been analyzed for the specific OHNP metabolites of 6-OHNP and 8-OHNP (Ramsay, 2014). Similar to the OHPAH data and air data, the face location had the largest geometric mean for post-shift urinary concentrations for 6-OHNP and 8-OHNP, and the surface locations had the smallest geometric mean for post-shift urinary concentrations. Additionally, the post shift urine concentrations were greater than the pre-shift urine concentrations for all locations except the surface (Ramsay, 2014).

As described previously, a mixed model was used to evaluate the association between OHPAH metabolites and OHNP urinary metabolites in SG corrected post-shift urine samples. Similar to the comparison made with air data, $n < 13$ was observed for 5 of the 6 metabolites because only urine concentrations measured via HPLC with fluorescence detection were examined. 1-OHP

had more samples than the other OHPAH metabolites because of combined data set from samples measured via HPLC with fluorescence and HPLC/MS/MS (n=104).. Table 14 displays the mixed model results comparing the urinary metabolites OHPAH and 6-OHNP.A positive relationship is observed between 1-OHP and 6-OHNP. On average, as 6-OHNP urinary concentration doubles, 1-OHP urinary metabolite concentrations increase by 46%. This relationship is statistically significant at $p < 0.05$ level ($p < 0.001$). Four of the six urinary PAH metabolites examined display a negative relationship with urinary 6-OHNP. These observations are most likely due to chance because of the small observed n.

Table 14. Results for the mixed effects model of association between log-transformed specific gravity adjusted OHPAH and 6-OHNP urinary metabolites

	n	**Percent Change in Concentration**	**p**	**95 % CI**	
Predicted from Doubling 6-OHNP Urinary Concentrations					
1-Hydroxypyrene	104	46%	< 0.001	24%	74%
2-Naphthol	11	38%	0.19	-17%	114%
1-Naphthol	5	-54%	0.23	-200%	31%
2+9-Hydroxy-Fluorene	12	-38%	0.16	-114%	14%
2-Hydroxy-Phenanthrene	10	-65%	0.014	-144%	-11%
3-Hydroxy-Phenanthrene	11	-13%	0.56	-72%	34%

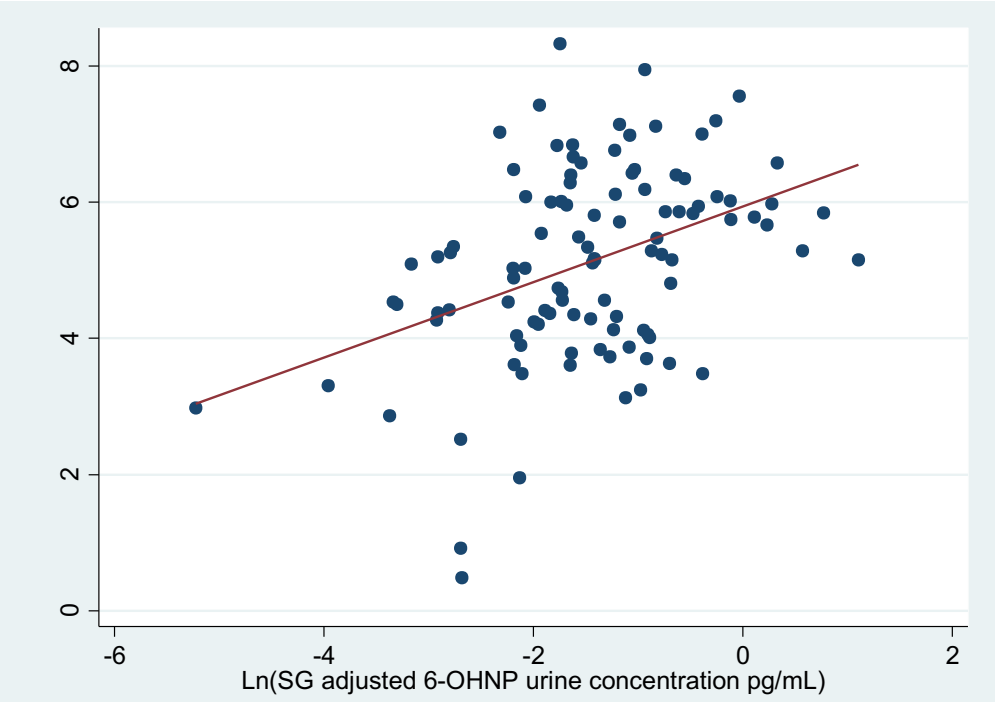

Figure 6. Scatter plot of SG adjusted Ln(6-OHNP in urine concentration pg/mL) vs. SG adjusted Ln (1-OHP urine concentration pg/mL)

Table 15 displays the mixed model results comparing the OHPAH urinary metabolites and 8-OHNP. Similar to the previous test, a small n was observed for 5 of the 6 metabolites because only urine concentrations measured via HPLC with fluorescence detection were examined. 1-OHP had more samples than the other OHPAH metabolites because of combined data set from samples measured via HPLC with fluorescence and HPLC/MS/MS (n=104).A positive relationship is observed between 1-OHP and 8-OHNP.On average, as 8-OHNP urinary concentration doubles, 1-OHP urinary metabolite concentrations increase by 51%. This relationship is statistically significant at $p < 0.05$ level ($p < 0.001$). Four of the six urinary PAH metabolites examined display a negative relationship with urinary 8-OHNP. These observations are most likely due to chance because of the small observed n.

Table 15. Results for the mixed effects model of association between log-transformed specific gravity adjusted OHPAH and 8-OHNP urinary metabolites

	n	Percent Change in Concentration	P	95 % CI	
Predicted from Doubling 8-OHNP Urinary Concentrations					
1-Hydroxypyrene	104	51%	< 0.001	26%	79%
2-Naphthol	11	13%	0.62	-42%	82%
1-Naphthol	5	-72%	0.19	-300%	31%
2+9-Hydroxy-Fluorene	12	-33%	0.23	-114%	20%
2-Hydroxy-Phenanthrene	10	-79%	0.007	-183%	-17%
3-Hydroxy-Phenanthrene	11	-16%	0.44	-69%	26%

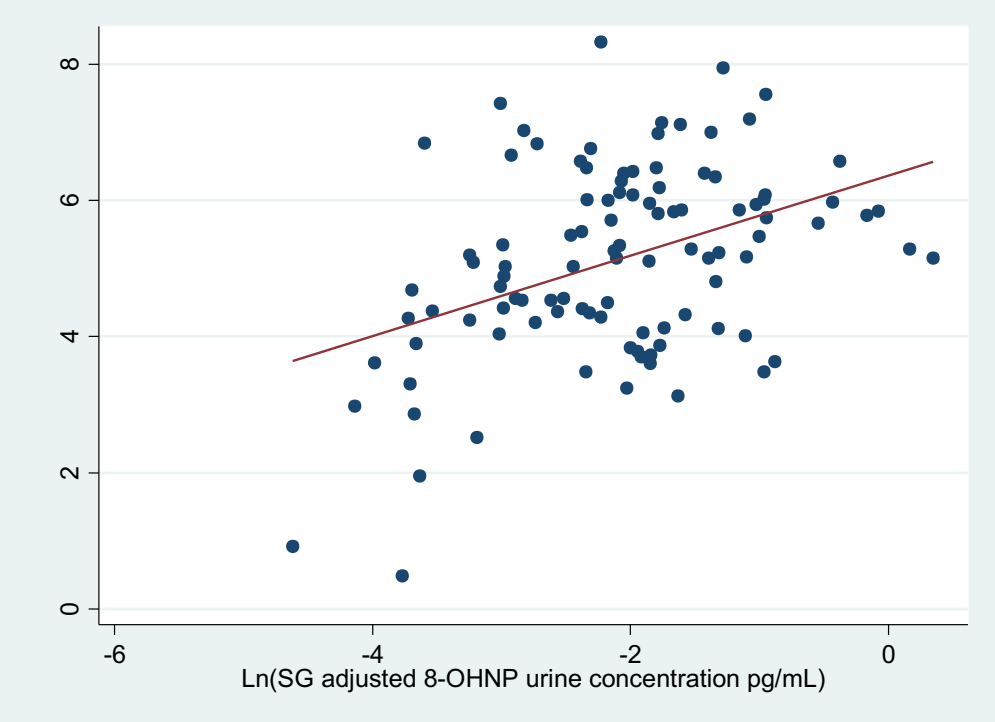

Figure 7. Scatter plot of SG adjusted Ln(8-OHNP in urine concentration pg/mL) vs. SG adjusted Ln (1-OHP urine concentration pg/mL)

Aim 3 - Temporal Variation in Exposures

In aim 3, temporal variations in urinary concentrations of the six OHPAH metabolites measured via HPLC with fluorescence detection were evaluated. Unlike the previous analyses, for these analyses the 1-OHP data measured via HPLC/MS/MS was not included. This was completed using two different statistical tests:

1. Evaluating the statistical significance of daily post-shift urinary concentrations/pre-shift urinary concentrations. If workers' daily exposure contributes to higher OHPAH metabolite levels, then the ratio of post-shift urinary concentrations/pre-shift urinary concentrations collected on the same day will be > 1.
2. Evaluating the statistical significance of post-shift urinary concentrations by day of the week. If daily exposures contribute to higher OHPAH metabolite levels and accumulate over the week, then day 2, day 3 and day 4 OHPAH urinary concentrations will be greater than day 1 OHPAH urinary concentrations.

The post-shift/pre-shift OHPAH metabolite ratios were evaluated using the mixed model, as described previously. As seen in table 16, the average daily cross shift ratio for all six OHPAH metabolites examined is greater than zero on the natural log scale. This means that the post-shift urinary OHPAH concentration is, on average, greater than the pre-shift urinary OHPAH concentration for samples collected on the same day. Although this relationship is observed on average, the only metabolite where the post-shift urinary OHPAH concentration divided by the pre-shift urinary OHPAH concentration is statistically significant at $p < 0.05$ level is 1-OHP ($p = 0.02$). For 1-OHP, the post-shift urinary concentration is on average 21% greater than the pre-shift urinary concentration (95% CI: 3% - 40%).

Table 16. Daily cross shift ratio (post/pre) of natural log transformed specific gravity adjusted OHPAH metabolites

	Percentage increase of post compared to pre	p	95 % CI	
2-Naphthol (n = 105)	26%	0.12	-6%	68%
1-Naphthol (n = 50)	51%	0.19	-23%	180%
2+9-Hydroxy-Fluorene (n = 110)	11%	0.40	-14%	39%
2-Hydroxy-Phenanthrene (n = 70)	2%	0.93	-36%	42%
3-Hydroxy-Phenanthrene (n = 72)	19%	0.29	-15%	63%
1-Hydroxypyrene (n = 110)	21%	0.02	3%	40%

The statistically significant daily cross shift ratio for 1-OHP was further broken down by worker location, seen in table 17. For face locations, the daily post-shift urinary OHPAH concentrations are greater than pre-shift urinary OHPAH concentrations by 51% for measurements collected on the same day ($p < 0.001$). The cross shift ratio for shop workers is > 0, but less than the cross shift ratio for face workers. For shop locations, the daily post-shift urinary OHPAH concentrations are greater than pre-shift urinary OHPAH concentrations by 4.1% for measurements collected on the same day ($p = 0.06$). The cross shift ratio for surface workers is < 0, meaning that on average pre-shift urine concentrations were larger than post-shift urine concentrations for urinary samples collected on the same day ($p < 0.001$).

Table 17. Daily cross shift ratio (post/pre) of natural log transformed specific gravity adjusted 1-OHP stratified by worker location

1-Hydroxypyrene	Percentage change post/pre	P
Face (n = 66)	51%	< 0.001
Shop (n = 22)	4.1%	0.06
Surface (n = 22)	-38%	< 0.001

Post-shift urinary concentrations were then evaluated to determine if sample concentrations varied by day of the week. As previously stated, if worker daily exposures contribute to higher OHPAH metabolite levels and accumulate over the week, then day 2, day 3 and day 4 OHPAH urinary concentrations will be greater than day 1 OHPAH urinary concentrations.

From the summary statistics seen in table 18, only the metabolites 2-naphthol and 2+9-hydroxyfluorene, display larger geometric means for urinary concentrations after day 1. In fact, contrary to the hypothesis that subsequent days following day 1 would have the higher OHPAH urinary concentrations, the metabolites 2-hydroxyphenanthrene and 1-hydroxypyrene display the highest geometric mean for urinary concentrations on day 1.

