Evaluation of PAH Metabolites as Biomarkers for Occupational Wood Smoke Exposure in Wildland Firefighters

Spencer Birch

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Committee:
Christopher Simpson
Lianne Sheppard
Catherine Karr

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Abstract

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Spencer Birch

Chair of Supervisory Committee:
Professor Christopher D. Simpson, PhD, MSc

Environmental and Occupational Health Sciences

Exposure to wood smoke has been associated with lung cancer and other adverse health outcomes, and therefore it is a great health concern for those in developing parts of the world as well as developed nations. Pyrene, naphthalene, fluorene, and phenanthrene are components of wood smoke and their metabolites have been frequently used as biomarkers of exposure to combustion emissions. In this study, we explored the use of polycyclic aromatic hydrocarbon (PAH) urinary metabolites to determine their effectiveness as biomarkers for wood smoke exposure.

We recruited ten professional firefighters and two volunteers certified to work as firefighters on prescribed burns who performed controlled burns on the United States Department of Energy's Savannah River Site (SRS) in North Carolina. We quantified metabolites in pre-shift and post-shift urine samples from the firefighters on 7 prescribed burn days and 3 non-burn days, which resulted in a total of 68 urine sample pairs (48 on burn days and 20 on non-burn days). We collected 69 personal air samples, including 48 samples on burn days and 21 samples on non-burn days; these were analyzed for $PM_{2.5}$, levoglucosan, and light absorbing carbon (LAC). We also measured personal carbon monoxide (CO) exposures in conjunction with the personal air filter samples. We investigated associations between cross-shift PAH metabolite concentrations and the 2 main tasks performed, holding, and lighting, as well as the association between the air contaminants and PAH metabolite concentrations. The subjects'

breathing rate, derived from accelerometry data, was used to calculate the inhaled dose of particulate matter, levoglucosan, and LAC.

We found a significant increase in geometric mean (GM) cross-shift metabolite concentration for all six metabolites on burn days and no significant change in GM cross-shift concentration for all six metabolites on non-burn days. 2-Napthol (2-Nap), 2+9-hydroxyfluorene (2+9-Flu), and 3-hydroxyphenanthrene (3-Phen) had the largest GM cross-shift ratios of 2.69, 2.61, and 3.19 respectively on burn days. When stratified by task, subjects that performed lighting had significant increases in the GM cross-shift ratio for all six metabolites (Range: 1.72-3.19), and for those who performed holding, all six metabolites exhibited an increase in GM cross-shift ratio, but none were significant. The burn day exposure data shows that those who performed lighting had a 4.2-fold higher GM LAC absorption coefficient, and a 2.6-fold lower levoglucosan/gravimetric $PM_{2.5}$ ratio. This pattern of higher LAC with lower relative levoglucosan provides strong evidence that subjects who perform lighting are exposed to much lower levels of wood smoke than those who are holding. It also suggests that the large increase in GM cross-shift metabolite ratio in lighters is most likely due to exposure to the combustion emissions from the drip torch. Since those who performed holding had much higher levels of wood smoke exposure and substantially lower GM cross-shift metabolite ratios, it appears OH-PAH metabolites may not be effective biomarkers of wood smoke exposure in this setting. Strong associations were observed between LAC and 2-Nap, 2+9-Flu, and 3-Phen.

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Background and Significance:

Wildland fighters are generally responsible for extinguishing wildfires, preparing for fires, and conducting controlled burns. They are subject to a number of hazardous exposures that cause both acute and chronic adverse health effects. Some of these exposures include heat, ergonomic stressors, and wood smoke. Wood smoke exposure is a prominent hazard for wildland firefighters performing controlled burns (Reinhardt et al., 2000; Therriault 2001; USDA-Forest Service 1989). During controlled burns, wildland firefighters often do not use respiratory protection as the work can be very physically strenuous (Naeher et al., 2007). Combining elevated wood smoke levels with high respiratory rate, wildland firefighter's dose is potentially quite high. Consequently, wildland firefighters have been shown to be at a higher risk of developing wood smoke exposure-related morbidities (Gaughan et al., 2014). To assess their exposure to wood smoke, an inexpensive, fast, and effective method of quantifying their dose needs to be determined that is more efficient and cost-effective than personal sampling. One potential method is to use biomarkers of wood smoke exposure.

Biomonitoring is an effective way to assess one's dose and offers many advantages over personal air sampling as it accounts for physiological differences and personal behaviors of a subject. Urinary OH-PAH metabolites have shown promising results as biomarkers for wood smoke exposure, as subjects exposed to wood smoke have shown an increase in cross-shift urinary concentrations (Strickland et al. 1999, Li et al., 2015, Adetona et al., 2015, Li et al., 2016). Our long-term goal is to discover an effective biomarker for wood smoke exposure that could be used to define biological exposure indices.

Wood smoke composition and health effects:

The wood smoke and other airborne contaminants firefighters are routinely exposed to may contribute to a variety of different health morbidities. Chronic exposure to wood smoke has been associated with

respiratory infections, impaired lung function, myocardial infarctions, asthma, bronchitis, and cancers (Fernando et al., 2016; Therriault 2001, Reinhardt et al., 2000, USDA-Forest Service 1989). Wood smoke contains a plethora of combustion and pyrolysis compounds including black carbon, carbon monoxide, methoxyphenols, and polycyclic aromatic hydrocarbons (PAHs). Some PAHs such as benzo(a)pyrene have been shown to be carcinogenic and mutagenic (Li et al., 2008). While the incidence of cancer in wildland firefighters has not been thoroughly investigated, several recent studies of structural firefighters have suggested an excess of colorectal, skin, brain, and myelogenous cancer deaths (USDA-Forest Service 1989).

CO is ubiquitous with the combustion of biomass and is produced in highest concentrations when fire is smoldering. Acute inhalation exposure to CO can cause a variety of short-term symptoms such as headaches, dizziness, visual impairment, reduced work capacity, and reduced manual dexterity. Other toxic combustion products include organic compounds acrolein, formaldehyde and benzene, as well as inorganic gases including oxides of nitrogen and ozone. Acrolein has an intense choking odor and even at low levels, acts as an eye and upper respiratory tract irritant. Acute low-level exposure to formaldehyde can cause irritation to the eyes, nose, and throat, and even the lower respiratory tract. Benzene and formaldehyde are also known carcinogens (Therriault 2001).

The airborne contaminant that likely poses the largest health risk is particulate matter (Therriault 2001). Particulate matter produced by the combustion of biomass is a mixture of solid particles and liquid droplets of small size. The size distribution of particulate matter in wood smoke was found to be bimodal, with one mode having a mean-mass diameter of 0.3 micrometers and the other with a mean-mass diameter of over 10 micrometers. 40-70% of the respirable particulate matter, particles with median aerodynamic equivalent diameter of less than 2.5 micrometers, is made of organic carbon material and many of the carcinogenic compounds are part of this fraction. Roughly 2-25% of the fine

particles are elemental carbon. The respirable particles penetrate the upper respiratory tract to the alveolar region of the lungs where the particles are generally retained. Acute and chronic exposure has been linked to adverse respiratory and cardiovascular effects such as loss of pulmonary function due to irritation and inflammation of lung tissue, heart disease, and increased susceptibility of viral and bacterial pathogens. Exposure is also known to exacerbate pre-existing cardiovascular and respiratory conditions such as COPD (Reinhardt et al., 2000; Therriault 2001; USDA-Forest Service 1989).

After exposure to respirable particles during a prescribed burn, one study reported an average cross-shift decrease in FEV_1 in one study of 0.05 L (95% CI (-0.05-0.16 L)). A typical FEV_1 is 4.5 L for a 30-year-old male (CDC). When stratified by age, the average decrease in FEV_1 was 0.10 L for those over 30 years old which may mean that those who are older may be more susceptible (Gaughan et al., 2014). There was a larger decrease seen for those exposed to a higher concentration of respirable levoglucosan, indicating the change in FEV_1 is attributed to wood smoke.

In a study examining the composition and abundance of 15 PAHs in wood smoke in Chile, the investigators found that pyrene, phenanthrene, fluorene, and naphthalene are some of the main PAH contaminants in the air. They estimated that 80-95% of PAHs measured in the air in this study were due to wood combustion (Pozo et al., 2015). Therefore, metabolites of these PAHs are potentially effective biomarkers as they are main components of the PAHs in wood smoke and may accurately reflect the amount of wood smoke exposure one receives.

A unique component of wood smoke is levoglucosan, which is a pyrolysis product of the combustion of vegetative biomass. It is specific to the combustion of biomass and is used as a tracer for wood smoke in ambient air samples. (Gaughan et al., 2014).

Past Wood Smoke and PAH Exposure Studies:

In one recent study where wildland firefighters were conducting controlled burns, 1-napthol, 1-hydroxypyrene and 2-hydroxyphenanthrene had cross-shift ratios of 4.23, 1.83 and 2.81 respectively and the proportion of subject-days with higher post-shift vs. pre-shift concentrations was 95, 88, and 95% respectively (Adetona et al., 2015). In another study where participants were exposed to campfire smoke in a controlled exposure study, hydroxylated naphthalene, fluorene, and phenanthrene metabolites were shown to have a significant increase in concentration in post-exposure samples (Li et al., 2015).

A recent comparison study in a large cohort compared exposure to wood smoke using traditional (open-fire stoves) with new chimney-equipped cook stoves, which were presumed to reduce wood smoke exposure. A statistically significant, 23% lower urinary concentration of 2-napthol was observed for those using the new style stove. CO measurements taken during the exposure periods confirmed lower exposures to biomass combustion emissions for those using the new style stove. (Li et al., 2016). Another study showed similar results, in which two people were exposed to wood smoke and had average OH-PAH urinary concentrations that were 4.4 times higher than NHANES (background) concentrations after exposure (Motorykin et al. 2015).

Other studies have evaluated PAH exposure through ingestion of PAH containing food to determine the efficiency at which they are metabolized and at what quantity they are detected in the urine. These studies too have found that OH-PAH metabolites are detectible in elevated quantities in urine in post-exposure compared to pre-exposure samples (Motorykin et al. 2015).

In other industries, urinary PAH metabolites have been shown to have an increase in cross-shift concentrations after inhalation exposure to PAHs as well. Workers exposed to a variety of airborne

PAHs in an aluminum smelter showed a statistically significant increase in urinary 1-OHP following exposure. The adjusted R^2 value between urinary 1-OHP and airborne pyrene, coal tar pitch volatiles, and benzo(a)pyrene were 0.84, 0.84 and 0.79 respectively indicating a strong correlation (Tjoe et al. 1993).

In a cohort of offshore oil production workers who were exposed to PAHs via inhalation when working inside crude oil cargo tanks, there was a statistically significant increase in cross-shift urinary PAH concentration. For tank workers, the pre-shift creatinine adjusted 1-OHP concentration was 0.137 µg/g creatinine and the creatinine adjusted post-shift 1-OHP concentrations was 0.281 µg/g (Hopf et al. 2010). These studies show the potential for PAH metabolites to be used as biomarkers for exposure for wood smoke and other sources of PAH exposure as well.