Table 18. Specific gravity adjusted post -shift OHPAH metabolite concentrations by day of the week

		All Days	Day 1	Day 2	Day 3	Day 4
		(n =119)	(n =26)	(n =28)	(n =33)	(n=32)
2-Naphthol (pg/mL)						
	Range	937 – 1,710,000	937 – 82,600	1,270 – 281,000	1,130 – 91,600	1,720 – 1,710,000
	AM(ASD)	40,700 (161,000)	18,900 (19,600)	27,100 (51,800)	23,800 (23,200)	87,900 (304,000)
	GM(GSD)	13,500 (3.7)	11,200 (3.1)	13,300 (3.2)	12,500 (3.7)	17,000 (4.6)
		(n =80)	(n =21)	(n =17)	(n =22)	(n=20)
1-Naphthol (pg/mL)						
	Range	308 – 18,900,000	13,100 – 2,240,000	13,300 – 521,000	7,340 - 18,900,000	308 - 12,400,000
	AM(ASD)	773,000 (2,710,000)	279,000 (483,000)	140,000 (127,000)	1,650,000 (4,390,000)	864,000 (2,740,000)
	GM(GSD)	115,000 (6.0)	114,000 (4.0)	96,900 (2.5)	178,000 (7.0)	81,300 (11.7)
		(n =125)	(n =27)	(n =29)	(n =35)	(n=34)
2+9 Hydroxy-Fluorene (pg/mL)						
	Range	22.7 – 15,800	39.6 – 15,800	22.6 – 6,740	103 – 14,800	167 – 8,090
	AM(ASD)	1,920 (2,650)	2,500 (3,750)	1,320 (1,480)	2,210 (3,050)	1,680 (1,770)
	GM(GSD)	1,050 (3.1)	1,080 (4.2)	782 (3.2)	1,250 (2.9)	1,090 (2.6)
		(n =94)	(n =19)	(n =24)	(n =23)	(n=28)

2-Hydroxy-Phenanthrene (pg/mL)						
	Range	31.5 – 8,930	31.5 – 8,930	38.2 – 2,530	79.2 – 7,490	49.9 – 2,130
	AM(ASD)	687 (1,300)	1,160 (2,070)	393 (509)	891 (1,630)	454 (509)
	GM(GSD)	326 (3.1)	426 (4.2)	233 (2.9)	419 (3.0)	296 (2.5)
		(n =95)	(n =57)	(n =19)	(n =19)	(n=56)
3-Hydroxy-Phenanthrene (pg/mL)						
	Range	11.6 – 1,580	22.4 – 1,580	11.6 - 604	30.8 - 931	23.6 - 392
	AM(ASD)	214 (217)	262 (343)	159 (137)	281 (217)	160 (110)
	GM(GSD)	145 (2.5)	159 (2.7)	114 (2.5)	205 (2.4)	119 (2.3)
		(n =122)	(n =27)	(n =29)	(n =33)	(n =33)
1-Hydroxypyrene (pg/mL)						
	Range	19.4 – 3,170	34.1 – 3,170	47.5 – 1,040	19.4 – 1,690	49.4 – 1,020
	AM(ASD)	387 (448)	502 (718)	323 (292)	437 (405)	301 (276)
	GM(GSD)	234 (2.8)	264 (3.0)	209 (2.7)	273 (2.9)	202 (2.5)

The variation in OHPAH concentrations by day of week was examined using mixed model described previously (table 19). 2-Naphthol and 2+9 hydroxyfluorene are the only metabolites where the geometric mean of post-shift urinary OHPAH metabolite concentrations were higher on day 4 compared day 1 of the workweek. However, it is important to note that OHPAH urinary concentration increases on subsequent days following day 1 were not statistically significant for any metabolite examined, seen in table 19.

Table 19. Post shift geometric mean of natural log-transformed specific gravity adjusted OHPAH metabolites by day of week

	Geomean urinary concentration	p	95% Confidence Interval	
2-Naphthol (n = 119)				
Intercept	10,900	0.0	6,630	18,000
Day 2	13,000	0.63	6,500	26,100
Day 3	12,200	0.76	6,250	23,900
Day 4	16,600	0.23	14,200	32,900
1-Naphthol (n = 83)				
Intercept	109,000	0.0	49,000	220,000
Day 2	98,700	0.87	32,900	297,000
Day 3	171,000	0.40	60,500	489,000
Day 4	81,600	0.59	26,900	236,000
2+9-Hydroxy-Fluorene (n = 125)				
Intercept	1,100	0.0	670	1,640
Day 2	1,500	0.30	440	2,040
Day 3	1,300	0.61	710	2,250
Day 4	1,100	0.96	630	1,980
2-Hydroxy-Phenanthrene (n = 95)				
Intercept	365	0.0	220	600
Day 2	230	0.18	120	460
Day 3	412	0.72	210	820
Day 4	293	0.51	150	570
3-Hydroxy-Phenanthrene (n = 96)				
Intercept	148	0.0	100	220
Day 2	116	0.36	70	200
Day 3	209	0.20	120	350
Day 4	122	0.46	70	2210
1-Hydroxypyrene (n = 122)				
Intercept	270	0.0	180	400
Day 2	215	0.39	130	370
Day 3	279	0.91	170	470
Day 4	206	0.31	120	350

CHAPTER 4: DISCUSSION

The geometric mean for the miners' OHPAH urinary biomarker concentrations of all metabolites examined in this study are greater than the 75th percentile for the US general public (non-smokers). In the metabolites, 1-naphthol and 2-hydroxyphenanthrene, the geometric mean for the miners' urinary biomarker concentrations are greater than the 90th percentile for the US general public (non-smokers). Thus, mine workers have elevated PAH exposure in comparison to the general public and that exposure is reflected in their elevated urinary OHPAH concentrations. Although this cohort has elevated urinary OHPAH concentrations compared to the general public, when compared to other occupations that are exposed to DE, the miners' OHPAH urinary concentrations are lower. This is most likely due to the extensive control methods used in this mine to reduce worker exposure. These control methods include vehicle exhaust filters, a substantial ventilation system, worker personal protective equipment and the use of biodiesel. In fact, in the subset of EC air samples used for statistical testing (n = 100), the highest measured EC concentration was 101 $\mu g/m^3$, which well below MSHA's compliance standard (160 $\mu g/m^3$ for TC and 176 $\mu g/m^3$ for EC after an error factor is applied).

It is known that these miners experience occupational exposures to PAHs, reflected in their elevated urinary OHPAH concentrations. Due to the increase of EC concentrations at mine locations where diesel vehicles and equipment are utilized frequently, a portion of the observed elevated urinary OHPAH concentrations is likely due to DE exposure. This assumption is reinforced by the associative relationship between the urinary biomarker 1-OHP and 1-NP in air and OHNPs in urine. Since 1-OHP in urine has an associative relationship with these diesel specific nitro-PAHs, it shows that as 1-NP and OHNP increase, 1-OHP also increases. Thus, a portion of the increase in urinary 1-OHP is attributed to DE exposure. It is important to note that there is unexplained variability in these data sets. This variability can possibly be explained by

differences in metabolism between study subjects. There are observed differences in metabolism between genders and tobacco users. Tobacco use can upregulate the enzymes which metabolize PAHs and thus result in higher urinary OHPAH concentrations.

1-OHP was also associated with EC. As previously stated, EC is used as a surrogate for DE exposure and in combination with total carbon (TC), is used for the determination of compliance with the Mine Safety and Health Administration PEL. This insight suggests that with continued research, urinary 1-OHP could potentially be used as a surrogate measure for EC. Again, there is unexplained variability which could be explained by the aforementioned differences in metabolism.

A general increase in OHPAH urinary concentrations for any day relative to day 1 was not observed. This means that OHPAH metabolites in urine are eliminated within 24 hours (time between the post-shift urine voids compared). Wide ranges of half-lives for OHPAHs have been previously reported, but the average is approximately 12 hours (ACGIH, 2017). Because miners in the cohort work 12-hour shifts, with an OHPAH urinary half-life approximately equal to 12 hours, the post shift urinary void should be representative of that same day's DE exposure. For the metabolite 1-OHP, the daily increase in metabolite concentration was statistically significant and especially high in workers at the mine face where the highest DE exposures occur. This reinforces that throughout the day, workers are exposed to DE, which results in higher post shift urinary OHPAH concentrations.

Although the measured urinary OHPAH concentrations were relatively low compared to other occupations and the ACGIH BEI of 2.5 µg/L, these concentrations can still be used to determine if effective control measures are in place. The Benchmark Guidance Value (BGV) sets achievable biomarker concentrations at the 90^{th} percentile of available biomonitoring results

from a representative workplace with good occupational hygiene practices (HSE, 1997). If a measured urinary value exceeds the BGV, this suggests exposure controls may not be adequate and should be improved (HSE, 1997). Thus, this mine would be able to determine which job tasks or mine locations need to improve exposure controls because of observed OHPAH urine samples exceeding the BGV. This is useful information for reducing worker exposure by improving exposure control methods.

Limitations

There are some limitations to note in this study. Firstly, it was not time or economically feasible to collect air samples for all miners on all days throughout each sampling campaign. Therefore, samples were only collected for 2 of the 4 days for each miner during each sampling campaign. Having more extensive air samples would have contributed additional, valuable information to the data set.

Secondly, the cohort only included 18 subjects. A larger sample size would have also given us additional information, but again, this was not economically feasible. We had planned on a cohort of 20 subjects, but cohort drop out was an additional issue that was encountered. During the first campaign, two cohort members had switched shifts to the night shift and thus, were no longer able to be sampled for the duration of the project. Additionally, events such as worker vacations, sick leave and family leave all contributed to the loss of samples.

Thirdly, this was a relatively clean mine. Occupational exposures to DE are very low compared to other mines. Higher exposures would have contributed more information regarding the relationship between exposure and dose. Also, because this mine was so clean, results from this study cannot be generalized to other mines where higher DE exposures occur.

Also, a proportion of the samples were below the limit of detection and adjustments needed to be made for them to be included in our data set. These adjustments will not represent the exact values of exposure concentrations, but will instead be close estimates.

Lastly, there was a lack of OHPAH data from HPLC/ fluorescence detection that corresponded with air data and OHNP urinary data. Because of this, 5 of the 6 metabolites had a very small number of samples for the statistical tests. To make it possible to compare 1-OHP to air data and OHNP urinary data, 1-OHP data from HPLC/ fluorescence detection and 1-OHP data from HPLC/MS/MS were combined into one dataset. 1-OHP data detected via mass spectrometry was adjusted for data combination. That adjustment may not represent the exact value of those urinary concentrations, but instead represent estimates of sample concentrations.

CHAPTER 5: CONCLUSIONS

Of the six OHPAH urinary metabolites examined in this study, it appears that 1-OHP has the greatest potential to be established as a reliable surrogate for DE exposure. When comparing OHPAH urinary metabolites to air exposure concentrations, 1-OHP displayed a positive association that was statistically significant to EC and 1-NP. And when comparing OHPAH urinary metabolites to OHNP urinary metabolites, 1-OHP was the only metabolite that displayed an association. Although 1-OHP has the greatest potential to be established as a reliable surrogate for DE, the variability in these relationships need to be further understood before this can occur.

When comparing daily cross shift OHPAH urinary metabolites, all OHPAH metabolites increased across the work shift, however this increase was only statistically significant for 1-OHP. No association between OHPAH metabolites in post-shift urine samples and day of week was observed. Consistent with reported urinary elimination half-lives for the OHPAH metabolites, this indicates that OHPAH metabolite concentrations do not accumulate across the workweek.

In conclusion, of the six different OHPAH urinary metabolites examined, 1-OHP was the most promising for the prediction of worker exposure to DE. In future research, the collection of more paired air in urine will aid in determining sources of variability in the exposure-biomarker relationship. In addition, differences in metabolism should also be studied to determine how this contributes to variability within the data. Doing these things would help to determine the generalizability and usability of 1-OHP as an exposure biomarker to DE in varying work environments under assorted working conditions.

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Appendix I. - Supplemental Table and Figures

Table 1. Data flags, flag meaning and the associated action taken

Data Flagging			
Symbol	**Meaning**	**Action**	**Flag code**
<IS	<25% expected ITSD	delete value/censor value cell	1
>IS	>125% ITSD	delete value/censor value cell	2
NS	ISTD Missing	delete value/censor value cell	3
ND	Compound not detected at 3 times S/N	add flag code, no change to value	4
AI	Area not valid ˆ Interference	delete value/censor value cell	5
N>	Conc is > UQL	add flag code, no change to value	6
RT	Unclear retention time	add flag code, no change to value	7

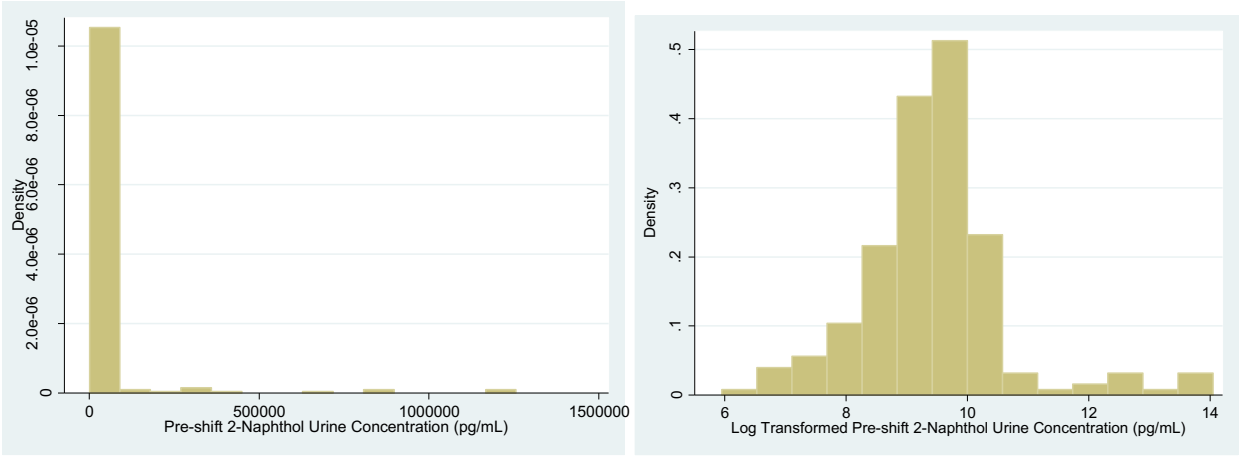


Figure 1. Specific gravity adjusted pre-shift 2-naphthol urine concentrations compared to ln transformed specific gravity adjusted pre-shift 2-naphthol urine concentrations

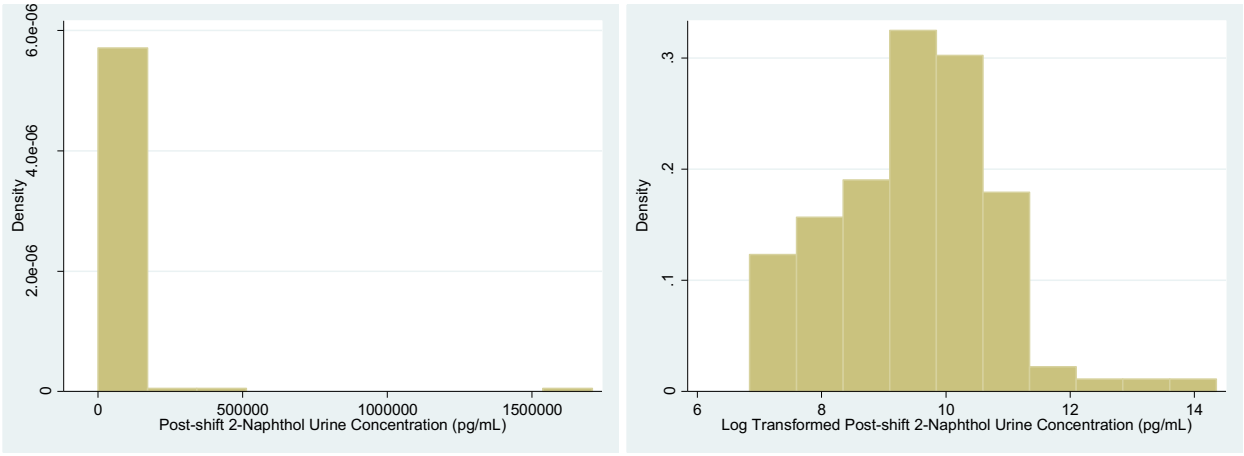


Figure 2. Specific gravity adjusted post-shift 2-naphthol urine concentrations compared to ln transformed specific gravity adjusted post-shift 2-naphthol urine concentrations

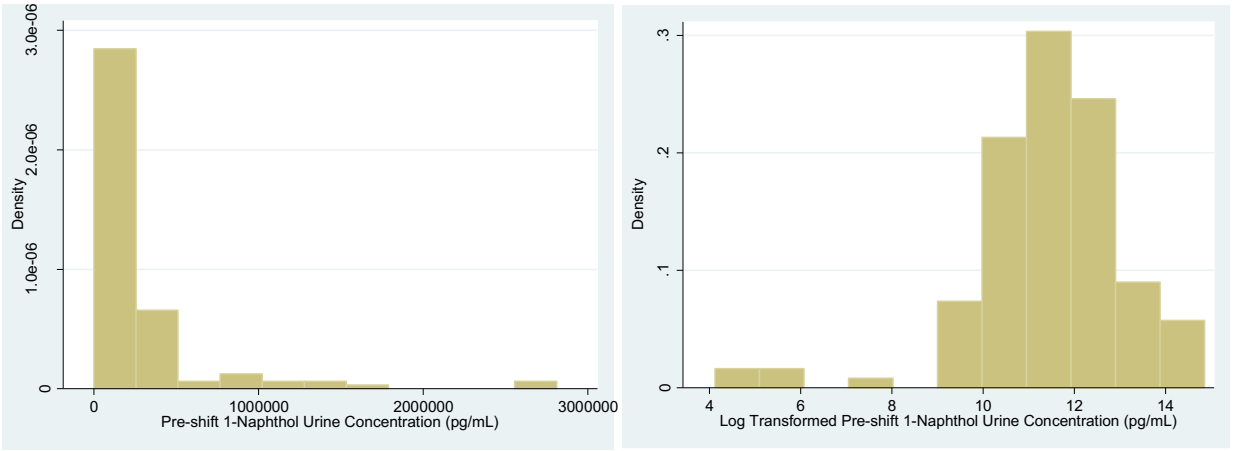


Figure 3. Specific gravity adjusted pre-shift 1-naphthol urine concentrations compared to ln transformed specific gravity adjusted pre-shift 1-naphthol urine concentrations

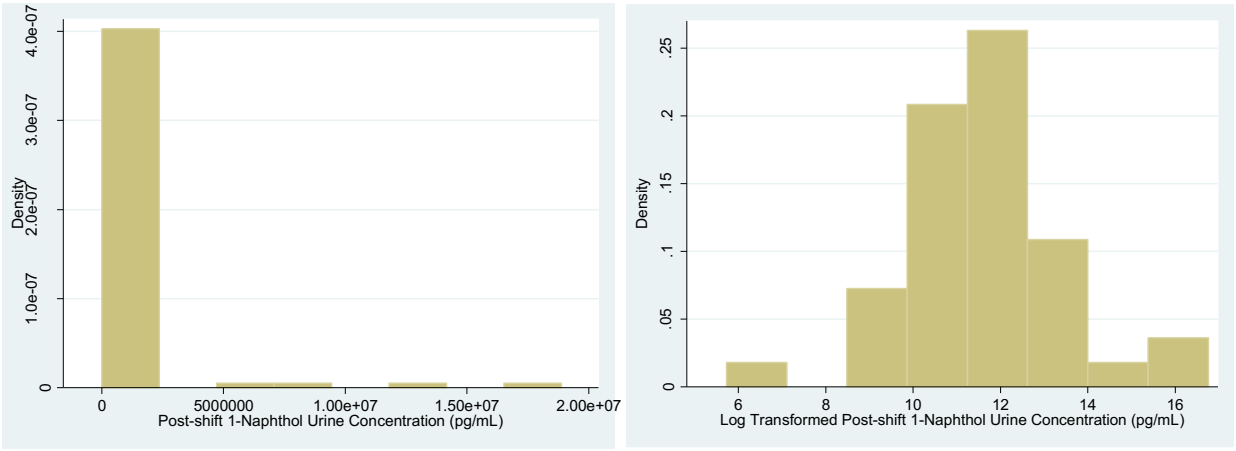


Figure 4. Specific gravity adjusted post-shift 1-naphthol urine concentrations compared to ln transformed specific gravity adjusted post-shift 1-naphthol urine concentrations

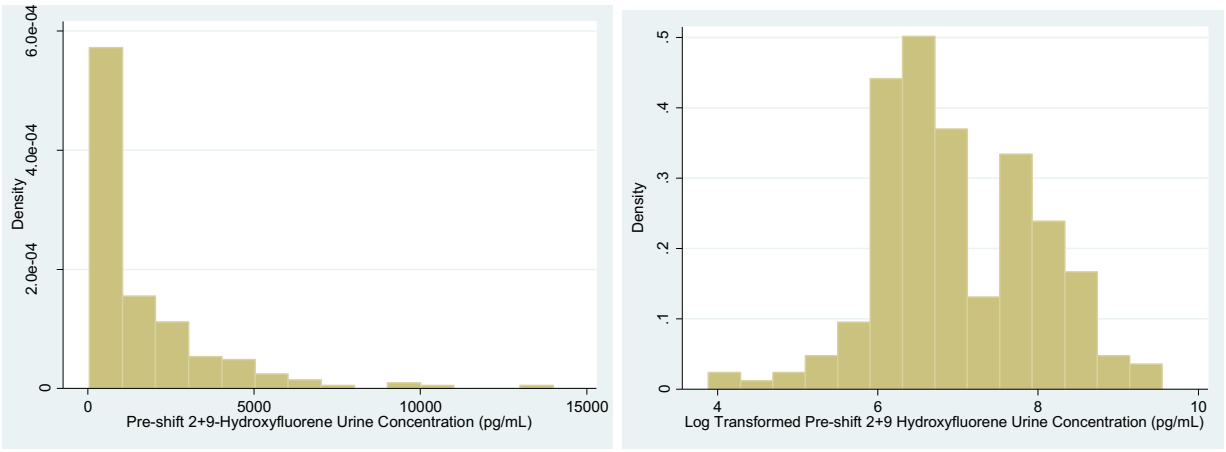


Figure 5. Specific gravity adjusted pre-shift 2+9 hydroxyfluorene urine concentrations compared to ln transformed specific gravity adjusted pre-shift 2+9 hydroxyfluorene urine concentrations

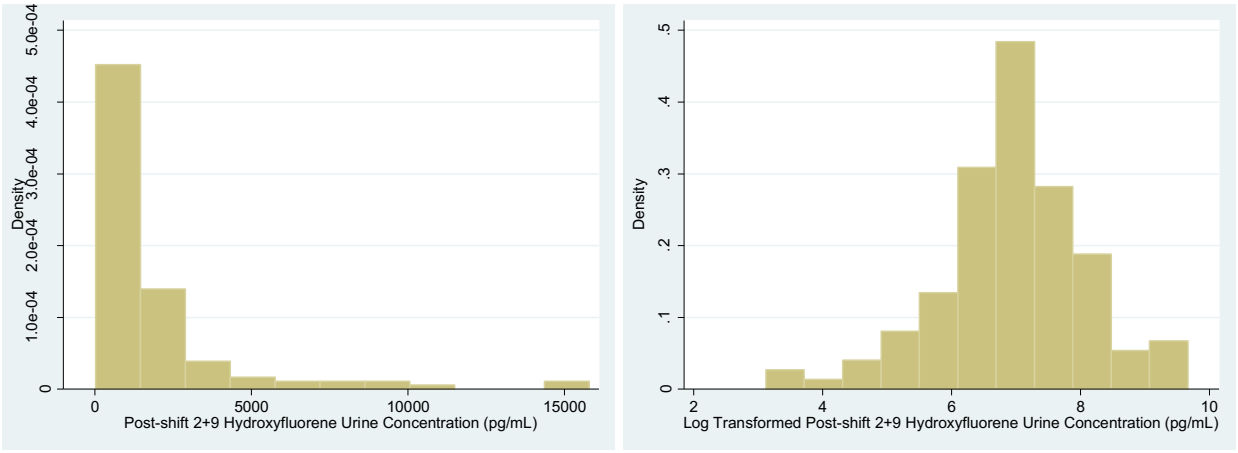


Figure 6. Specific gravity adjusted post-shift 2+9 hydroxyfluorene urine concentrations compared to ln transformed specific gravity adjusted post-shift 2+9 hydroxyfluorene urine concentrations