PAH metabolites, bioavailability, stability, and half-life:

After introduction into the body, PAHs can be metabolized to reactive epoxide intermediates through phase 1 biotransformation by cytochrome P450 enzymes. The epoxide groups can then be reduced or hydrolyzed to hydroxyl functional groups. During phase 2 biotransformation, the hydroxyl functionalized PAHs undergo a conjugation reaction with either glucuronic acid or sulfate. This increases the compound's affinity for polar substances and thus is more prone to be excreted from the body in the urine. It has been found that compounds with either 2 or 3 aromatic rings are more likely to be excreted through the urine and larger molecular weight compounds, such as 1-OHP, are more prone to be excreted through the feces (Li et al., 2008).

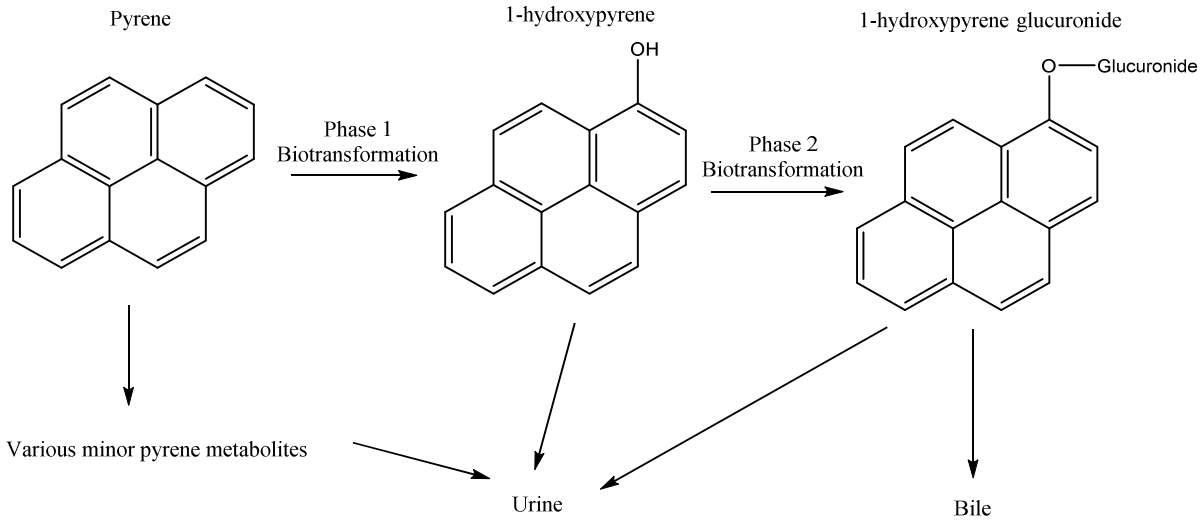

Figure 1: Metabolism of 1-OHP (Derived from ACGIH 2003)

PAH metabolites feature several characteristics that potentially make them effective urinary biomarkers of wood smoke exposure. First, these PAHs are typically retained in the lungs and metabolized at appreciable levels. Using 1-hydroxypyrene (1-OHP) as an example, it was found that 61% of inhaled pyrene was retained in the lungs and that the metabolic efficiency of pyrene was 29%. This means that 29% percent of the pyrene retained in the lungs was detected in the urine as 1-OHP (Brzeźnicki et al., 1997). Another source stated that 2-20% of inhaled pyrene was excreted into the urine as 1-OHP (ACGIH, 2003). Another favorable feature is the stability of the biomarkers. Once collected, the samples can be kept for up to a year without significant degradation if frozen at the correct temperature and kept away from light (Jongeneelen et al., 1987).

The half-life of the OH-PAH metabolites make it possible to obtain recent quantitative information regarding a person's respiratory exposure to PAHs (Li et al., 2008, Li et al., 2015). If the half-life is too short, on the order of minutes or an hour or two, the metabolite concentration would decrease too quickly and it would be difficult to detect in the next collected urine sample. If it is too long, on the order of days or more, recent exposures could not be easily monitored as they would be confounded by past exposures.

In one study, the half-life of 1-OHP was found to be 9.8 hours after acute respiratory exposure when described as a one compartment model (Brzeźnicki et al., 1997). However, the half-life of 1-OHP varies depending on route of exposure as well as the distribution of 1-OHP into blood, lean tissue, and adipose tissue (ACGIH, 2003). Inhalation exposure typically has the shortest half-life, followed by ingestion, then dermal exposure (Li et al., 2008.) The half-life in one simulation study that utilized a three-compartment model was 5.5, 23, and 384 hours and the distribution was 60%, 38%, and 2% for 1-OHP in blood, lean tissue and adipose tissue respectively after several weeks of constant 5-day-a-week exposure (ACGIH, 2003). Reported half-lives of other OH-PAHs tested in this study are listed in Table 1.

Table 1: Estimated Half-lives of OH-PAH metabolites

Metabolite	Median 1/2 life, (95% CI) hours
2-Napthol	6.3 (4.9-9.05)
1-Napthol	6.6 (4.8-10.5)
2-hydroxyfluorene	8.4 (6.0-14.2)
9-hydroxyfluorene	7.7 (5.7-11.6)
2-hydroxyphenanthrene	9.9 (6.1-24.8)
3-hydroxyphenanthrene	11.0 (7.2-23.5)
1-hydroxypyrene	23.5 (13.5-92.0)

(Li et al., 2015)

Wildland Firefighter Tasks:

There could be a great amount of variability in total exposure and dose within the two main tasks of lighting and holding that are performed by the wildland firefighters while conducting controlled burns (Reinhardt et al., 2004). The first task, lighting, is the task that involves igniting designated areas of vegetative biomass. This is done with a liquid fuel that is made up of 3 parts diesel fuel and 1-part gasoline with a device called a drip torch while the firefighters are on foot. If lighting needs to be done on a larger scale, it is done from helicopters. The other main task is holding, where the firefighter keeps the fire burning within a designated area. This is done by clearing paths of biomass fuel around burn

sites, using shovels, bulldozers, fire trucks, mule trucks, and water hoses to constrain the fire within specific boundaries.

A third task called “mop-up,” entails completely extinguishing the embers and residual fire after the vast majority of the biomass has been burned. In one study, the geometric mean (GM) total and respirable levoglucosan concentrations for those performing mop-up (1.13 and 0.28 $\mu g/m^3$ respectively) were significantly lower than those who were working at the fire-line (3.64 and 2.59 $\mu g/m^3$ respectively) indicating a substantially lower wood smoke exposure (Gaughan et al., 2014). Thus, the exposures associated with mop-up are smaller compared to the two other main tasks. No samples were collected in the current study from subjects that performed “mop-up.”

The composition of wood smoke has been shown to vary for the other two tasks. In preliminary studies, there was a difference in CO concentrations between burn-day tasks, with holding being significantly higher than lighting. Also, there is a difference in the particle mass light absorption efficiency, between lighting and holding, with lighting having a mean value of nearly three times that of holding. Interestingly, there was no detectable difference in the $PM_{2.5}$ exposures between the two burn-day tasks (Adetona, 2016). This is important information to know as different exposures will require different controls to mitigate the hazards, and may be differentially associated with health outcomes. Quantifying levoglucosan exposure as will be done in the current study, will help to differentiate the source of the PAHs, as they also can be produced by the drip torch and fire engines, in addition to wood smoke.

Wildland firefighters performing lighting are responsible for starting the fires and not controlling them and generally do not remain in the burn area as long as someone who is holding. Therefore, it is hypothesized that those performing lighting may have lower wood smoke exposures than those who are

holding and therefore lower PAH metabolite levels. Since LAC (light absorbing carbon), $PM_{2.5}$, levoglucosan, and CO are products of combustion of biomass, we hypothesize that there will be a positive association with these air contaminants and PAH metabolite levels. In this study, we will use LAC as a surrogate measure for black carbon, which is a product of incomplete combustion.

Preliminary Studies:

Preliminary research completed by researchers at the University of Georgia shows that the composition of the smoke varies depending on the task the firefighter is performing (Figure 2). This is the same study in which the urine samples analyzed in the current study were collected. These data suggest that the urinary PAH metabolite levels may show a differential response to exposure for these two tasks, since the smoke composition varies significantly with respect to the three listed air contaminants.

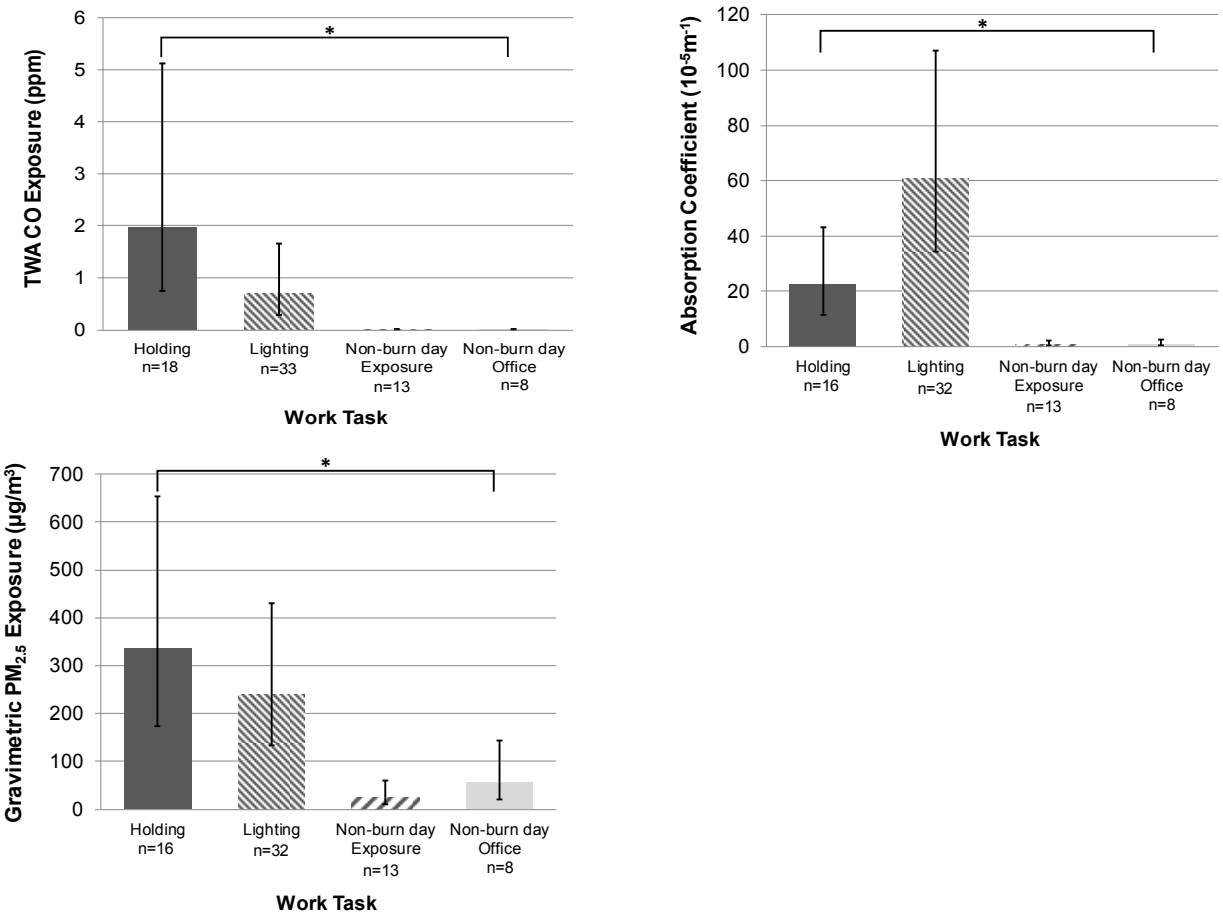

Figure 2: Exposure to Components of Wood Smoke Differentiated by Task (from Adetona et al, 2016)

* work tasks are statistically significantly different from each other ($p<0.05$) (These values were obtained from a mixed model output)

Methods:

Setting, cohort, sample collection:

The sampling was conducted from January 2015 to July 2015 in the United States Department of Energy's Savannah River Site (SRS) in North Carolina. This is a repeated measures design. The SRS is a nuclear materials production plant and occupies 310 mi^2 of land. Before being acquired in 1950, the SRS land consisted of 33% crop use and 67% forested area. Every year the SRS conducts prescribed burns on 20,000 acres of the property to reduce the risk of a forest fire, and to renew ecological processes – fire is a natural part of forest ecosystems in the southeast United States (Workman et al., 1990). The main types of biomass on the SRS are hickory, oak, holly, gum, red maple, mixed hardwoods, and cypress. This is important to note as the type of biomass burned can influence the level of exposure to PAHs one receives (Motorykin et al., 2015).