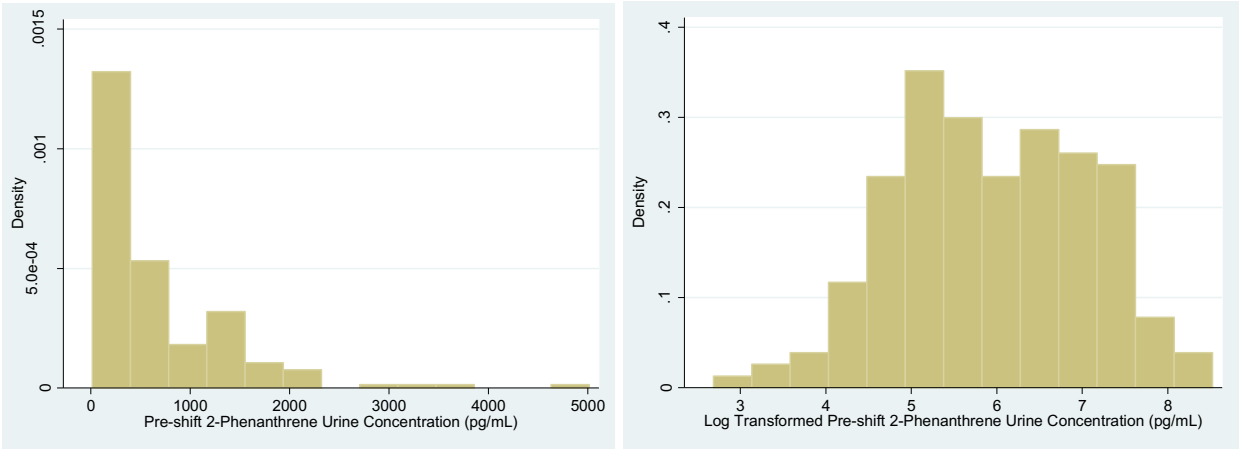


Figure 7. Specific gravity adjusted pre-shift 2 phenanthrene urine concentrations compared to ln transformed specific gravity adjusted pre-shift 2 phenanthrene urine concentrations

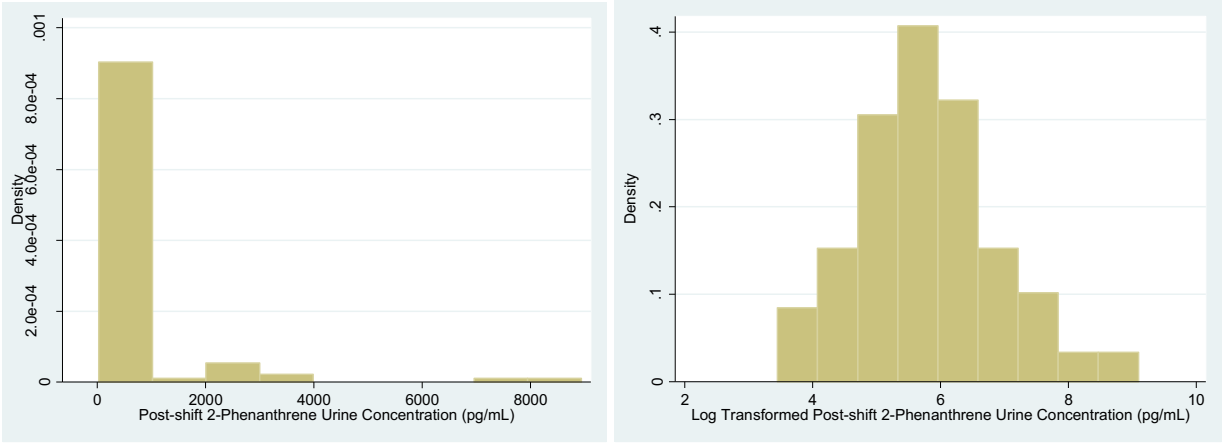


Figure 8. Specific gravity adjusted post-shift 2 phenanthrene urine concentrations compared to ln transformed specific gravity adjusted post-shift 2 phenanthrene urine concentrations

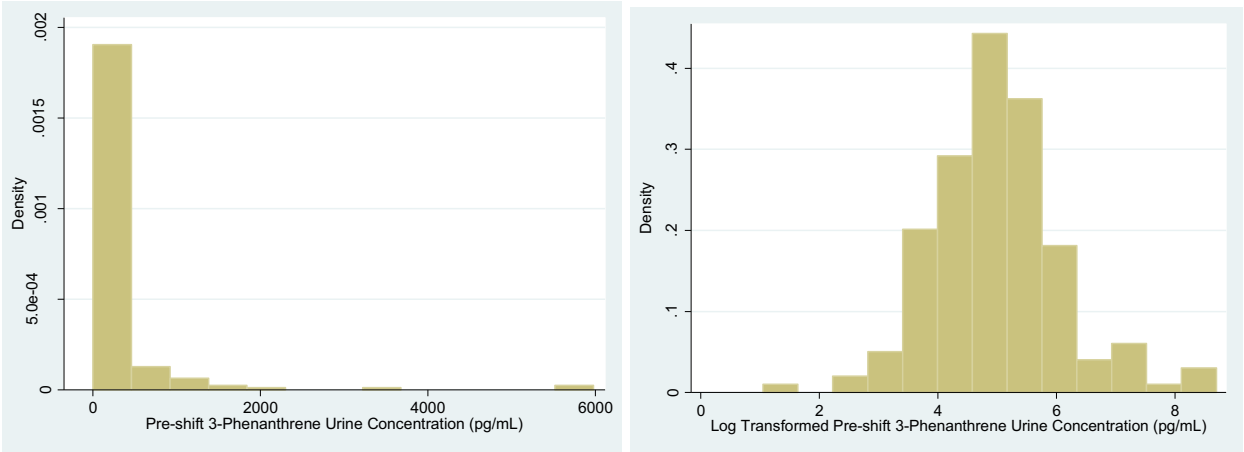


Figure 9. Specific gravity adjusted pre-shift 3 phenanthrene urine concentrations compared to ln transformed specific gravity adjusted pre-shift 3 phenanthrene urine concentrations

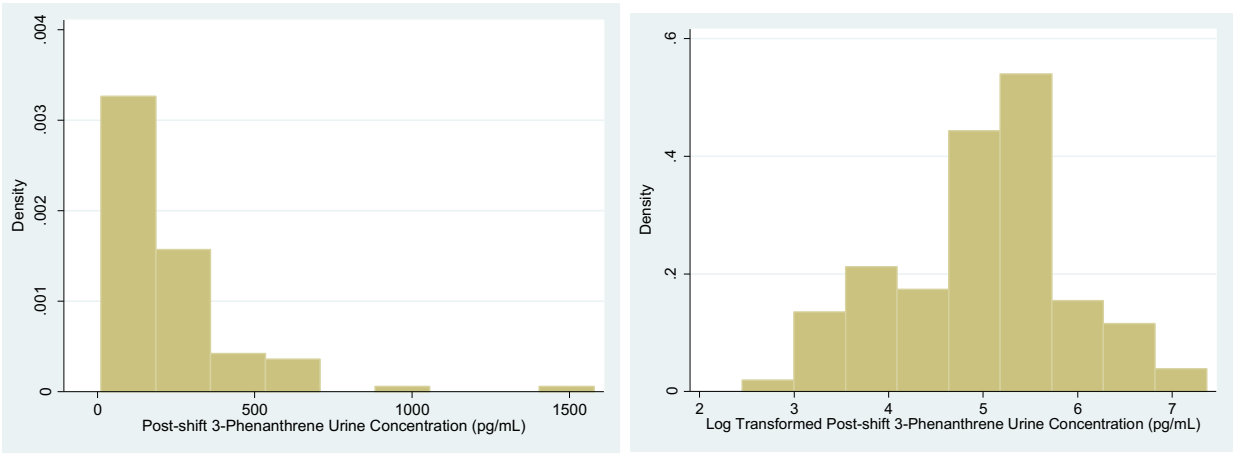


Figure 10. Specific gravity adjusted post-shift 3 phenanthrene urine concentrations compared to ln transformed specific gravity adjusted post-shift 3 phenanthrene urine concentrations

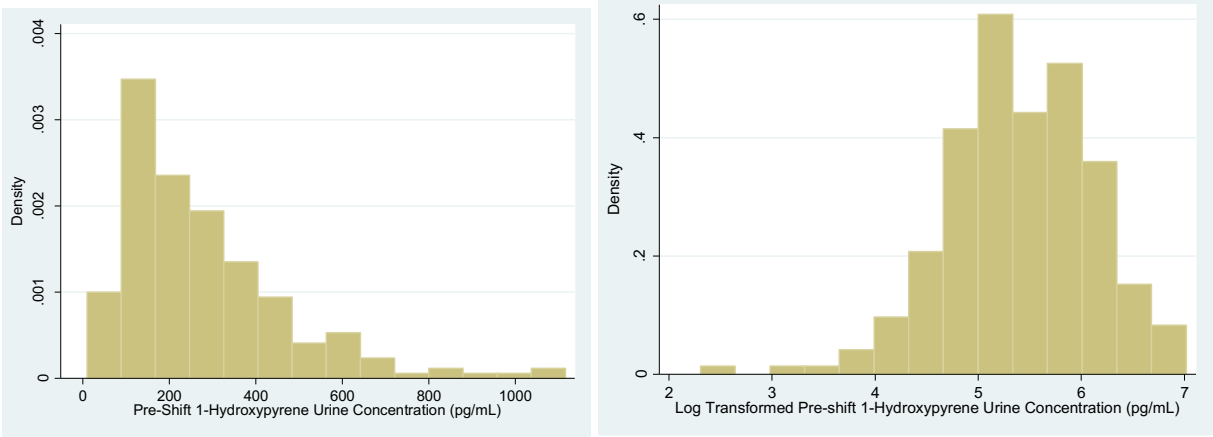
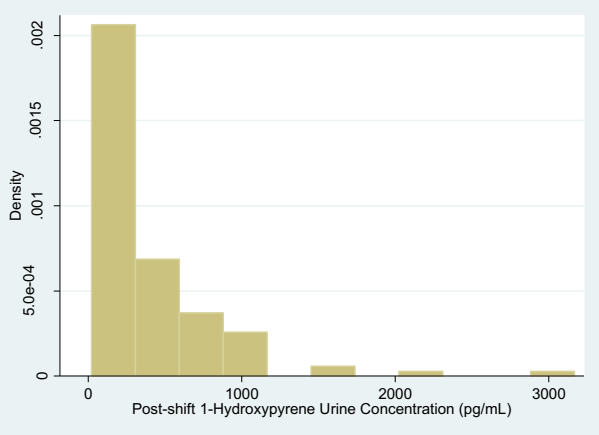


Figure 11. Specific gravity adjusted pre-shift 1-OHP urine concentrations compared to ln transformed specific gravity adjusted pre-shift 1-OHP urine concentrations



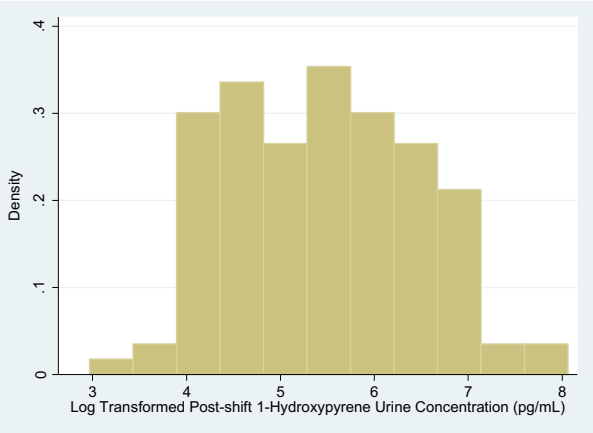
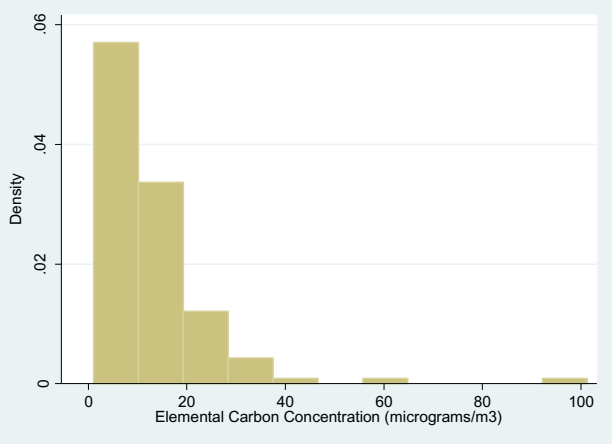


Figure 12. Specific gravity adjusted post-shift 1-OHP urine concentrations compared to ln transformed specific gravity adjusted post-shift 1-OHP urine concentrations



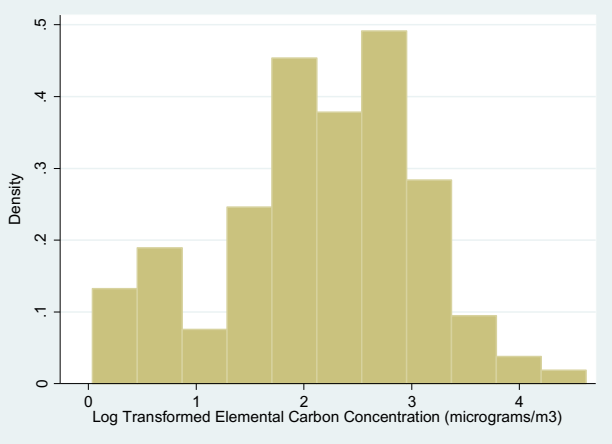


Figure 13. Elemental carbon air concentrations compared to ln transformed elemental carbon air concentrations

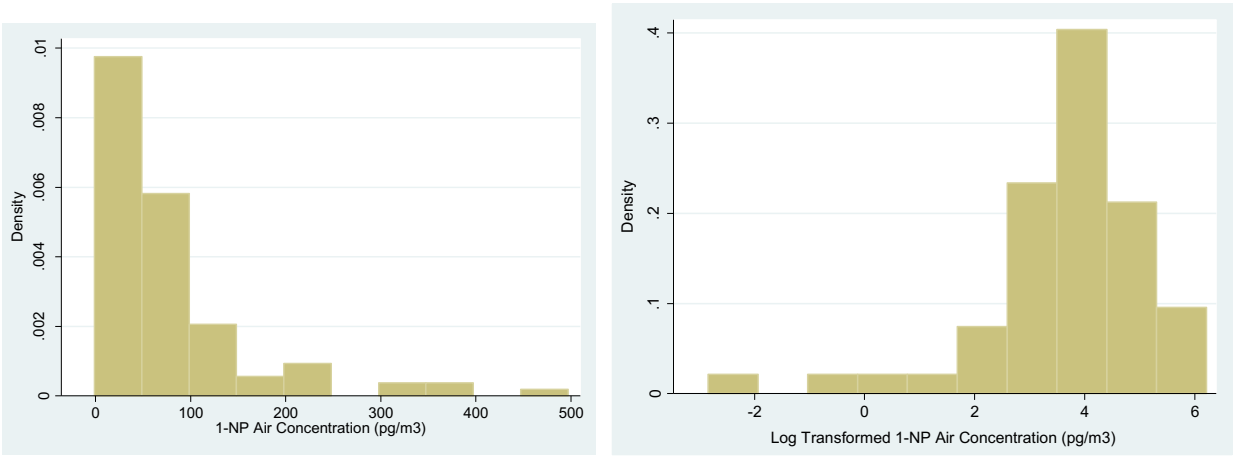


Figure 14. 1-Nitropyrene air concentrations compared to ln transformed 1-nitropyrene air concentrations

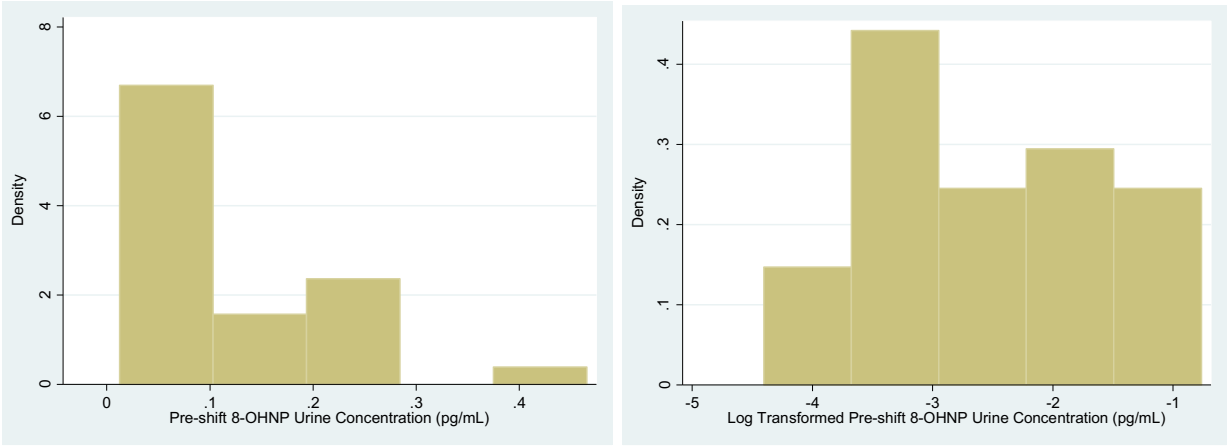


Figure 15. Specific gravity adjusted pre-shift 8-OHNP urine concentrations compared to ln transformed specific gravity adjusted pre-shift 8-OHNP urine concentrations

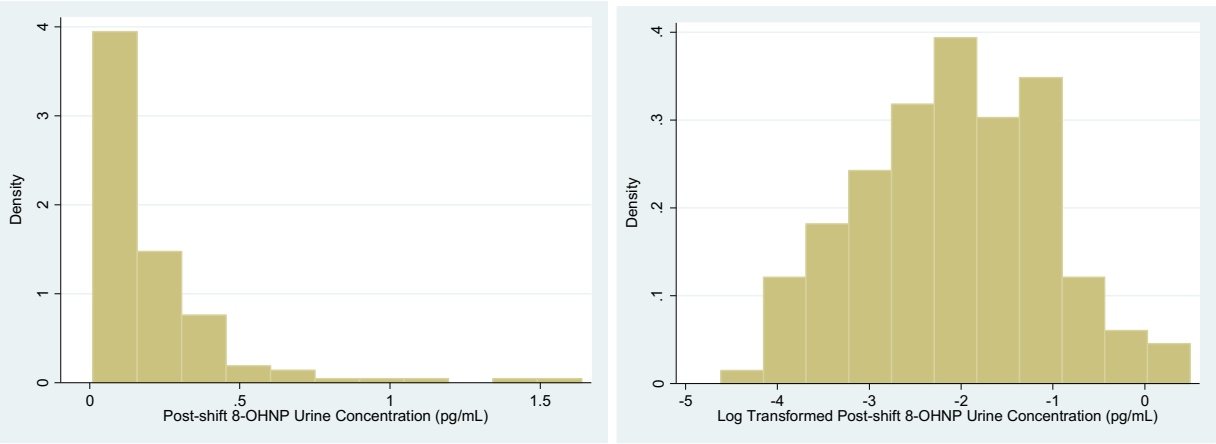


Figure 16. Specific gravity adjusted post-shift 8-OHNP urine concentrations compared to ln transformed specific gravity adjusted post-shift 8-OHNP urine concentrations

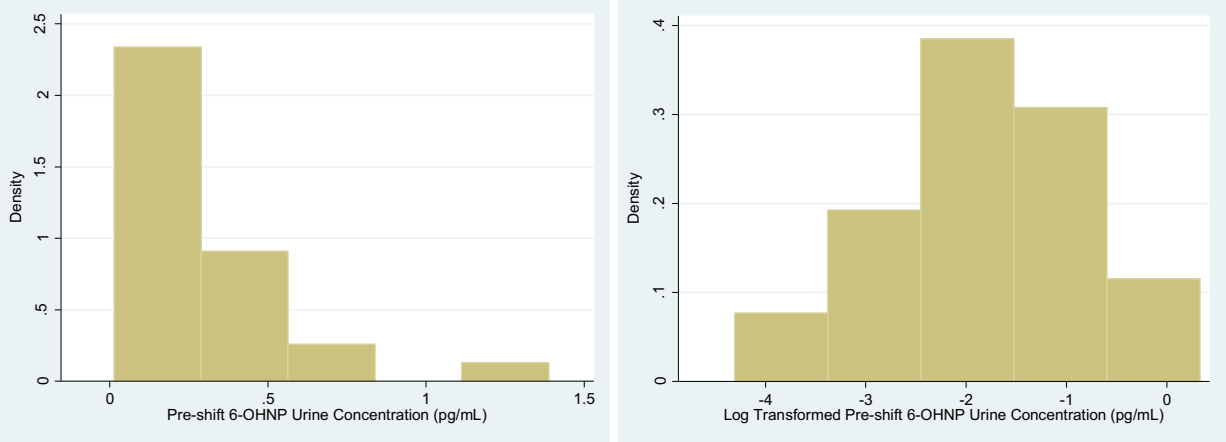


Figure 17. Specific gravity adjusted pre-shift 6-OHNP urine concentrations compared to ln transformed specific gravity adjusted pre-shift 6-OHNP urine concentrations

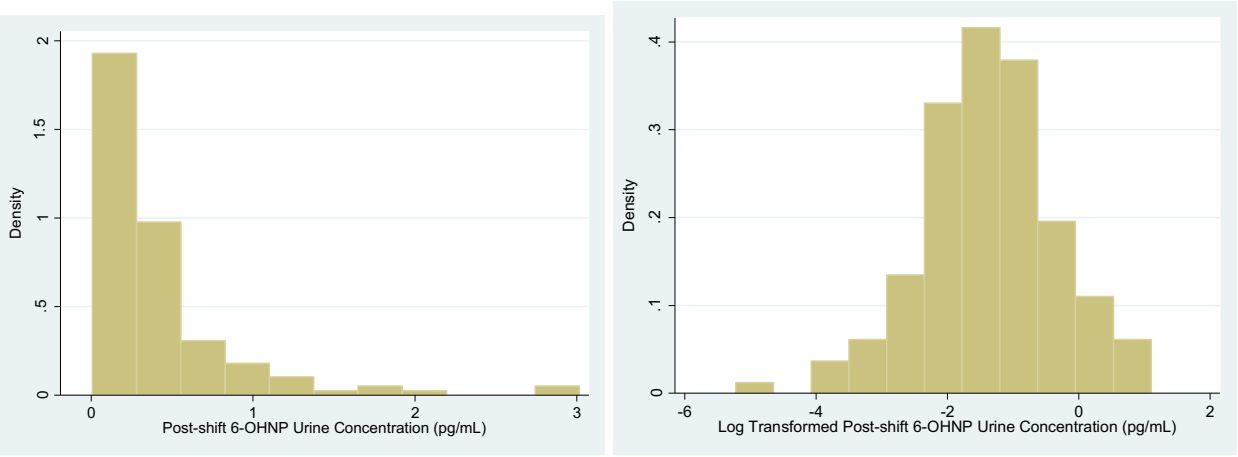
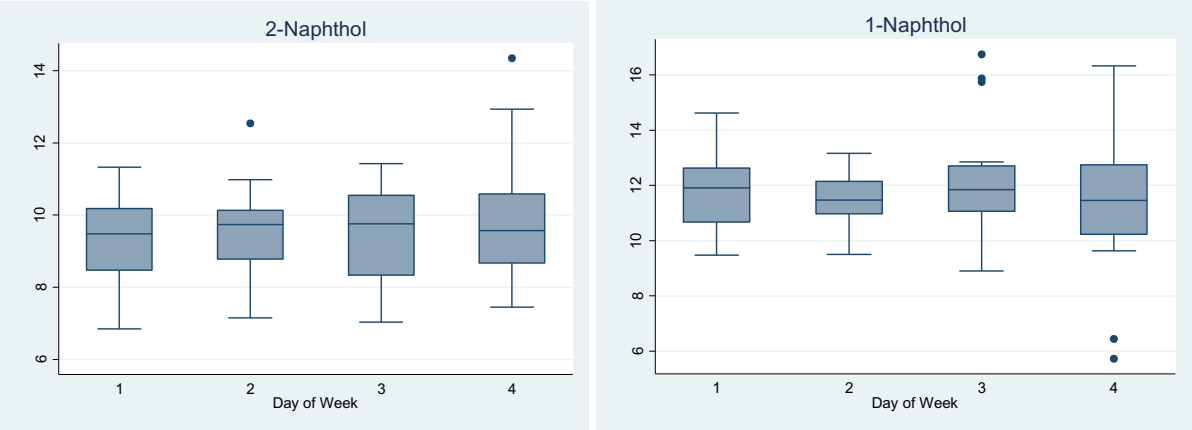


Figure 18. Specific gravity adjusted post-shift 6-OHNP urine concentrations compared to ln transformed specific gravity adjusted post-shift 6-OHNP urine concentrations