The cohort included twelve subjects who volunteered for the study. Ten were wildland forest firefighters who were employed by the United States Forest Service-Savannah River (USFS-SR) in South Carolina and the other two were volunteer firefighters who were certified to work on controlled burns. The subjects were not currently smokers, none were pregnant, were at least 18 years of age, and were in good health overall. They signed an IRB consent form for the study processed through the University of Georgia, Athens, Georgia.

For burn days, the two tasks of holding and lighting are considered. The non-burn day tasks are separated into two categories, "non-burn day exposures" and "non-burn day office." For "non-burn day exposure," the subjects performed tasks such as patrolling, engine maintenance, and field prep work, which may expose them to vehicle exhaust and dust from field prep work.

From January 2015 to March 2015, air sampling and urine sample collection were performed for four prescribed burn days and two non-burn days. From May 2015 to July 2015, air sampling and urine sample collection were performed for three prescribed burn days and one non-burn day. The personal exposure monitoring was conducted for workdays when prescribed burns were performed and workdays when no prescribed burns were performed.

The work days monitored were not the only days these subjects worked during this period from January 2015 to July 2015. The work days in which sampling was done were spread out so that there was little to no carryover of a previous exposure in the urine samples. In other words, we did not want past exposure of wood smoke to confound the samples collected for this study. However, there were seven subjects that had worked a prescribed burn only one day before they were monitored for a work day in our study, and one subject who worked a prescribed burn day two days prior to being monitored for a work day in our study. A controlled wood smoke exposure study examining the half-lives of metabolites measured for this study shows that the metabolites return to background levels approximately 20 hours after exposure stops. Therefore, the level of confounding from these samples should be minimal.

The personal air sampling was measured for each shift of each firefighter; it consisted of real-time carbon monoxide (CO), gravimetric $PM_{2.5}$, real-time $PM_{2.5}$, levoglucosan, and LAC. All of these measurements were taken in the breathing zone. In addition, subjects wore accelerometers, from which estimates of breathing rate were obtained.

From seven prescribed burn days, 54 person-days of air samples were collected from 12 subjects. Five of the gravimetric filter samples and five real-time $PM_{2.5}$ samples were not included in the analysis due to pump failure in the field. One additional gravimetric filter sample was excluded due to a problem with

the o-ring on the cassette. One CO sample was not collected for a burn day due to the sampling device being lost during the work shift.

From three non-burn work days, 21 person-days of air samples were collected from eight subjects. Collectively, a total of 69 personal air filter samples from both burn and non-burn days were collected. There were also 11 field blanks collected, one for each day sampling was conducted plus one for a MicroPEM.

Table 2: Number and Type of Air Samples Collected per Subject

	Burn day			Non-burn day		
	Filters	Real-time	CO	Filters	Real-time	CO
Subject 1	5	5	5	3	3	3
Subject 2	5	5	6	3	3	3
Subject 3	5	5	5	3	3	3
Subject 4	4	4	5	3	3	3
Subject 5	7	7	7	3	3	3
Subject 6	5	6	6	2	2	2
Subject 7	5	5	6	2	2	2
Subject 8	1	1	1	0	0	0
Subject 9	5	5	6	2	2	2
Subject 10	3	3	3	0	0	0
Subject 11	1	1	1	0	0	0
Subject 12	2	2	2	0	0	0
Total	**48**	**49**	**53**	**21**	**21**	**21**

A total of 223 total urine samples were collected including 202 unique samples and 21 duplicate samples. 144 of the samples were collected on burn days and 58 were collected on non-burn days. Pre-shift and post-shift urine samples were collected for each of the 75 person-days that personal air samples were collected. The pre-shift and post-shift urine samples were collected just before the subjects began their work shift, and as soon as possible after they completed their work shift respectively. However, some urine samples are missing as some subjects could not provide a sample when needed, resulting in a few incomplete sample pairs. Urine samples were also collected on the day

following the personal sampling (morning after samples) for each subject that was involved in the previous day's work. Again, some of these samples were not able to be collected.

Table 3: Number of Urine Samples Collected on Burn Days per Subject

	Complete pre-post pairs	Sampling day pre-shift	Sampling day post-shift	Morning after (MA)	Complete MA-Pre pairs
Subject 1	2	4	3	3	2
Subject 2	6	6	6	4	4
Subject 3	5	5	5	3	3
Subject 4	5	5	5	5	5
Subject 5	7	7	7	7	7
Subject 6	6	6	6	5	5
Subject 7	5	5	5	5	5
Subject 8	1	1	1	1	1
Subject 9	6	6	6	6	6
Subject 10	3	3	3	2	2
Subject 11	1	1	1	1	1
Subject 12	1	1	2	2	1
Total	**48**	**50**	**50**	**44**	**42**

Table 4: Number of Urine Samples Collected on Non-burn Days per Subject

	Complete pre-post pairs	Sampling day pre-shift	Sampling day post-shift	Morning after (MA)	Complete MA-Pre pairs
Subject 1	3	3	3	1	1
Subject 2	3	3	3	3	3
Subject 3	3	3	3	1	1
Subject 4	2	3	2	3	3
Subject 5	3	3	3	3	3
Subject 6	2	2	2	2	2
Subject 7	2	2	2	2	2
Subject 8	0	0	0	0	0
Subject 9	2	2	2	2	2
Subject 10	0	0	0	0	0
Subject 11	0	0	0	0	0
Subject 12	0	0	0	0	0
Total	**20**	**21**	**20**	**17**	**17**

Table 5: Number of Person-days of Samples by Task

	Task	Person-days	Air filter samples
Burn day	Holding	19	16
	Lighting	33	30
	Lighting by helicopter	2	2
Non-burn day	Non-burn day exposure	13	13
	Non-burn day office	8	8
	Total	**75**	**69**

Table 6: Time of Urine Sample Collection

	Burn days		Non-burn days	
	Ave	Range	Ave	Range
Pre-shift	8:13	6:45-10:00	8:46	7:10-11:00
Post-shift	15:33	12:00-19:25	16:14	14:05-17:55
Morning After	8:31	7:20-12:06	9:11	7:55-10:40

Questionnaire Info:

After enrolling and prior to performing any work in the study, each subject filled out a "baseline questionnaire." They were asked about their personal characteristics such as their age, weight, height, work history, diet, physical activity, current medications, non-occupational wood smoke exposure, smoking habits and other questions related to pre-existing health conditions and possible sources of confounding for the measured PAH metabolite concentration in urine. This questionnaire was only administered once prior to the start of the study.

On each sampling day, for all burn days and non-burn days, the subjects filled out a "daily questionnaire" that gathered information regarding the tasks they performed such as the proportion of time spend doing each task, what their primary work task entailed, what other tasks were performed, the number of days since the last prescribed burn they worked, respirator use, and information about various possible confounding exposures such as second-hand smoke. If a subject spent more than 50% of their time working the same task, then that task was considered their primary task. A field technician

recorded the duration of each subject's exposure along with the area of the prescribed burn and the time and date of the burn.

Each subject also filled out a "morning questionnaire" the morning after they completed their work shift that was monitored in this study. This questionnaire asked for information regarding their activities, possible confounding exposures including non-occupational wood smoke exposure, cigarette smoke, certain foods, and medications/supplements taken.

Instrumentation/Methods for Collecting Air Samples:

The air samples were collected by University of Georgia researchers. A sampling device called a MicroPEM (RTI International, Research Triangle Park, NC, USA) was used to measure the gravimetric $PM_{2.5}$ and the real-time $PM_{2.5}$ levels in the breathing zone. It used an impactor that had a cut point for particles with a median aerodynamic diameter of 2.5 microns. The gravimetric samples were collected on pre-weighed 25 mm PTFE filters (Pall Life Sciences, Ann Arbor, MI, USA) with 3 µm porosity. The light-scattering response for the MicroPEMs were zero-calibrated before use by using a low-pressure drop HEPA filter. The flow was calibrated using an air flow meter (TSI 4100 flowmeter; TSI Incorporated, Shoreview, Minnesota, USA). The MicroPEM ran for 20 seconds every minute during the sampling period at a flow rate of 0.4 L/minute, giving a 33.3% duty cycle. At the completion of each sampling day, the real-time $PM_{2.5}$ data was downloaded to a software program (MicroPEM Docking Station version 3.2, RTI International, Research Triangle Park, NC, USA) and the data log was reset on the monitoring device. The continuous $PM_{2.5}$ measurements were averaged over the entire sampling period. The filters in the MicroPEMs were also analyzed for levoglucosan and LAC.

Accelerometer:

The MicroPEM also had a built in triaxial accelerometer that measures activity of the subject to data log the movement of the worker throughout the work-shift so that the pulmonary ventilation rate can be

estimated. Since the exposure and thus dose can be vastly influenced by respiratory rate, it is essential to determine the respiratory rate of the subjects in order to calculate an accurate dose. A linear regression is used to convert the activity measurements from the MicroPEM to a ventilation rate (Adetona et al., 2016)

CO data collection:

Continuous CO data was obtained by using a Dräger Pac III single gas monitor which recorded the concentration in air every 30 seconds for the entire sampling period in the subject's breathing zone. Each Dräger Pac III monitor was calibrated to zero ppm using pure nitrogen gas and then was calibrated with 100 ppm CO span gas. TWA calculations were calculated by averaging the recorded CO levels over the sampling period.

Sample Analysis:

Urine analysis: The samples were collected in the field by University of Georgia researchers and then shipped to the University of Washington where they were stored frozen. The samples were thawed and prepared for analysis by performing hydrolysis and solid phase extraction before being analyzed by HPLC-fluorescence. The full procedure used for analyzing the urine samples in listed in Appendix 2. In brief, PAH metabolites were identified by matching the retention times of analyte peaks in the samples, to those of a series of authentic standards. The metabolites quantified included 2-napthol, 1-napthol, 2-hydroxyfluorene, 9-hydroxyfluorene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, and 1-hydroxypyrene. The urine samples were analyzed in 20 individual batches, with an average of 16 samples per batch. The pre/post/morning after triplets were not kept in the same batch.