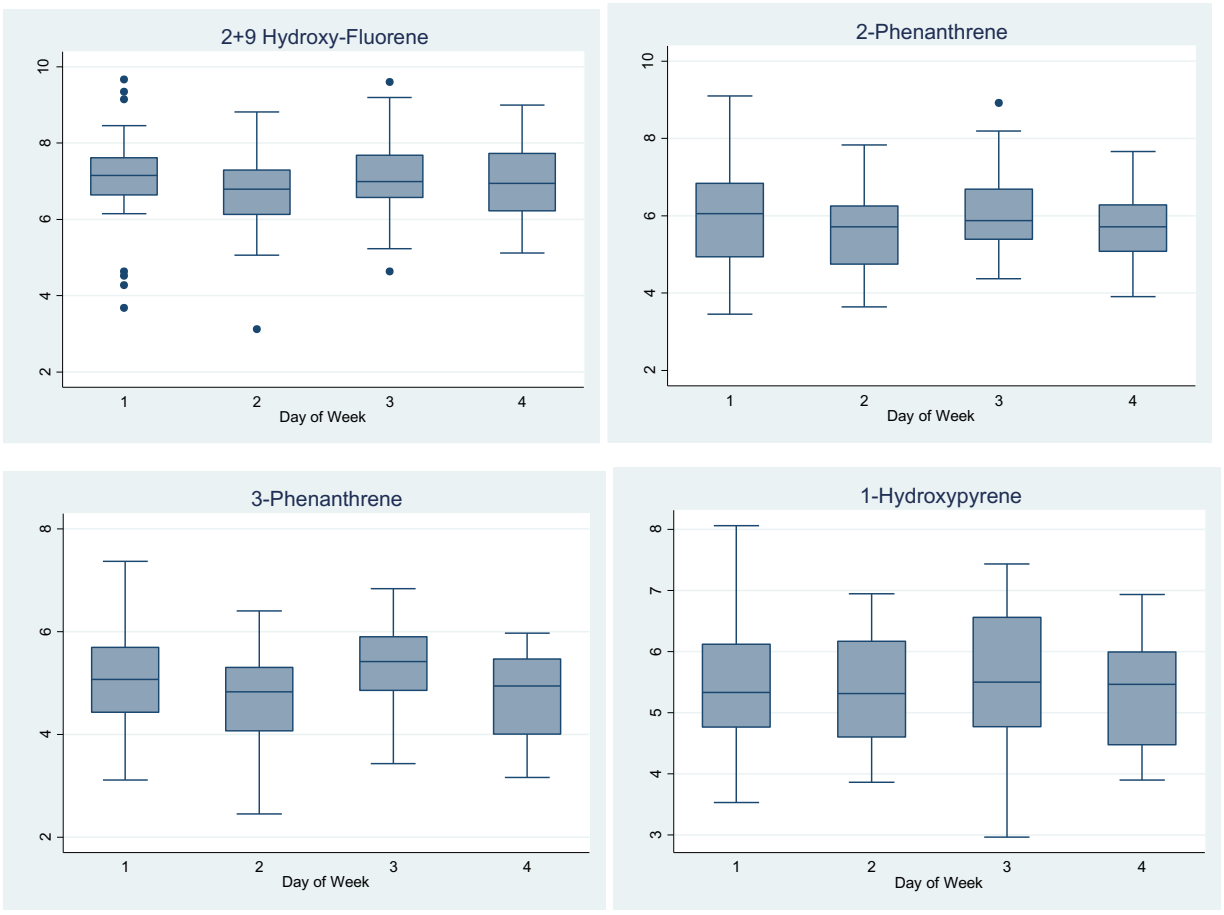


Figure 19. Ln transformation of specific gravity adjusted post shift metabolite concentrations by day of the week

Appendix II. - Job Task and Activity Questionnaire

DE Exposure – Subject Daily Activity Log

Part 1: Technician - Complete time activity log each day based on observation and questioning of subjects. Estimate in 30-min time increments. List other activities in the Notes section at the end of log.

Activity	AM						PM						
	6	7	8	9	10	11	12	1	2	3	4	5	6
Work shift prep (inside miner change room)													
Travel from surface to underground mine site													
Working in areas with active DE emissions													
Working in areas having no active DE emissions													
Lunch/break in areas with active DE emissions													
Lunch/break in areas with no active DE emissions													
Note time, duration & cause of any work stoppage due to air quality problems													

Notes:

Part 2: Technician – Complete each day based on observation and questioning of subjects.

1. What type of job activity did you perform today? What was the % time you spent on each activity? (Check all that apply and estimate to the nearest 5% - overall % must equal 100)

1a. ☐ Ore channeling_____% 1b. ☐ Jack leg drill operation _____% 1c. ☐ Load-Haul-Dump operation _____%

1d. ☐ Cage Tending _____% 1e.☐ Diesel Engine Repair _____% 1f. ☐ Above ground office work _____%

1g. ☐ Other:

_____

_____%

2. What percentage of the time that you were exposed to DE emissions did you wear a respirator?_____%

Part 2: Technician – Complete each day based on observation and questioning of subjects.

1. What type of job activity did you perform today? What was the % time you spent on each activity? (Check all that apply and estimate to the nearest 5% - overall % must equal 100)

1a. ☐ Ore channeling_____% 1b. ☐ Jack leg drill operation _____% 1c. ☐ Load-Haul-Dump operation _____%

1d. ☐ Cage Tending _____% 1e.☐ Diesel Engine Repair _____% 1f. ☐ Above ground office work _____%

1g. ☐ Other:

_____

_____%

2. What percentage of the time that you were exposed to DE emissions did you wear a respirator?

_____**%**

Technician: Questions related to biosampling

Part 3: Technicians: Ask these questions at the end of the day about the previous day's activities, using the phrase,

"In the last 24 hours have you…"

1. Smoked cigarettes?

☐ Yes ☐ No ☐ Don't know

→ _____ (number of cigarettes smoked)

2. Used chewing tobacco?

☐ Yes ☐ No ☐ Don't know

→ _____ (number of cans of chewing tobacco used)

3. Performed any off the job activities where you may have been exposed to DE emissions?

☐ Yes ☐ No ☐ Don't know

→ 3a.If yes, how long were you exposed? _____ (hrs, min)

3b.What types of activities were you doing?

Activities:

_____

_____

3c.What percent of your time spent doing these activities did you use a respirator?

_____%

Part 4: Technicians: Ask these questions at the end of the day about the previous day's activities, using the phrase,
"On the day before yesterday, have you…"

1. Smoked cigarettes?

☐ Yes ☐ No ☐ Don't know

→ _____ (number of cigarettes smoked)

2. Used chewing tobacco?

☐ Yes ☐ No ☐ Don't know

→ _____ (number of cans of chewing tobacco used)

3. Performed any off the job activities where you may have been exposed to DE emissions?

☐ Yes ☐ No ☐ Don't know

→3a.If yes, how long were you exposed? _____ (hrs, min)

3b.What types of activities were you doing?
Activities:

_____
_____

_____
_____

3c.What percent of your time spent doing these activities did you use a respirator?

_____**%**

Notes:

Appendix III. – SOP for extraction and analysis of PAH metabolites

STANDARD OPERATING PROCEDURE (SOP) FOR EXTRACTION AND ANALYSIS OF PAH METABOLITES IN URINE USING HPLC-FLUORESCENCE

Prepared by:	Mike Paulsen Niloufar Ghodsian	Date:	1/26/16
Revised by:	Niloufar Ghodsian	Date:	1/19/2017
Revised by:		Date:	
Revised by:		Date:	
Revised by:		Date:	
Revised by:		Date:	
Revised by:		Date:	
Reviewed by:		Date:	
Approved by:		Date:	

List of Tables

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Changes for Version 3:

1. Shorter incubation time (3 hours)
2. Lower amount of glucuronidase (20% of the original amount)
3. 9 mL of acetonitrile instead of 7 mL during the evaporation step

Supplies:

1. Silanized glassware: amber HPLC vials, HPLC vial micro inserts, Pasteur pipettes, TurboVap tubes
2. HCl, Fisher, A144s-500
3. Sodium Acetate anhydrous, Sigma S8750-1KG
4. Acetic acid
5. β-Glucuronidase/Arylsulfatase
   a. Sigma, G0751 (H-1), ≥ 300,000 U/gram glucuronidase and ≥10,000 U/gram arylsulfatase
6. Glucuronidase lot information
   a. New (11.2016) No.1: SLBP2483V, 2MU, 3.79 g solid, 527350 U/g
7. L (+)-Ascorbic acid, ACROS 401475000, CAS 50-81-7, Lot A0320409, Received 12/9/13
8. TurboVap tubes, 5 ml size (silanized and methanol-rinsed)
9. Sep-Pak
10. Methanol, Fisher, optima, A454-4
11. Syringe for extract filtration, disposable, 1 cc, BD, with slip tip, 309602
12. Syringe filters, Pall Acrodisc 13 mm PTFE 0.2 µm, #4542
13. HPLC vials with caps
14. D9-Hydroxypyrene-glucuronide, Toronto Research Chemicals (TRC), Cat. No. H952752, Lot.
    4-PMk-170-2
15. 1-Naphthol, Pestanal, Sigma Aldrich Inc., P.N. 31097-1G
16. 2-Naphthol, 99%, Sigma Aldrich Inc., P.N. 185507-5G
17. 2-Hydroxyfluorene, 98%, Sigma Aldrich Inc., P.N. 299847-100MG
18. 9-Hydroxyfluorene, 96%, Sigma Aldrich Inc., P.N. H31204-5G
19. 2-Hydroxyphenanthrene MRIGlobal Chemical Carcinogen Repository, P.N. 1284
20. 3-Hydroxyphenanthrene MRIGlobal Chemical Carcinogen Repository, P.N. 1132
21. Silanized 4 ml Amber screw cap vial, 15 X 45, Sigma Aldrich Inc., P.N. 27217
22. Solid cap W/PTFE liner, 13 mm, Sigma Aldrich Inc., P.N. 27141
23. SPE columns, Supelclean LC-18 500 g/ml, Sigma Aldrich Inc., P.N. 57012
24. 1,1,1,3,3,3, -Hexamethyldisilizane, Sigma Aldrich Inc., P.N. 379212-100mL

Equipment:

1. Shaking Water-Bath
2. Low volume TurboVap (5 ml size)
3. Sonic Bath
4. SPE Vacuum Manifold
5. HPLC-FLD, Agilent 1100

Preparation of Solutions:

1. Buffer for hydrolysis:
   a. 1 M acetic acid: 57 µl/ml water X 100 ml = 5.7 ml
   b. 1 M sodium acetate: 82.03 g/mol X 1 mol/L X 0.1 L = 8.2 g/100 ml

c. Combine 1 M acetic acid and 1 M sodium acetate to reach pH 5.5 [approximately 15 ml acid per 100 ml acetate]

2. D9-Hydroxypyrene-glucuronide
   a. Dissolve 0.5 mg standard in 100 mL MeOH (5 ng/µl). Transfer to three 40 mL silanized amber vials. Aliquot one of the three vials to silanazed amber autosampler vials. Store at -80ºC. Starting 4/26/2016 dilute the spiking solution 1:10 in MeOH and store at -80ºC.
   b. 20 µl of 0.5 ng/µl stock for d9-OHP-glucuronide, 10 ng/10 ml = 1 ng/ml
   c. 563 (OHP) pg/ml urine (563 X 10)/220 µl) = 25.59 pg/µl

   Note: estimated 100 µl remainder after evaporation
3. Glucuronidase: prepare fresh and only the amount needed. Note that each lot will have a different activity and the actual activity may be much higher than the minimum level stated on the product information sheet. The actual concentration must be obtained from the company web site so the correct amount of enzyme is used.
   a. Weigh enzyme into 1.5 ml or 4 ml glass vial. Use static discharger
   b. Pour powder into a glass Wheaton bottle (OK to reuse after rinsing with water and methanol)
   c. Add 1 M sodium acetate buffer, pH 5.5
   d. Cap and mix by hand
4. Ascorbic acid, 250 mg/ml in water (need 100 µl per 10 ml urine sample → 2.5 mg/ml urine). Prepare in 4 ml amber vial. Prepare fresh daily.
5. Mobile phase A: Water with 10 mM sodium acetate, pH 5.0
   a. 10 mM acetic acid: 570 µl/L
   b. 10 mM sodium acetate: 82.03 g/mol X 0.01 mol/L = 820 mg/L
   c. Combine 10 mM acetic acid and 10 mM sodium acetate to reach pH 5.0
6. Mobile phase B: Methanol

Table 1: Preparation of Stock Solutions

Analyte	Amount Purchased (mg)	Amount Used (mg)	volume (ml)	Stock Conc. (ppm)	Manufacture	Lot. No	Date Received	Date Prepared
1-OH-NAP	1000	40	20	2000	Fluka	SZBC247XV	1.29.16	2.5.16
2-OH-NAP	5000	25.8	25	1032	Aldrich	STBF1199V	1.29.16	3.29.16
2-OH-FLU	100	24.6	25	984	Aldrich	MKBT2365V	1.29.16	3.29.16
9-OH-FLU	5000	25.8	25	1032	Aldrich	STBF0066V	1.29.16	3.29.16
2-OH-PHEN	5.944	5.944	25	238	MRI Global	1284	2.2.16	3.29.16
3-OH-PHEN	5.512	5.212	25	208	MRI Global	1132	2.2.16	3.29.16
1-OHP		11.1	25	444	Aldrich	36,151-8		6.2.16

Urine Extraction

1. Thaw urine samples
2. Transfer 10 ml urine to 40 ml amber silanized glass vials. Total volume should be 10 ml. If the sample volume is less than 10 ml, add DI water to make a total volume of 10 ml.
3. Transfer 10 ml water to 40 ml amber silanized glass vials
   i. D+H Spike (N=2)
   ii. D Spike (N=2)
   iii. Blank (N=1)
4. Add 100 µl ascorbic acid solution to all samples (250 mg/ml in water)
5. Add 5 ml of 1 M acetate buffer, pH 5.5 containing β-Glucuronidase/aryl sulfatase to all samples
6. Add 20 µl of 1:10 diluted D-OHP-glucuronide spike to all samples except for the blank, 0.5 ng/µl → 10 ng
7. Add 25 µl of H Spike to D+H samples
8. Incubate at 37°C for 3 hours in a shaking water-bath. Set vials in a rack positioned on an include to improve mixing and set oscillation speed to 100. Water level should <u>not</u> be above the cap.

Solid Phase Extraction

1. Add 1.6 ml methanol to each sample (10% final volume)
2. Condition SPE columns with 3 x 3 ml methanol followed by 3 x 3 ml water
3. Add samples to SPE columns with a flow rate of approximately 0.5 to 1 ml/minute
   a. Let the solid in urine samples to settle down in the bottom
   b. Keep the solid until the end to mix with the rinse solution
4. Rinse vials that previously contained samples with 5 ml water and add to the corresponding SPE columns
5. Add 5 ml 40% methanol to columns to wash
6. Elute columns by adding 5 ml 80% methanol
7. Evaporate to approximately 2 ml in TurboVap at 55º C
8. Add 4.5 ml acetonitrile and invert several times to mix
9. Evaporate to 2 ml again, and add another 4.5 ml acetonitrile
10. Evaporate to approximately 1 ml
11. Add 20 µl DMSO and vortex for 1 minute
12. Evaporate to near dryness (20 µl DMSO remaining after evaporation)
13. Reconstitute in 200 µl 60:40 water: MeOH
14. Vortex five minutes at setting of 1
15. Sonicate five minutes
16. Filter into HPLC vials with silanized inserts
17. Analyze by HPLC-FLD using method OHP34.M

Quality Control Samples

1. Water Blanks
   a. N=2 per batch
   b. 10 ml water process like urine, but no spike solutions are added
2. Deuterated spiked water
   a. N=2 per batch
   b. 10 ml water process like urine
   c. Spike with 20 µl D-OHP-glucuronide spike, 0.5 ng/µl → 10 ng
3. D and H spiked water
   a. N=2 per batch
   b. 10 ml water process like urine
   c. Spike with 20 µl D-OHP-glucuronide spike, 0.5 ng/µl → 10 ng and with 25 µl protonated spike mix
4. 100% Controls: protonated spike solution controls. Spike solution added directly to solvent for injection
   a. N=2 per batch
   b. 25 µl protonated spike plus 120 µl water and 55 methanol
5. Benchmark urine
   a. N=2 per batch

Instrumental Analysis

1. Analyze by HPLC Fluorescence (Agilent 1100 HPLC)
2. Method: OHP34.M
3. Install column: Agilent Poroshell 120, 2.1 X 100 mm, 2.7 µm particles, P.N. 685775-902, S.N. USCFH01115, Guard column P.N: 821725-912
4. Purge pumps with mobile phase for 10 minutes at 2.5 ml/ min, 50% each channel A and channel B
5. Sample naming (examples, where MMDDYY is the preparation date of the sample or standard and AA is the run number):
   a. Std 10 MMDDYY AA.d
   b. D H2O Spk #1 MMDDYY AA.d
   c. D+H H2O Spk #1 MMDDYY AA.d
   d. D+H 100% Ctl #1 MMDDYY AA.d
   e. H2O Blk #1 MMDDYY AA.d
   f. BM #1 MMDDYY AA.d
   g. Sample ID MMDDYY AA.d

6. Sample log: Analyze the samples and standards in the following order
   a. Check Std
   b. Standards, in order from low to high concentration
   c. Blank
   d. Blank
   e. QC and Benchmark sample extracts plus samples to make 15 runs
   f. Check Std
   g. Blank
   h. 15 Samples
   i. Check Std

j. Blank
k. 15 Samples
l. Check Std
m. Blank
n. Repeat pattern
o. Reanalysis of 1 QC (D+H H2O Spk)
p. Reanalysis of urine extracts (1 per 15 samples analyzed; for long sequences, can re-analyze earlier in the sequence)
q. Check Std
r. Blank (shutdown method)

Reporting, Data Analysis and QC Review

1. Set up calibration curves using 1/x weighting
2. Print the worklist
3. Generate quantitative analysis report
4. Print each report
5. Review reports
   a. Were peaks correctly chosen?
   b. Were peaks free of apparent interfering peaks?
   c. Were peaks integrated properly?
6. HOW TO EXPORT BATCH DATA FROM THIS CHEMSTATION VERSION
7. Open file in Excel for assigning QC flags

Preparation of Calibrants and Spike Solutions

1. Calibrants:
   a. Prepare high standard according to Table 2
      i. Start with adding 2-OH-Flu (67.77 µl x 3) and go down the list of less concentrated stock solutions: 9-OH-Flu, 2OH-Phen, 3-OH-Phen, OHP, 2-OH-Nap
      ii. Add 1-OH-Nap at the end
      iii. Add water after adding all the analytes using the manual adjustable pipette
   b. Prepare calibrants 2 through 6 by diluting the high standard according to the dilution table below
      i. First, add 60:40 H_2O:MeOH to the rest of vials according to the dilution table below
      ii. Use positive displacement pipette for transferring 400 µl of calibrant 1 to calibrant 2
   c. Store at -20°C in freezer FZ-S2

Table 2 Preparation of Calibration Standards

PAH Metabolite	Stock Solution Conc. (ng/µl)	Volume into Calibrant 1 (µl)	Calibrant 1 (pg/µl)	Calibrant 2 (pg/µl)	Calibrant 3 (pg/µl)	Calibrant 4 (pg/µl)	Calibrant 5 (pg/µl)	Calibrant 6 (pg/µl)
1-OH-Nap	2000	2000	1,000,000	100,000	20,000	10,000	2,000	400
2-OH-Nap	1032	38.8	10,000	1,000	200	100	20	4.00
2-OH-Flu	9.84	203.3	500	50	10	5.0	1.00	0.20
9-OH-Flu	10.32	96.9	250	25	5.0	2.5	0.50	0.10
2-OH-Phen	11.888	42.1	125	12.5	2.5	1.25	0.25	0.05
3-OH-Phen	10.424	48.0	125	12.5	2.5	1.25	0.25	0.05
OHP	20	100.0	500	50	10	5.0	1.00	0.20

		400 µl of Cal. 1 (1:10)	80 µl of Cal.1 (1:50)	40 µl of Cal 1 (1:100)	80 µl of Cal. 2 (1:50)	80 µl of Cal. 3 (1:50)
Total vol of stocks	2528.9					
MeOH Vol	1471.1	3600	3920	3960	3920	3920
Total Vol	4000	4000	4000	4000	4000	4000

Spike Solutions

Table 3: H Spike Solution Concentration

PAH Metabolite	Stock Solution Conc. (ng/µl)	Volume into Spike Solution (µl)	Spike Solution Conc. (ng/µl)	Final Vol (µl)	Vol Spike into Sample (µl)	Vol Extract (µl)	Conc. in Extract (pg/µl)
1-OH-Nap	2000	438.0	219.000	4000	25	200	27375
2-OH-Nap	10.32	823.2	2.124	4000	25	200	265.5
2-OH-Flu	9.84	68.8	0.169	4000	25	200	21.2
9-OH-Flu	10.32	65.6	0.169	4000	25	200	21.2
2-OH-Phen	11.888	36.0	0.107	4000	25	200	13.4
3-OH-Phen	10.424	41.2	0.107	4000	25	200	13.4
OHP	20	17.5	0.088	4000	25	200	10.94

Total Vol (µl)	1490.3
Vol MeOH (µl)	2509.7

Table 4: HPLC Method OHP14.M

==========

1100 Capillary Pump 1

==========

Pump Mode
Mode : Normal

Control
Column Flow : 125.000 µl/min
Stoptime : 70.00 min
Fast Reconditioning : Off
Posttime : Off

Solvents
Solvent A 2 : 50.0 % (H2O)
Solvent B 1 : 50.0 % (MEOH)

PressureLimits
Minimum Pressure : 0 bar
Maximum Pressure : 400 bar

Auxiliary
Compressibility A : 46*10^-6/bar
Minimal Stroke A : Auto
Compressibility B : 120*10^-6/bar
Minimal Stroke B : Auto

Store Parameters
Store Ratio A : Yes
Store Ratio B : Yes
Store Flow : Yes
Store Pressure : Yes

Agilent 1100 Contacts Option
==========

Contact 1 : Open
Contact 2 : Open
Contact 3 : Open
Contact 4 : Open

Timetable

Time	Solv.B	Flow	Pressure
0.00	50.0	125.000	
60.00	63.0		
60.10	90.0		
65.00	90.0		
65.10	40.0		

==========

Agilent 1100 Fluorescence Detector 1

==========

Signals (Multiple Emission)
- Excitation : 240 nm
- Emission A : 388 nm
- Emission B : 336 nm
- Emission C : 355 nm
- Emission D : 375 nm
- Acquire Em. Spectra : None

Time
- Stoptime : As pump
- Posttime : Off

Peakwidth : > 0.2 min
PMT-Gain : 10

Analog Outputs
- Zero offset output 1 : 5 %
- Zero offset output 2 : 5 %
- Attenuation output 1 : 100 LU
- Attenuation output 2 : 100 LU

Phosphorescence Detection : Off

Fluorescence Scan :
- Excitation Range : 220 to 380 nm, Step 5 nm
- Emission Range : 300 to 500 nm, Step 5 nm

Lamp
- Only On During Run : Yes
- Economy Mode : No
- Lamp Energy Reference : On

Enable analysis
when lamp is off : No

Special Setpoints
Baseline Behavior : Append
Signal Polarity : Positive
Fit Spectral Range : Yes

Agilent 1100 Contacts Option
==========

Contact 1 : Open
Contact 2 : Open
Contact 3 : Open
Contact 4 : Open

==========
Agilent 1100 Autosampler 1
==========

Injection
Injection Mode : Needle Wash
Injector volume : 10.00 µl
Wash Vial : 91
Optimization : none

Auxiliary
Drawspeed : 100 µl/min
Ejectspeed : 100 µl/min
Draw position : 0.0 mm

Time
Stoptime : As Pump
Posttime : Off

Agilent 1100 Contacts Option
==========

Contact 1 : Open
Contact 2 : Open
Contact 3 : Open
Contact 4 : Open

Table 5: HPLC Method OHPOFF.M

==========

1100 Capillary Pump 1

==========

Pump Mode
Mode : Normal

Control
Column Flow : 0.000 µl/min
Stoptime : 30.00 min
Fast Reconditioning : Off
Posttime : Off

Solvents
Solvent A 1 : 0.0 % (Buffer)
Solvent B 2 : 100.0 % (ACN)

PressureLimits
Minimum Pressure : 0 bar
Maximum Pressure : 400 bar

Auxiliary
Compressibility A : 46*10^-6/bar
Minimal Stroke A : Auto
Compressibility B : 120*10^-6/bar
Minimal Stroke B : Auto

Store Parameters
Store Ratio A : Yes
Store Ratio B : Yes
Store Flow : Yes
Store Pressure : Yes

Agilent 1100 Contacts Option
==========

Contact 1 : Open
Contact 2 : Open
Contact 3 : Open
Contact 4 : Open

Timetable

Time	Solv.B	Flow	Pressure
0.00	100.0	0.000	
2.00	100.0	150.000	
12.00	100.0	200.000	

==========

Agilent 1100 Fluorescence Detector 1

==========

Signal
Excitation : Zero Order
Emission : Zero Order

Time
Stoptime : As pump
Posttime : Off

Peakwidth : > 0.2 min
PMT-Gain : 10

Analog Outputs
Zero offset output 1 : 5 %
Zero offset output 2 : 5 %
Attenuation output 1 : 100 LU
Attenuation output 2 : 100 LU