Urine sample data work up: The 2-napthol, 1-napthol, 2-hydroxyfluorene + 9-hydroxyfluorene (they elute as one peak), 2-hydroxyphenanthrene, and 3-hydroxyphenanthrene data were not adjusted for 1-

hydroxypyrene-d^9-glucuronide, only 1-hydroxypyrene was. The reason for this is that when 1-hydroxypyrene-d^9 glucuronide is hydrolyzed, it produces 1-hydroxypyrene, and it is not known how much different the hydrolysis efficiency or recovery is for the other metabolites compared to 1-hydroxypyrene. In our preliminary analysis, we observed that adjusting the 1-hydroxypyrene concentration based on the recovery of the 1-hydroxypyrene-d^9 glucuronide internal standard improved precision for measurement of 1-hydroxypyrene; but did not consistently improve precision for the other PAH metabolites (Appendix 1, Table 40).

The raw extract concentrations were corrected for injection volume since injection volume varied between batches. The urine concentration was calculated from the extract concentration by using the following formula:

$$\frac{\text{extract concentration } \left(\frac{\text{pg}}{\mu\text{L}}\right) * 220\ \mu\text{L extract vol}}{\text{urine vol used (mL)}} \textbf{ = pg/mL urine metabolite concentration}$$

With each batch of samples, benchmark urine samples were run along with the study samples. They were urine samples that were used to monitor performance and consistency of the assay and instrument. "Hi Miner" was used for batches 6-8, "Lo Miner" for batches 9-13, and "5+16" for batches 14-20. A benchmark adjustment was made to all data that was collected in sample batches that contained benchmark samples so that any assay or instrument differences between batches could be adjusted for. The formula used:

urine metabolite concentration (pg/mL)* $\frac{\text{Overall average of benchmarks}}{\text{Average benchmark for a particular batch}}$ **= benchmark adjusted urine concentration (pg/mL)**

- The "Overall average of benchmarks" is the average of all benchmarks of a particular benchmark type. Taking "Hi-Miner" as an example, it is the average across all batches of samples where the "hi miner" benchmark sample was used.

- Average benchmark for a particular batch is the average metabolite concentration for the benchmarks samples used in that batch.

To determine if there was substantial variation between groups of batches, a covariate was tested in the Aim 1 models that was coded 0 for no bench mark used, 1 for "Hi Miner," 2 for "Lo Miner," and 3 for "5+16" benchmark. Only negligible changes were observed, so this covariate was not included in any analyses.

Gravimetric $PM_{2.5}$: The filters were equilibrated for 48 hours in a climate controlled laboratory prior to pre-weighing. After removal from the MicroPEMs, the PTFE filters were stored in a laboratory at 20°C and 40% humidity for 48 hours prior to post-weighing. The filters were pre-weighed twice and post-weighed twice at the University of Georgia's Department of Environmental Health Science laboratory using a Cahn C-35 microbalance (Thermo Electron, Waltham, MA, USA). The sensitivity of the scale is ±1.0 µg. Afterwards the filters were shipped to the University of Washington, and stored at -20°C in order to preserve the LAC and levoglucosan from degradation.

LAC: The light absorbing carbon was used as a surrogate for black carbon, and measurements were made by reflectance analysis. The instrument used was the Evans Electroselenium Limited smoke stain reflectometer (Model 43D, Diffusion Systems Ltd, London, United Kingdom). When light is shined on the sample, some is absorbed, and the rest is reflected and this quantity of reflected light is measured from a 0-100% linear scale. The instrument was calibrated before and after the measurement of each sample, using a calibration plate provided by the manufacturer. The calibration plate has two separate circular areas, one white and the other gray. The white area is used to zero calibrate the instrument and the gray area is used to calibrate the instrument with a standard, known absorbance value. The following equation (ISO 9835) was used calculate the absorption coefficient:

$$a = \left(\frac{A}{2V}\right)\ln\left(\frac{R_b}{R_s}\right)$$

a= absorption coefficient (m^{-1})

A= area of the sample (m^2)

V= volume of air sample (m^3)

R_b= reflectance of a field blank filter

R_s= reflectance of sample filter

Since reflectance measurements were not made on the filters prior to using them for sampling, the average reflectance of the 11 field blanks was used. The average % reflectance of the blanks was 69.3% (SD: 0.9%, 1.3% CV). For convenience, the calculated values of *a* were multiplied by 10^5.

Levoglucosan analysis: After the air filter samples were analyzed for LAC, we analyzed them for their levoglucosan content. The full procedure is in Appendix 3. In brief, the filters were cut and placed into a test tube, spiked with a d^7-levoglucosan internal standard and extracted by sonication in methylene chloride. After evaporating the methylene chloride, the samples were reconstituted in 0.5 mL of an ethyl acetate, trimethylamine, and anhydroheptulose solution. Next, derivatization was performed by adding 10 µL of pyridine, 50 µL of N-trimethylsilyl-N-methyl trifluoroacetamide + 1% trimethylsilyl chloride solution, and 50 µL of sample aliquot into GC vials and analyzing with GC-MS. The levoglucosan was derivatized to trimethylsilyl derivatives to enhance the sensitivity and instrument performance. QC samples, the field blanks, and calibration standards were run along with the filter samples.

Creatinine: Aliquot (0.4 mL) of the urine samples were sent to a clinical laboratory in the University of Washington Medical Center for analysis, using a Beckman Coulter AU Analyzer.

Specific gravity: An ATAGO A300CL Handheld Clinical Refractometer was used to measure the urine specific gravity. It has a range of 1.000-1.050, can read specific gravity in 0.001 increments, and has an accuracy of ±0.001. Prior to making measurements, the refractometer was zero-calibrated with water. Roughly 180/202 of the urine samples submitted to the University of Washington Laboratory for creatinine analysis were returned to our laboratory and these were used for the specific gravity analysis. The rest of the specific gravity measurements were made using urine from the 15 mL plastic screw top test tubes that the urine samples were sent to us in by the UGA. 2-3 drops of the urine were placed on the glass lens of the refractometer for each measurement. The refractometer was rinsed with deionized water and dried with a Kimwipe after each measurement.

Data Manipulation and Statistical Data Analysis:

The distribution for the urine metabolite data and the air data was assessed with Q-Q plots for both the native and natural log-transformed versions of the data (Refer to Appendix 1, Figure 5). The between and within variance of the urine metabolite data was determined numerically from the mixed models. All further analyses were performed with natural log-transformed urine metabolite and air data.

Specific gravity measurements were used as the urine dilution correction in this analysis. Specific gravity correction has been shown to be a more robust correction than creatinine adjustment (Suavé et al., 2015). Frequently, creatinine adjusted urine dilution has been used based on the assumption that creatinine is excreted from the body at a constant rate for all individuals, but this is not always the case. Recent research has shown that the rate of creatinine excretion can vary depending on a range of factors including age, sex, muscle mass, and time of day. For example, the difference in median creatinine measurements between men and woman was 14.4 mmol/L and 10.2 mmol/L respectively, whereas the specific gravity was 1.024 for men and 1.020 for women, which is a smaller percent difference. These factors have a much smaller influence on urine specific gravity (Suavé et al., 2015).

There is no universally agreed upon value for an average or "typical" urine specific gravity. One study examining the effect of specific gravity normalization in xenobiotic biomarkers of a cohort of smokers states that the mean specific gravity ranges from 1.016 to 1.024 (Heavner et al., 2006). Another study that assessed the effect of specific gravity normalization for urinary biomarkers of isocyanates stated that 1.020 is the mean urine specific gravity value (Gaines et al. 1020). The value we used in the current study is 1.020.

The data analysis was performed using Stata version 13. All data used in the analysis was inputted into excel files and then imported into Stata and merged by using subject ID, date of sample collection, or sample number, depending on which were available for each set of data. The urine metabolite data and air data separately were assessed descriptively to obtain summary statistics. The summary statistics include the arithmetic mean, arithmetic standard deviation, geometric mean, geometric standard deviation, number of observations, and range. Subject ID was included as a random effect in all statistical models to control for subject to subject variation in these repeated measures analyses.

These model covariates are used in some or all of the models:

- β_0 = average cross-shift ratio
- β_j = random effect of subject j: This is necessary since repeated measures were taken from each subject.
- β_d = day type: non-burn day or burn day
- β_t = task
- β_a = air contaminant conc.
- ε = experimental error

Specific Aims

1. Determine whether cross-shift urinary PAH metabolite concentrations are elevated in wildland firefighters following inhalation exposure to wood smoke.

To address this aim we will analyze pre-shift and post-shift urine samples of wildland firefighters for PAH metabolites and determine whether there is a significant increase in cross-shift concentrations. We hypothesize that there will be a substantial increase based on previous studies. We use a linear mixed model with α=0.05 to evaluate this hypothesis.

Overall Cross-shift change (Burn day vs. non-burn day)

- Determine if there is a statistically significant increase in cross-shift concentrations for the PAH metabolites for burn days and non-burn days separately. Include day type as a covariate. Use linear combination of constant and "day type" to combine results.

$$PAH\ metabolite\ ln(Post-shift)/(Pre-shift) = \beta_0 + \beta_d + \beta_j + \varepsilon$$

The "day type" variable was coded "zero" for non-burn days and "one" for burn days.

Post-shift (Burn day vs. non-burn day)

- Sub aim 1: Determine if there is a statistically significant difference in the PAH metabolite concentrations between burn day post-shift samples and non-burn day post-shift samples.

$$PAH\ metabolite\ ln(Post-shift) = \beta_0 + \beta_d + \beta_j + \varepsilon$$

The "day type" variable was coded "zero" for non-burn days and "one" for burn days.

- Sub aim 2: Determine if PAH metabolite concentrations have returned to background levels the next morning after a work shift.

$$PAH\ metabolite\ ln(Morning\ after)/(Pre-shift) = \beta_0 + \beta_j + \varepsilon$$

2. Determine whether cross-shift PAH metabolite concentrations are different between the two work tasks, holding and lighting.

For the second aim, we compared the PAH metabolite cross-shift concentration changes for the two main tasks of holding and lighting. Only burn-day samples are included as these tasks were not performed during non-burn days. We hypothesize holding will have the highest wood smoke exposures and therefore the strongest association with PAH metabolites. A mixed model with α=0.05 was used to determine if urinary PAH metabolite levels differ across tasks.

$$PAH\ metaabolite\ ln(Post-shift)/(Pre-shift) = \beta_0 + \beta_t + \beta_j + \varepsilon$$

Holding was coded as number zero and is represented by the "intercept", and lighting was coded as number one. The 2 subject-days of samples collected for those who performed lighting by helicopter were excluded leaving only the tasks lighting and holding.

3. Determine the association of PAH metabolites with measured air concentrations of CO, $PM_{2.5}$, levoglucosan, and LAC. The association of PAH metabolites with LAC dose, levoglucosan dose, and $PM_{2.5}$ dose derived from accelerometry data will also be determined.

The cross-shift metabolite levels were regressed in a mixed model against the air contaminants and dose estimates. Task was included as a covariate in a secondary analysis, as the smoke composition likely varies between the two tasks and will likely show a different association. We hypothesize that cross-shift increases in urinary PAH metabolite concentrations will be positively associated with inhalation exposure to LAC, $PM_{2.5}$, levoglucosan, and CO. Respiratory rate derived from accelerometry data will be used to calculate the dose of $PM_{2.5}$, levoglucosan, and LAC for each subject for each day worked. The association of these dose values with the cross-shift increase in biomarker levels will also be evaluated.