Phosphorescence Detection : Off

Fluorescence Scan :
Excitation Range : 220 to 380 nm, Step 5 nm
Emission Range : 300 to 500 nm, Step 5 nm

Lamp
Only On During Run : Yes
Economy Mode : No
Lamp Energy Reference : On
Enable analysis
when lamp is off : No

Special Setpoints
Baseline Behavior : Append
Signal Polarity : Positive

Fit Spectral Range : Yes

Agilent 1100 Contacts Option
==========

Contact 1 : Open
Contact 2 : Open
Contact 3 : Open
Contact 4 : Open

==========

Agilent 1100 Autosampler 1

==========

Injection
Injection Mode : Standard
Injector volume : 5.00 µl
Optimization : none

Auxiliary
Drawspeed : 100 µl/min
Ejectspeed : 100 µl/min
Draw position : 0.0 mm

Time
Stoptime : As Pump
Posttime : Off

Table 5 Data QC Flags

Symbol	Meaning	Action
ND	Compound not detected	Replace value with <LOD
NQ	Compound not quantifiable but detected	delete value/censor value cell
NR	Concentration has error	delete value/censor value cell
NS	ISTD Missing	delete value/censor value cell
N<	Conc. is < LQL	no action

AI	Area not valid ˆ Interference	delete value/censor value cell
N>	Conc. is > UQL	no action
AX	Area not valid	delete value/censor value cell
N1	Compound not analyzed for	no action
RY	Recovery outside QA limits or was indeterminate	delete value/censor value cell
MR	Value less than minimum reported level	replace with <LOD
R2	Calibration outside QA limit	delete value/censor value cell
M03	outlier	no action

Appendix IV. Data Traceability

Prepared by Emily Zamzow: May 7th, 2017

I. Raw Data Location

Electric files containing raw data documents stored in *S:\lab\EZ_Diesel2014*

QUESTIONNAIRE DATA

- **Folder location:**
  - *S:\lab\ EZ_Diesel2014\Questionnaire Data*
    original surveys are currently stored in the Stillwater Mine Study binder in the Simpson Lab
- **Files in folder**:
  - *survey data table_03may2015*
  - *Survey Data Dictionary_03may2015*
- **File Contents:**
  - *survey data table_03may2015:* compilation of information collected from the post-shift questionnaire that were previously collected in the field. This included information on daily tasks, time worker performed task, tobacco usage and respirator use.
  - *Survey Data Dictionary_03may2015:* defines all of the variables in *survey data table_03may2015* file, and the adjustments/changes made to the spread sheet *survey data table_03may2015.*
- **File Source:** The original surveys were manually entered into excel by Joemy Ramsay in 2014/2015. The original file is found in *S:\lab\Nitropyrene\Miner Study 2014\Final Data\Final Processed Data*. She also found it necessary or helpful for later analysis to either alter survey entries upon input, or to guess entry values for blank survey fields. When survey data was altered upon entry into the excel spreadsheet, a flag variable corresponding to that field was given a value of "1" or "3". A description for the replacement of written fields or guessing for empty fields is provided in the survey data dictionary (*S:\lab\Nitropyrene\Miner Study 2014\Final Data\Survey Data Dictionary_03may2015.* These files were copied to *S:\lab\EZ_Diesel2014* for logistical reasons of having all data in a central location.

AIR DATA

- **Folder location:**
  - *S:\lab\ EZ_Diesel2014\Air Data*
- **Files in folder:**
  - *compiled air data_2july2015*
  - *Air Data Dictionary_30april2015cs*
- **File Contents:**
  - *compiled air data_2july2015*: all of the air data including: 1-NP on Teflon filter, 1-NP on quartz filter, EC, OC, TC, equipment failure, damaged filters, flags in data, filter breakthrough, any associated notes
  - *Air Data Dictionary_30april2015cs*: description of all the variables in file *compiled air data_2july2015*
- **File Source:** The original file for *compiled air data_2july2015* is found in *S:\lab\Nitropyrene\Miner Study 2014\Final Data\Final Processed Data*. The original file

for *Air Data dictionary_30april2015cs* is found in *S:\lab\Nitropyrene\Miner Study 2014\Final Data*. These files were copied to *S:\lab\EZ_Diesel2014* for logistical reasons of having all data in a central location.

URINARY PAH DATA

- **Folder location:**
  - *S:\lab\ EZ_Diesel2014\Urinary PAH Data*
- **Files in folder:**
  - *urine full dataset JMR 5_22_15*
  - *PAH Urine Integration Results*
  - *consolidated urinary PAH data*
- **File Contents:**
  - *urine full dataset JMR 5_22_15*: all urinary biomarker samples for Stillwater Mine study 2014, compiled by Joemy Ramsay. This file also contains a data dictionary which defines all terms/abbreviations used.
  - *PAH Urine Integration Results*: all extract concentrations typed directly from the printed integration results. Each tab in this file is labeled in order, by batch number and batch date.
  - *consolidated urinary PAH data*: due to the fact that each batch is separated into different sheets in *PAH Urine Integration,* all urinary samples analyzed for PAH metabolites were combined into one sheet in this file. This file contains the unadjusted raw extract concentrations (copied from the spreadsheet *PAH Urine Integration Results)* converted to urine concentration in the excel file for each analyte. This file will also contain the associated data dictionary defining each label used.
- **File Source:**
  - The original file (*urine full dataset JMR 5_22_15*) is found in *S:\lab\Nitropyrene\Miner Study 2014\Final Data\Final Processed Data*. This file was created by Joemy Ramsay. This file was copied to *S:\lab\EZ_Diesel2014* for logistical reasons of having all data in a central location.
  - **To create the file** *consolidated urinary PAH data* OHPAH extract concentrations for all samples were first copied manually from the instrument print outs to *PAH Urine Integration Results*. The extract concentrations from this intermediate file were separated in sheets by batch/extraction date. The unadjusted extract concentrations were copied into the *consolidated urinary PAH data* file for a more centralized location consisting of one sheet for all data. The folder containing the original instrument print outs can be found in the Simpson Lab.

II. Electronic Input and Manipulation

QUESTIONNAIRE DATA

Written survey entries from PDF scans of paper surveys were inputted manually into an excel spreadsheet by Joemy Ramsay in late 2014/early 2015. In some cases, she found it necessary or helpful for later analysis to either alter survey entries upon input, or to guess entry values for blank survey fields. When survey data was altered upon entry into the excel spreadsheet, a flag variable corresponding to that field was given a value of "1" or "3". A description for the replacement of written fields or guessing for empty fields is provided in the survey data dictionary (*S:\lab\Nitropyrene\Miner Study 2014\Final Data\Survey Data Dictionary_21april2015.xlsx*), and is summarized below:

- Field entries were entered as written, and corresponding flags were left blank if the field was filled out in the subject's handwriting, or in the handwriting of a QC person in the field (who is assumed to have gotten the value by word-of-mouth from the subject on the spot).
- If a value was guessed by Chris Simpson after the campaigns had ceased (for the June campaign surveys, in particular), the value was guessed using his knowledge of the subject's other information and previous survey entries; each guessed value is given a justification note on the paper survey. The corresponding flag variable was given a value of "1" by Joemy during entry into the excel spreadsheet.
- If a value was guessed by Joemy Ramsay during entry of data from the PDF scans into the excel spreadsheet, the value was guessed using her knowledge of the subject's other information and previous survey entries, and the corresponding flag variable was given a value of "1" by Joemy during entry into the excel spreadsheet.
- Certain specific fields were filled in by subjects in ways that were either erroneous (for example, the % of time spent on each activity, summed together, does not add up to 100%), or vague (for example, if an "other" task was written in as a strangely-worded task name). In these cases, Joemy made alterations to the survey field contents during entry into the excel spreadsheet. She gave each corresponding flag variable a value of "1" for a corrected erroneous entry, and a value of "3" for a clarification of the task name. Specific details are outlined in the survey data dictionary.

AIR/URINARY DATA

Raw Data Files, in Original Forms, are Messy and Disorganized

Emily Carpenter entered in all of the air data. Joemy Ramsay entered in the 1-NP urinary data. And I (Emily Zamzow) entered in all of the additional urinary PAH data. All information is contained in excel workbooks found on the server. Each raw data excel file consists of a workbook containing multiple sheets of data. Since the files come from a variety of sources and laboratories, excel sheet and workbook formatting varies widely between the files.

For example, any given pump calibration field log file contains one sheet of data containing 4 separate tables, one for each sampling day, in a 2x2 grid. Therefore, there are multiple columns containing air volume data, and any given row contains data for more than one sample. This would make any attempt to extract the data directly into another program difficult.

Alternatively, for any given PAH analysis report, there are multiple sheets containing (separately) calibration curve data, QC tables, and the final extract concentrations, etc. The sheet containing extract concentrations lists a different sample per row, and variable names in a header row, but also contains many columns with peak areas, etc. so the data of interest is difficult to locate and would make data extraction directly into another program difficult.

Creating Consistency between Raw Datafiles

Without altering the formatting or contents of the original datasheets, into each raw data file was added a new worksheet universally labeled "input". Each "input" sheet contains a single header row containing variable names for variables of interest, and a unique row for each sample. To avoid copy/paste errors, sample names (usually the 12-digit filter ID) and corresponding data are

linked directly with cells in the raw data sheets scattered elsewhere in the workbook. The raw data sheets are locked from editing to prevent accidental alteration.

III. Compilations of Multiple Electronic Files into a Master File

Justification:

The data for PM 2.5, EC/OC/TC, 1-NP and other NPAHs in raw datafiles are in units of mass per filter or mass per sample (extract concentration for 1-NP and other NPAHs). To convert these to units of mass per volume of air, this data needed to be merged with air volume data from the pump calibration field logs. The urinary PAH data and the questionnaire data needs to be combined with the air exposure data also, for analysis. This will allow for comparisons within and between samples of all available measurements.

Method:

Stata 13 will be used to import and merge the raw and processed data. The Stata code for input and merge commands are in various ".do files." . Merge commands will be saved in executable files (.do files) and thus every step of the air data compilation is traceable and reproducible. They will be stored here: *S:\lab\EZ_Diesel2014.*

Data Compilation (Urine, questionnaire and air):

Each distinguishing urine sample id will be separated in excel using the text to column feature. This will separate each separate component of the sample id into its own column (i.e. month, day, type of sample, subject id and time of void). These newly generated columns will make it possible to match data with other spreadsheets (1-NP urinary data, air data and questionnaire data) based on subject id and the date of sample collection.

Urinary PAH concentrations will be imported to Stata from the master input file found at *S:\lab\ EZ_Diesel2014\Urinary PAH Data\consolidated urinary PAH data.* The corresponding Stata code will also be downloaded. This code is not yet written, but once it is, it will be stored in *S:\lab\ EZ_Diesel2014\Urinary PAH Data*. This will be saved in an intermediate Stata file.

urine full dataset JMR 5_22_15 will be merged with the urinary PAH values. This code will match samples based on the unique combination of subjects id and sample date (ddd.mm), the commonality between all workbooks. Note that there are not 1-NP metabolite values for all of samples, because due to budget limitations, not all urine samples could be analyzed for 1-NP metabolites. This will be saved in an intermediate Stata file.

Next the questionnaire data will be combined with the urine data. The input sheet containing the questionnaire data (*survey data table_03may2015)* will be imported into Stata and saved as an additional intermediate file. This will be merged with the file containing all of the compiled urine data by matching the subject ID and date of the samples. This intermediate data set will be saved.

Lastly the air data will be combined with the urine/questionnaire Stata file. It will be merged with by matching the date and subject ID, and will be saved as **"Final data.dta"** to make the final, complete dataset.

All intermediate Stata files will be saved to *S:\lab\ EZ_Diesel2014*

List of intermediate Stata do files:

Input do file
Data Analysis

List of imported .xlsx data:
Consolidated urinary PAH data
urine full dataset JMR 5_22_15
survey data table_03may2015
compiled air data_2july2015

intermediate .xlsx files used for compilation of data for master file:
EZ_data – Urinary PAH data
1NP_data – Urinary 1-NP data
PAH_1NP_Urinedata – Combined urinary PAH and 1-NP data
questionairredata – Questionnaire data
PAH_1NPUrine_questionairredata – Combined urinary PAH and 1-NP data and questionnaire data
air_data – Air data
PAH_1NPUrine_questionairre_airdata – Combined urinary PAH and 1-NP data, questionnaire and air data used for data analysis