If there is a strong correlation between the PAH metabolites and the measured air contaminants, then the PAH metabolites could potentially be used as a surrogate of exposure for those contaminants.

$$PAH\ metabolite\ ln(Post-shift)/(Pre-shift) = \beta_0 + \beta_a + \beta_t + \beta_j + \varepsilon$$

Other covariates to be analyzed in aim 3 and justification for their use:

- Effect of duration of sample collection (minutes) k: The longer a subject is sampled, they are more likely to be exposed to air contaminants for a longer duration, and thus may have a higher dose.
- Number of acres burned: The larger the burn area, the higher the exposure and dose for a subject is likely to be
- Season: The season of the burn may have an influence in the foliage density. Performing controlled burns later in the growing season when there is more foliage may lead to higher exposures. The weather may be different during the different seasons too.

After the statistical models were run, the output for Aim 1 and Aim 2 were converted to the native scale by exponentiating the beta coefficients and the 95% CIs (Ex: e^{β}). For Aim 3, Table 20, all original β coefficients and the 95% CIs were converted by doing 1.5^{β}.

Results:

Table 7: Comparison of NHANES to Pre-shift and Post-Shift Burn Day Samples (ng/g)

	GM pre-shift burn day (Cr. adj.)	GM post-shift burn day (Cr. adj.)	NHANES GM (Cr. adj.)	NHANES 75th percentile (Cr. adj.)
2-Nap	1911	3988	3710	6690
1-Nap	19716	31190	2140	4680
2+9-Flu	287	577	516	834
2-Phen	92	133	67	98
3-Phen	58	155	75	114
1-OHP	158	227	125	202

The pre-shift and post-shift creatinine adjusted concentrations are comparable to the NHANES values except for 1-Nap. For 1-Nap, there might be interferences in the retention window in which it elutes since even the pre-shift levels are several-fold higher than the NHANES GM.

The post-shift samples for 2-Nap and 2+9-Flu are above the NHANES GM, and it was expected that they would have been much higher, but there is still a substantial cross-shift increase. On the other hand, 2-Phen, 3-Phen, and 1-OHP are higher than the 75th percentile NHANES values (Table 7), and suggests that these three metabolites may be the most sensitive biomarkers for wood smoke exposure.

Normality of urine data:

Upon plotting the six urinary metabolites with quantile-quantile plots, it was found that all six metabolites were heavily right skewed and needed a transformation to become normal. A natural log transformation was chosen and the resulting Q-Q plots were closer to normality. (Appendix 1, Figure 5)

The normality was also assessed for the eight types of air contaminant data and it was found that all eight were highly right skewed. A natural log transformation was used to improve the normality of the distribution for all eight. The transformed versions of the data were used for all statistical analyses. The native version of the data was used for all descriptive analyses.

Table 8: Exposure Data from Personal Air Samples (Burn Days)

	All Tasks				Holding				Lighting			
	AM (ASD)	GM (GSD)	Range	N	AM (ASD)	GM (GSD)	Range	N	AM (ASD)	GM (GSD)	Range	N
Gravimetric PM2.5 (µg/m3)	371 (388)	239 (2.73)	11.2-1859	48	439 (483)	259(2.99)	44.8-1565	16	357 (337)	268 (2.15)	45-1859	30
Real-Time PM2.5 (µg/m3)	227 (157)	175 (2.2)	8.0-787	49	295 (208)	230 (2.2)	45.9-787	17	199.9 (103.8)	171 (1.8)	32.8-371.9	30
CO (ppm)	1.57 (1.75)	0.89 (3.41)	0.018-8.98	53	2.8 (2.4)	1.5 (4.4)	0.018-8.98	18	1.00 (0.72)	0.76 (2.27)	0.114-3.25	33
Absorption Coefficient (10-5 m-1)	77 (76)	46.5 (3.25)	1.63-339.1	48	28.7 (22.2)	21.1 (2.36)	4.5-79.4	16	108.1 (80.1)	87.6 (1.9)	29.4-339	30
Levoglucosan (µg/m3)	18.4 (33.8)	5.35 (6.4)	0.03-166	48	37.8 (52.5)	12.1 (6.5)	0.62-165.9	16	9.37 (10.5)	4.83 (3.6)	0.44-41.3	30
Levoglucosan/Grav PM2.5 ratio (%)	4.96	2.24	N/A	48	8.61	4.67	N/A	16	2.62	1.80	N/A	30
PM2.5 Dose	32.87 (59.3)	16.2 (3.65)	0.75-407.4	48	21.8 (21.7)	11.2 (4.2)	0.75-81.9	16	40.8 (72.5)	23.0 (2.8)	2-407	30
Burn size (acres)	286 (318)	173 (2.7)	38-1000	54	310.9 (374.5)	165.9 (3.1)	38-1000	19	230 (230)	160 (2.3)	38-1000	33
Duration of Sampling (min)	265 (113)	244 (1.5)	112-566	54	279 (127)	253 (1.6)	112-566	19	245 (96)	230 (1.42)	113-558	33

Table 9: Exposure Data from Personal Air Samples (Non-burn Days)

	All Tasks				Non-burn day exposures				Non-burn day office			
	AM (ASD)	GM (GSD)	Range	N	AM (ASD)	GM (GSD)	Range	N	AM (ASD)	GM (GSD)	Range	N
Gravimetric PM2.5 (µg/m3)	55 (39)	42.5 (2.15)	14-158.3	21	44.5 (34.2)	33.8 (2.2)	14-110.8	13	72.0 (42.8)	61.6 (1.85)	22.6-158.4	8
Real-Time PM2.5 (µg/m3)	15.2 (8.0)	12.7 (1.94)	3.5-31.15	21	15.7 (8.8)	12.8 (2.05)	3.5-31.1	13	14.4 (7.1)	12.5 (1.84)	4.8-22.9	8
CO (ppm)	0.057 (0.18)	0.006 (7.97)	0.0007-0.84)	21	0.028 (0.049)	0.008 (6.4)	0.007-0.18	13	0.11 (0.29)	0.003 (11.2)	0.0007-0.84	8
Absorption Coefficient (10-5 m-1)	1.02 (0.73)	0.75 (2.48)	0.11-3.3	21	0.92 (0.56)	0.70 (2.45)	0.11-1.65	13	1.18 (0.95)	0.85 (2.7)	0.11-3.3	8
Levoglucosan (µg/m3)	0.0002 (0.12)	0.007 (3.41)	-0.03-0.02	21	-0.0004 (0.013)	0.007 (3.76)	-0.027 (0.024)	13	0.001 (0.012)	0.006 (3.65)	-0.018-0.019	8
Levoglucosan/Grav PM2.5 ratio (%)	0.0004	0.02	N/A	21	-0.0009	0.02	N/A	13	0.0014	0.01	N/A	8
PM2.5 Dose	3.41 (2.7)	2.5 (2.27)	0.74-10.5	21	2.80 (2.28)	2.1 (2.2)	0.75-7.4	13	4.4 (3.1)	3.4 (2.3)	0.74-10.5	8
Burn size (acres)	0	0	0	21	0	0	0	13	0	0	0	8
Duration of Sampling (min)	374.2 (45.3)	371.2 (1.15)	234-465	21	384.3 (38.2)	382.5 (1.1)	302-465	13	357.8 (53.5)	353.5 (1.2)	234-412	8

Table 10: Summary Statistics of Metabolite Levels (Burn Days Only)

	Post-Pre AM (pg/mL)	Post/Pre Ratio GM	Proportion of Sample Pairs with Higher Post-shift than Pre-shift conc. (%)	n (pairs)
All tasks				
2-Nap	8182	2.79	81%	47
1-Nap	47412	1.74	58%	31
2+9-Flu	815	2.35	67%	47
2-Phen	97	1.64	70%	43
3-Phen	286	3.12	82%	44
1-OHP	293	1.66	71%	48
Holding				
2-Nap	2797	1.70	69%	16
1-Nap	90971	2.05	58%	12
2+9-Flu	306	1.35	82%	17
2-Phen	71	1.71	69%	16
3-Phen	114	1.60	69%	16
1-OHP	67	1.32	65%	17
Lighting				
2-Nap	11886	3.87	90%	29
1-Nap	31034	1.78	65%	17
2+9-Flu	1207	3.60	86%	28
2-Phen	116	1.61	68%	25
3-Phen	414	5.32	92%	25
1-OHP	448	1.96	76%	29

For metabolites below the LOD, no LOD correction was made for this table. These values were derived descriptively, they were not calculated from regression output.

Table 11: OH-PAH Conc. Burn Day (Specific Gravity Adjusted, pg/mL)

	Pre-shift				Post-shift				Morning After			
All tasks	AM (ASD)	GM (GSD)	Range	n	AM (ASD)	GM (GSD)	Range	n	AM (ASD)	GM (GSD)	Range	n
2-Nap	5152 (5293)	2869 (3.51)	69-24771	49	12598 (21801)	6989 (3.13)	223-155622	50	4665 (3613)	3164 (3.0)	27-15742	44
1-Nap	50477 (66163)	29700 (2.67)	7268-353859	38	96256 (135508)	53732 (2.96)	0-733892	43	55468 (53266)	36488 (2.73)	0-180689	34
2+9-Flu	705(860)	431 (2.77)	28-4666	49	1531 (1593)	1010 (2.7)	49-8516	50	732 (743)	487 (2.6)	24-3793	43
2-Phen	315 (462)	139 (3.9)	6.9-2306	45	423 (462)	231 (3.33)	18-2023	49	394 (1144)	141 (3.63)	0-7169	39
3-Phen	176 (388)	87 (3.4)	0-2610	46	448 (653)	272 (2.48)	66-3691	49	125 (123)	86 (2.6)	3-718	43
1-OHP	362 (521)	237 (2.3)	67-3531	50	665.6 (813)	398 (2.75)	67-4561	50	403 (322)	305 (2.13)	66-1527	44
Holding												
2-Nap	3188 (2325)	2290 (2.54)	320-7050	16	5616 (5122)	3375 (3.39)	223-18487	18	3027 (1791)	2100 (3.54)	27-6186	17
1-Nap	41304 (53323)	24910 (2.57)	8621-193604	14	126484 (201534)	60889 (3.16)	9686-733892	15	61426 (59891)	41577 (3.05)	0-163779	12
2+9-Flu	445 (222)	384 (1.86)	63-932	17	826 (617)	562 (2.88)	49-2904	18	524 (438)	359 (2.77)	24-1465	17
2-Phen	232 (425)	92 (3.83)	8.4-1778	17	303 (400)	159 (3.25)	27-1635	17	576 (1763)	126 (4.69)	0-7169	16
3-Phen	107.5 (43.3)	97.6 (1.65)	25.3-184.5	17	222 (239)	159.5 (2.14)	66-1044	17	147 (170)	99 (2.34)	26-719	17
1-OHP	173 (113)	150 (1.69)	67-536	17	264 (173)	213 (1.99)	67-664	18	265 (170)	218 (1.92)	66-640	17
Lighting												
2-Nap	6015 (5975)	3227 (3.93)	69-24771	31	17290 (27015)	11350 (2.29)	2168-155622	30	5502 (4118)	3900 (2.51)	729-15742	25
1-Nap	53486 (76122)	30302 (2.76)	7268-353859	22	85691 (82444)	52681 (2.85)	8802-311001	26	54280 (52452)	34738 (2.72)	4153-180689	20
2+9-Flu	822 (1041)	440 (3.27)	28-4666	30	2003 (1861)	1496 (2.11)	327-8516	30	864 (876)	610 (2.25)	145-3793	24
2-Phen	386 (496)	189 (3.84)	6.87-2306	26	507 (494)	300 (3.24)	18-2022	30	270 (333)	149 (3.2)	12-1427	21
3-Phen	222 (504)	77 (4.82)	0-2610	27	598 (783)	389 (2.27)	126-3692	30	115 (81)	83 (2.78)	3-348	24
1-OHP	464 (635)	297 (2.43)	74-3531	31	928 (960)	586 (2.74)	94-4561	30	504 (373)	390 (2.11)	100-1527	25

Table 12: OH-PAH Conc. Non-burn Day (Specific Gravity Adjusted, pg/mL)

All tasks	AM (ASD)	GM (GSD)	Range	n	AM (ASD)	GM (GSD)	Range	n	AM (ASD)	GM (GSD)	Range	n
2-Nap	4222 (3411)	3025 (2.39)	554-11697	21	3625 (2655)	2785 (2.18)	562-11053	20	4892 (4551)	3371 (3.05)	0-17211	17
1-Nap	88378 (75307)	51761 (3.5)	3405-244629	18	71425 (51749)	50472 (2.57)	10208-164108	19	96296 (113943)	53601 (3.28)	4730-416878	14
2+9-Flu	657 (655)	430 (2.73)	34.4-2835	21	513 (322)	436 (1.78)	147-1463	20	763 (744)	502 (2.58)	90-2425	17
2-Phen	246 (217)	159 (2.83)	21-726	18	141 (132)	84 (4.14)	0.8-525	17	237 (322)	101 (4.97)	0-1115	14
3-Phen	113 (95)	82 (2.43)	7-383	20	142 (199)	87 (2.65)	8-917	19	214 (285)	134 (2.42)	45-1125	16
1-OHP	282 (117)	259 (1.55)	112-549	21	253 (128)	226 (1.61)	98-557	20	282 (232)	203 (2.37)	46-863	17
Non-burn day exposures												
2-Nap	4964 (3897)	3465 (2.59)	555-11697	13	3447 (2428)	2602 (2.31)	562-7753	12	6481 (4651)	5066 (2.22)	901-17211	10
1-Nap	91426 (88368)	45536 (4.28)	3405-244629	10	69194 (55861)	47296 (2.68)	10208-164108	12	103356 (141012)	44652 (4.18)	4730-416878	9
2+9-Flu	688 (744)	451 (2.56)	98-2836	13	565 (361)	445 (1.78)	197-1464	12	774 (757)	523 (2.52)	127-2425	10
2-Phen	269 (250)	168 (2.98)	21-726	11	135 (102)	104 (2.23)	28-379	10	339 (380)	177 (3.58)	45-1115	8
3-Phen	117 (81)	95 (1.97)	31-320	12	120 (73)	104 (1.75)	41-275	11	274 (345)	168 (2.57)	75-1125	10
1-OHP	302 (136)	271 (1.66)	112-549	13	271 (139)	241 (1.66)	110-557	12	271 (196)	206 (2.27)	55-572	10
Non-burn day office												
2-Nap	3017 (2130)	2427 (2.04)	854-7074	8	3893 (3119)	3084 (2.06)	1137-11053	8	2621 (3187)	1710 (3.68)	0-8923	7
1-Nap	84568 (60853)	60752 (2.76)	7469-188768	8	75250 (47812)	56420 (2.56)	11077-126742	7	83588 (45962)	74467 (1.71)	36172-158660	5
2+9-Flu	606 (521)	399 (3.2)	34-1743	8	494 (274)	423 (1.86)	147-860	8	748 (785)	474 (2.87)	90-2268	7
2-Phen	211 (162)	145 (2.8)	43-449	7	149 (175)	61 (7.8)	0.8-525	7	101 (171)	41 (5.99)	0-440	6
3-Phen	108 (119)	66 (3.2)	7-383	8	171 (304)	69 (3.95)	8-916	8	116 (105)	92 (2.0)	45-325	6
1-OHP	250 (72)	239 (1.4)	132-323	8	226 (115)	206 (1.57)	98-487	8	297 (292)	199 (2.66)	46-863	7

Statistical Model Results:

Overall Cross-shift Change. (Burn Day and Non-burn Day Comparison):

Table 13: Regression Results for Burn Days (Post-shift/Pre-shift)

	GM Cross-shift Ratio	p	95% Conf. Interval	N (pairs)
2-nap	2.69	0.000	(1.73, 4.22)	47
1-nap	1.95	0.038	(1.04, 3.71)	45
2+9-flu	2.61	0.000	(1.80, 3.74)	48
2-phen	2.14	0.012	(1.19, 3.82)	48
3-phen	3.19	0.000	(1.94, 5.31)	46
1-OHP	1.72	0.000	(1.33, 2.23)	48

Regression results for linear combination of intercept and day type covariate converted to the native scale.

Table 14: Regression Results for Non-burn Days (Post-shift/Pre-shift)

	GM Cross-shift ratio	p	95% Conf. Interval	N (pairs)
2-nap	0.96	0.880	(0.54, 1.70)	20
1-nap	1.31	0.558	(0.53, 3.29)	19
2+9-flu	1.03	0.915	(0.58, 1.80)	20
2-phen	0.53	0.176	(0.21, 1.32)	20
3-phen	0.94	0.887	(0.44, 2.03)	20
1-OHP	0.92	0.632	(0.66, 1.28)	20

Regression results for the intercept converted to the native scale.

Detailed output results from this model is in Appendix 1, Table 21.

All metabolites showed a statistically significant increase in their GM post-shift to pre-shift ratio on burn days. 2-napthol, 2+9-flu, and 3-phen showed the highest GM post-shift to pre-shift ratios at 2.69, 2.61, and 3.19 respectively, which is substantial (Table 13). On non-burn days, none of the metabolites showed a statistically significant increase in cross-shift concentration, which is expected since the subjects were not exposed to wood smoke exposure during these shifts. The GM cross-shift ratios were generally close to 1 for all six metabolites, and their confidence intervals encompass 1 which indicates no increase in the metabolite levels across the shift (Table 14).

Post-shift (Burn Day vs. Non-burn Day):

Table 15: Post-shift (Burn Day vs. Non-burn Day) Regression Results

	Factor of Difference	P	95% Conf. Interval	n (pairs)
2-nap	2.51	0.000	(1.51, 4.18)	70
1-nap	0.79	0.520	(0.37, 1.65)	69
2+9-flu	2.32	0.000	(1.51, 3.53)	70
2-phen	4.44	0.000	(1.99, 9.87)	70
3-phen	3.42	0.000	(1.92, 6.17)	70
1-OHP	1.77	0.003	(1.21, 2.48)	70

Factor of difference is the numerical-fold difference between the GM post-shift concentrations for non-burn days and burn days. It is the output from the "day type" covariate in the mixed model that has been converted to the native scale from the natural log scale.

Detailed regression output is in Appendix 1, Table 24.

The metabolite concentrations ranged from a 0.79-fold change, to a 4.44-fold change between post-shift samples collected on non-burn days and burn days. All metabolites showed a meaningful elevation in concentration and did not include 1 in the 95% CI, except for 1-nap (Table 15).

Return to Pre-shift Levels from Elevated Metabolite Levels:

Table 16: Return to Pre-shift Levels from Elevated Metabolite Levels (Burn days only)

	GM Morning After to Pre-shift ratio	P	95% Conf. Interval	N (pairs)
2-nap	1.08	0.612	(0.79, 1.49)	41
1-nap	1.30	0.487	(0.62, 2.72)	38
2+9-flu	1.09	0.718	(0.67, 1.79)	42
2-phen	0.73	0.455	(0.33, 3.16)	42
3-phen	0.92	0.722	(0.54, 1.52)	40
1-OHP	1.38	0.055	(0.99, 1.90)	42

Regression results for intercept on the native scale converted from the natural log scale.

Table 16 reports the ratio of the morning after urine samples to the pre-shift samples was assessed for burn days. All six metabolites showed a non-significant change in their GM ratio concentration. The morning after to pre-shift ratios ranged between 0.73 (1-nap) and 1.38 (1-OHP) and all the 95% CIs included 1, which indicates no change. This shows that if someone is exposed to wood smoke, the next day the metabolite levels are back to their pre-exposure level. 1-OHP has a morning-after to pre-shift ratio of 1.38, which is the highest of the six metabolites. The overall cross-shift ratios for 1-OHP on burn days was 1.72 (Table 13). This implies that there may be some carryover from the previous PAH exposures. While this ratio was not statistically significantly different from one, it may suggest that the levels are have not yet entirely returned to pre-exposure levels (Table 16).

Detailed regression output is in Appendix 1, Table 25.

Sensitivity Analysis of Aim 1:

The specific gravity adjustment and creatinine adjustment should give the same or very similar outcomes. Since all descriptive and statistical analysis were done with the specific gravity adjusted data, a comparison was necessary to confirm this assumption. An analysis comparing GM cross-shift metabolite ratios between burn days and non-burn days was run using creatinine adjusted concentrations. Overall, the cross-shift ratios were slightly higher when using specific gravity adjustment. All p-values regarding the difference between burn days and non-burn days (day type p-value) were nearly identical to those obtained using specific gravity adjusted values. Also, the p-values for determining if non-burn day (intercept p-value) cross-shift ratios were greater than one were nearly identical as well (Appendix 1, Table 22).

The only difference is in the linear combination table that identified whether the cross-shift burn day metabolite ratio was different from one (Appendix 1, Table 23). There was a non-significant result (0.136 p-value) for 1-nap when using the creatinine adjusted data and a 0.038 p-value for 1-nap when using specific gravity correct data. The GM cross-shift ratios were 1.95 and 1.65 for specific gravity and creatinine adjusted 1-nap respectively, which is not a large difference. The 1-nap data is poor quality so this discrepancy is not a concern (Appendix 1, Table 23). In summary, this sensitivity test confirms that our aim 1 findings are robust to the choice of creatinine or specific gravity for dilution correction. This likely due to the strong correlation of creatinine and specific gravity (see Appendix 1, Figure 4).

The Effect of Task on Metabolite Levels (Lighting vs. Holding):

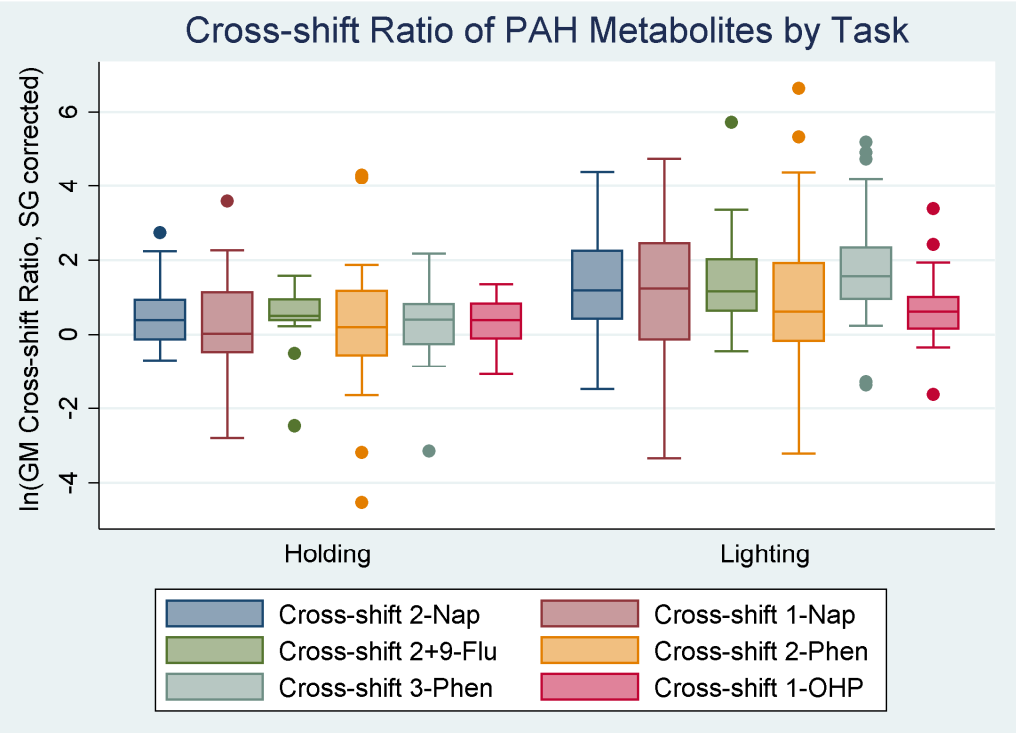

Figure 3: Box plot of Cross-Shift Metabolite Ratios by Task

Table 17: Change in Cross-shift Metabolite ratio for lighting vs holding

	Factor of Difference	p	95% Conf. Interval	n (pairs)
2-nap	2.77	0.003	(1.42, 5.47)	45
1-nap	2.46	0.146	(0.73, 8.25)	44
2+9-flu	3.10	0.004	(1.43, 6.69)	46
2-phen	2.24	0.189	(0.66, 8.25)	46
3-phen	4.95	0.000	(2.01, 12.18)	44
1-OHP	1.39	0.080	(0.96, 2.01)	46

The factor of difference is the numerical-fold difference between the GM cross-shift ratios for holding and lighting. It is the output from the "task" covariate in the mixed model that has been converted to the native scale from the natural log scale.

Table 18: Cross-shift Metabolite Ratios for Lighting

	GM Cross-shift ratio	p	95% Conf. Interval	N (pairs)
2-nap	3.97	0.000	(2.34, 6.82)	29
1-nap	2.89	0.007	(1.33, 6.23)	28
2+9-flu	4.18	0.000	(2.64, 6.69)	29
2-phen	2.94	0.006	(1.38, 6.36)	29
3-phen	6.36	0.000	(3.60, 11.25)	27
1-OHP	1.99	0.000	(1.46, 2.69)	29

Table 18 reports the GM of the cross-shift metabolite rations for lighting. These were derived from the regression results for the linear combination of intercept and the covariate "task," converted from natural log sale to the native scale.

Table 19: Cross-shift Metabolite ratios for Holding

	GM Cross-shift ratio	p	95% Conf. Interval	N
2-nap	1.43	0.282	(0.75, 2.72)	16
1-nap	1.17	0.750	(0.43, 3.22)	16
2+9-flu	1.35	0.331	(0.73, 2.48)	17
2-phen	1.27	0.642	(0.47, 3.46)	17
3-phen	1.28	0.499	(0.63, 2.61)	17
1-OHP	1.43	0.054	(0.99, 2.08)	17

Table 19 reports the GM of the cross-shift metabolite rations for holding. These were derived from the regression results for the intercept from the regression converted from the natural log scale to native scale.

For subjects who performed holding, all of the metabolites showed a positive post-shift/pre-shift ratio between 1.17-1.43 (i.e. metabolite levels increased across the work shift), however, the confidence intervals on the geometric mean cross-shift ratios for all six metabolites included one, which is consistent with no increase in the cross-shift ratios for these compounds. 1-OHP had the largest cross shift ratio (1.43) which was close to a significant result however, and it is possible that with a larger

sample size the cross-shift change for 1-OHP associated with holding may have been significant. (Table 19). For subjects who were performing lighting, the GM cross shift ratio ranged between 1.99 (1-OHP) to 6.36 (3-phen), and was statistically significant for all six metabolites (Table 18).

When comparing the metabolite levels between the two tasks, the difference in GM cross shift ratio for lighting vs holding was positive for all six metabolites. 1-OHP showed a 1.39-fold higher cross-shift ratio, the smallest increase of the six metabolites, and 3-phen showed a 4.95-fold increase in cross-shift ratio which indicates that lighting was associated with higher GM cross-shift ratios for all six metabolites. This difference in GM cross shift ratios for holding vs lighting were statistically significant for 2-nap, 2+9-flu, and 3-phen, and approached significance for 1-OHP (Table 17).

Detailed regression output is in Appendix 1, Table 26.

Association of Air Contaminant Data with Biomarker Levels:

Table 20: Summary of Coefficients, p-values, and 95% CIs for Air Contaminants and Metabolite Association (Basic Models)

	2-Nap β (p-value) (95% CI)	1-Nap β (p-value) (95% CI)	2+9-Flu β (p-value) (95% CI)	2-Phen β (p-value) (95% CI)	3-Phen β (p-value) (95% CI)	1-OHP β (p-value) (95% CI)
- CO (ppm)	0.92 (0.135) (0.82, 1.02)	1.00 (0.981) (0.82, 1.22)	0.99 (0.954) (0.87, 1.14)	0.92 (0.408) (0.75, 1.12)	1.01 (0.926) (0.86, 1.19)	1.01 (0.828) (0.95, 1.07)
- Real-Time $PM_{2.5}$ ($\mu g/m^3$)	0.95 (0.587) (0.80, 1.14)	1.10 (0.563) (0.80, 1.51)	1.07 (0.449) (0.90, 1.27)	1.01 (0.965) (0.74, 1.36)	1.14 (0.280) (0.90, 1.45)	1.06 (0.245) (0.96, 1.16)
- Gravimetric $PM_{2.5}$ ($\mu g/m^3$)	1.04 (0.617) (0.90, 1.21)	1.15 (0.321) (0.87, 1.51)	**1.14 (0.044) (1.00, 1.29)**	1.03 (0.829) (0.79, 1.34)	1.16 (0.154) (0.95, 1.43)	1.06 (0.177) (0.98, 1.15)
- Grav $PM_{2.5}$ Dose (μg/kg bodyweight)	1.05 (0.435) (0.93, 1.18)	1.10 (0.390) (0.89, 1.36)	**1.12 (0.024) (1.02, 1.23)**	1.03 (0.789) (0.84, 1.26)	1.16 (0.061) (0.99, 1.36)	**1.07 (0.038) (1.00, 1.13)**
- LAC (10^{-5} m^{-1})	**1.14 (0.033) (1.01, 1.28)**	1.17 (0.184) (0.93, 1.48)	**1.17 (0.001) (1.07, 1.29)**	1.13 (0.249) (0.92, 1.39)	**1.27 (0.003) (1.08, 1.48)**	**1.07 (0.050) (1.00, 1.14)**
- LAC Dose ($10^{-5}m^{-1}$/kg bodyweight)	**1.10 (0.044) (1.00, 1.21)**	1.10 (0.278) (0.92, 1.32)	**1.13 (0.002) (1.05, 1.22)**	1.09 (0.327) (0.92, 1.28)	**1.21 (0.003) (1.07, 1.36)**	**1.06 (0.015) (1.01, 1.12)**
- Levoglucosan ($\mu g/m^3$)	0.97 (0.406) (0.90, 1.05)	1.01 (0.869) (0.87, 1.18)	1.04 (0.287) (0.97, 1.11)	0.96 (0.595) (0.84, 1.10)	1.01 (0.886) (0.91, 1.12)	1.03 (0.147) (0.99, 1.08)
- Levoglucosan Dose ($\mu g/m^3$/kg bodyweight)	0.98 (0.633) (0.91, 1.05)	1.02 (0.831) (0.89, 1.17)	1.04 (0.165) (0.98, 1.11)	0.97 (0.673) (0.86, 1.10)	1.03 (0.574) (0.93, 1.14)	**1.04 (0.052) (1.00, 1.08)**

A 50% increase in the exposure or dose estimate will increase the metabolite concentration by a factor of the listed coefficient. Bold font indicates a significant result, or a nearly significant result.

In the basic models, each individual biomarker was regressed against each individual type of air contaminant. No covariates were included. To create the full models, three covariates were added to the basic models including acres burned, duration of work shift (sampling time), and the season. The addition of the covariates did not make any appreciable change in the associations between exposure (or dose) and biomarker, so they will not be considered further (Appendix 1, Table 28).

The associations between CO and the metabolites were poor overall (Table 20).

All associations between real-time $PM_{2.5}$ and metabolites were poor, and suggests that $PM_{2.5}$, when measured using real-time instruments, is not associated with these urinary biomarkers. There was an

association between gravimetric $PM_{2.5}$ and 2+9-fluorene, and a possible correlation between 3-phen and gravimetric $PM_{2.5}$. Interestingly, there were no strong associations between levoglucosan and the metabolites. There may be a possible association between 1-OHP and levoglucosan. (Table 20).

LAC displayed the strongest associations between the metabolites of all the air contaminants measured. 2-nap, 2+9-flu, 3-phen, and 1-OHP showed particularly strong associations. There were also strong associations between LAC dose and the metabolites, which reflects the strong associations seen between the metabolites and LAC. There were also associations between 2+9-flu, 3-phen, and 1-OHP with inhaled $PM_{2.5}$ dose (Table 20).

In the air contaminant and metabolite association models that were stratified by burn day task, there were fewer significant associations than the models with all tasks combined (Appendix 1, Table 27).

Discussion:

Overall Cross-shift Change for All Burn Day Tasks:

For burn days, when not factoring in task, we found increases in cross-shift concentration ratio for all metabolites. (Table 13). This initially suggests that all metabolites can potentially be useful biomarkers for occupational exposures to wood smoke in this setting. 2-nap, 2+9-flu, and 3-phen had the largest modeled GM post-shift to pre-shift ratios at 2.70, 2.61, and 3.19. Therefore, these three metabolites may be the most sensitive of the metabolites to wood smoke exposure, and may be the most effective biomarkers of wood smoke exposure.

When looking at cross-shift ratios of non-burn day samples, none of the results showed meaningful cross-shift changes (Table 14). This was expected as there should have not been any occupational exposure to wood smoke during these work shifts.

Post-shift (Burn Day vs. Non-burn Day):

We did a sub analysis of the post-shift urine samples from burn days and non-burn days in order to confirm that the increase in concentration was due to occupational exposure, and not other factors, like natural time-dependent processes in the body. Also, this analysis may have been better suited to minimizing any confounding from past exposures since a small number of subjects worked burn days the previous day. All the metabolites showed a meaningful difference between non-burn day post-shift samples to burn day post-shift samples, except for 1-nap (Table 15). 2-nap, 2-phen, and 3-phen burn day post-shift concentrations were 2.51, 4.44, and 3.42-fold higher than for non-burn days. As an example of the concentration difference for 3-phen, the GM concentration was 72.9 pg/mL on non-burn days, and 249.6 pg/mL on burn days. This indicates that the exposures from the sampling day were

responsible for the increase in metabolite concentration and also suggests that these metabolites may be effective biomarkers of wood smoke exposure.

Return to Pre-shift Levels from Elevated Metabolite Levels:

We compared metabolite levels in the urine samples collected the morning after a sampling day, to pre-shift levels in a ratio. Assuming the metabolites completely return to pre-shift levels, their concentrations in the morning after samples should be no different from the concentrations in the pre-shift samples, and the pre-shift to morning after ratio should equal one. The data in table 16 are consistent with this expectation. However, 1-OHP had a p-value of 0.055 and a morning after to pre-shift ratio of 1.38 (95% CI: 0.99-1.90) which indicated that the levels may still be elevated in the morning after samples (Table 16). Looking at the half-life data from Li et al. 2015, 1-OHP had a median half-life of 23.5 hours, which is more than twice the half-life of the metabolite with the 2nd longest half-life, 3-phen at 11 hours. This provides further evidence that 1-OHP may indeed remain elevated in the morning after samples. This information suggests that for situations in which daily monitoring of consecutive days needs to be done, 1-OHP may not be the most suitable choice.

The Effect of Task on Metabolite Levels (Lighting vs. Holding):

It is important to determine which task contributes to a higher PAH dose so that effective, task-specific interventions can be implemented if someone is found to be overexposed. Subjects who performed lighting had significantly higher GM cross-shift ratios for 2-nap (2.77-fold), 2+9-flu (3.10-fold), 3-phen (4.95-fold), and possibly 1-OHP (1.39-fold) (95% CI: 0.96, 2.01) than those who performed holding (Table 17). This is not reflective of previous findings where metabolite levels in those who perform lighting and holding were not significantly different. (Adetona et al., 2015). However, this discrepancy may be due to how tasks were defined. For example, in the current study, if the subjects spent more than 50% of their

time performing a task, that task was considered their primary task, whereas the threshold in the study by Adetona et al. was 75%. Also, the number of acres burned, the weather, and sample size among other things may have been different between the two studies.

For subjects that performed holding, the GM cross-shift ratio metabolite concentration for all metabolites was roughly 1.3. This suggests that urinary metabolite concentrations increased as a consequence of the work shift exposure, albeit the increases were not statistically significant for any of the metabolites (Table 19). However, all six metabolites showed a significant increase in the GM cross-shift ratio for subjects performing lighting (Table 18), with the GM cross-shift ratio ranging between 1.99 (1-OHP) to 6.36 (3-phen).

This information presented so far initially leads to the assumption that those who perform lighting are exposed to larger quantities of wood smoke. However, the GM levoglucosan concentration for those performing holding and lighting is 12.1 and 4.83 $\mu g/m^3$ respectively. In addition, the GM levoglucosan: gravimetric $PM_{2.5}$ ratio is 4.67 and 1.80 for holding and lighting respectively (Table 8). Since levoglucosan is unique to the combustion of biomass, these results show that those who perform holding actually have substantially higher wood smoke exposures than those who performed lighting.

Furthermore, the GM absorption coefficients for LAC for those who performed holding and lighting are 21.1 and 87.6 10^{-5} m^{-1} respectively (Table 8). LAC is produced from both the incomplete combustion of biomass and the diesel/gasoline fuel mixture used in the drip torch. LAC, a component of soot, also contains organic carbon such as PAHs. In one study of open burning of jet fuel, which is very similar in composition to diesel fuel, each gram of soot emitted was found to contain 44.5 mg of PAHs (EPA, 1993). Therefore, it is likely that the drip torches can be a major source of PAH exposure.

The 4.2-fold higher GM LAC absorbance, 2.5-fold lower GM levoglucosan concentration, and 2.6-fold lower GM levoglucosan:$PM_{2.5}$ ratio for those who were lighting compared to those who performed holding strongly suggests that the large increase in cross-shift metabolite levels seen for subjects performing lighting are due to exposure to the combustion emissions of drip torch fuel, and not the wood smoke. Although the mass of emissions from the drip torches is likely much lower than the mass of emissions from the burning biomass, the workers who use the drip torches are in very close proximity to the source of the emissions and are likely exposed to a substantial fraction of the emissions from this source. Any maintenance of the drip torch, and refilling the drip torch will also expose those who are lighting to the fuel mixture. Similarly, these findings also strongly suggest that these 6 metabolites are not effective biomarkers for wood smoke exposure in this setting, since those who were holding had substantially higher wood smoke exposure than those lighting, yet the increases in GM cross-shift ratios for holding were modest, and were not statistically significant. However, since there are only 16 observations for holding, there may not be enough statistical power to detect a significant change when there is one present.

Interestingly, in a similar study conducted on the SRS in 2008-2009, the same trends regarding levoglucosan concentration among burn day work tasks was present. There was approximately a 2-fold higher levoglucosan concentration for those who performed holding versus those who performed lighting (approximately 28 $\mu g/m^3$ to 14 $\mu g/m^3$ respectively) (Adetona et al., 2013).

Comparison of Urinary Metabolite Levels to ACGIH BEI

The ACGIH has developed a biological exposure index (BEI) for the compound 1-OHP. The BEI is 2.5 µg/L urine concentration (ACGIH 2017). Converting this BEI to the units used in this study yields 2500 pg/mL. Of the 70 post-shift urine samples collected (including burn days and non-burn days), the 1-OHP concentrations in three samples exceeded the BEI. The values were 2521, 2569, and 4561 pg/mL (specific gravity corrected). Seven samples had concentrations from 1000-2500 pg/mL. All of the

samples over 1000 pg/mL concentration are from burn days, and all from subjects who performed lighting. This suggests that subjects who perform lighting are at risk of overexposure to 1-OHP, and likely other PAHs, some of which are carcinogens. This may necessitate the development of interventions such as respiratory protection to mitigate the hazards of PAH exposure.

Association of Air Contaminant Data and Metabolite Data:

Determining the association between air contaminant data and metabolite data can provide useful information that would allow the air contaminant to potentially be used as a surrogate of exposure and dose for certain PAHs. The first stage of this analysis for the association between air contaminant concentration and metabolite levels included only burn day samples and all burn day tasks pooled together. Associations were found between the metabolites, 2-nap (95% CI: 0.03, 0.61), 2+9-flu (95% CI: 0.16, 0.62), 3-phen (95% CI: 0.19, 0.96), and 1-OHP (95% CI: 0.00, 0.32) and measured LAC (Table 20). Another way of looking at the associations listed is by determining the increase in metabolite concentration from a set fold increase in exposure or dose measure. For table 20, the set fold increase of exposure and dose data was 1.5, and the coefficients represent the coefficient-fold increase in metabolite concentration in pg/mL. The increase in metabolite concentration is 1.14, 1.17, 1.27, and 1.07-fold for 2-nap, 2+9-flu, 3-phen and 1-OHP respectively, for a 50% increase in the absorption coefficient (Table 20). 3-phen appears to be the most sensitive to increases in LAC levels, and suggests that LAC is especially rich in 3-phen. Overall, this data suggests that LAC contains substantial PAH content, or there are substantial amounts of PAHs present when LAC is present, and that these exposures cause a linear increase in concentrations of urinary PAH metabolites. This is also reflected in the large cross-shift metabolite ratios for subjects that are lighting which also have a very high exposure to LAC. Lighting makes up 30/46 of the observations in the models, so it is likely heavily influencing the results in the LAC metabolite association models. There were marginal associations between LAC and 2-phen or 1-nap, which is consistent with the fact that these two metabolites had low increases in GM cross shift ratios with wide confidence intervals surrounding them (Table 13).

There were no associations with levoglucosan and metabolite levels (Table 20). This is consistent with a previous study conducted in the SRS where none of the metabolites tested in this study were significantly associated with levoglucosan (Adetona et al, 2015). In the prior study, there was a significant association between 4-phen and levoglucosan, however 4-phen was not quantified in our current study. These findings suggest that the wood smoke the subjects were exposed to was not as high in concentration with PAHs as LAC and therefore, urinary PAH metabolites cannot be used as a surrogate of exposure to wood smoke in this setting. These conclusions are also reflected by the aim 2 results where those who performed holding, who were exposed to high levels of wood smoke, did not have significant cross-shift increases in their urine PAH metabolite levels.

Many of the associations between air contaminant and urinary metabolites listed above were attenuated when the analysis was repeated, stratified by task (Appendix 1, Table 27). This is likely due to the small sample size for both lighting and holding in the stratified analysis.

Gravimetric $PM_{2.5}$, levoglucosan, and LAC dose associations with metabolite concentrations:

There were associations between $PM_{2.5}$ dose calculated from the accelerometry data and the levels of several metabolites, and no associations between $PM_{2.5}$ exposure concentrations and metabolite levels, except for 2-9-flu. (Table 20). There are stronger associations for $PM_{2.5}$ dose than for $PM_{2.5}$ air concentrations for all metabolites except for 1-nap. The associated coefficients for $PM_{2.5}$ dose and metabolites are 0.24, 0.30, 0.46, and 0.15 for 2-nap, 2+9-flu, 3-phen, and 1-OHP respectively. This means that as the $PM_{2.5}$ dose increases by a factor of 1.5, the following fold-increases in urine concentration are seen: 2-nap 1.0 5 (95%CI: 0.93-1.18); 1-nap 1.10 (95%CI: 0.89-1.36); 2+9-Flu 1.12 (95%CI: 1.02-1.23); 2-phen 1.03 (95%CI: 0.84-1.26); 3-phen 1.16 (95%CI: 0,99-1.36); 1-OHP 1.07 (95%CI:

1.00-1.13). 3-phen shows the largest response to $PM_{2.5}$ dose, which suggests that 3-phen is the most abundant in $PM_{2.5}$ of the metabolites.

The estimated ventilation rate may have been the cause of the stronger association between the inhaled $PM_{2.5}$ and the metabolites overall. It is possible that particular PAH metabolites may be used as a surrogate measure of exposure and dose including LAC, LAC dose, and $PM_{2.5}$ dose.

Similar to the exposure measurement of levoglucosan, the levoglucosan dose associations with the metabolites were generally poor, with 1-OHP (p-value 0.052) being the only metabolite with an association that may be of interest.

Similar to the associations observed between measured LAC absorbance and cross-shift metabolite concentrations, the same metabolites found to have a significant association were found to have a significant association with LAC dose. These include 2-nap, 2+9-flu, 3-phen, and 1-OHP. (Table 20). For the 4 metabolites listed, the associations were generally stronger for LAC dose, rather than LAC, based on the narrower 95% CIs. For example, the regression coefficients for the associations were 2-nap-LAC (95% CI: 0.03, 0.61) and 2-nap-LAC dose (95% CI: 0.01, 0.46). Also, 1-OHP-LAC (95% CI: 0.00, 0.32) and 1-OHP-LAC dose (95% CI: 0.03, 0.28). Inclusion of the estimated ventilation rate for each subject on each day was likely the cause of the stronger associations between LAC dose and the metabolites.

This study was an addition to a study conducted by the University of Georgia (UGA), which looked to determine differences in the airborne exposures and ventilation rate with regard to task. The UGA study only used external measures of exposure, whereas our study measured internal dose, which is ultimately more important from a health effects perspective.

The dose estimates of the air contaminants calculated from the accelerometry data is an important addition to this study as associations between air contaminant dose and metabolite levels were determined. In future studies this could be used to create predictive models that use metabolite levels as a surrogate of exposure and dose. For example, measured metabolites such as 2-nap or 1-OHP could be used to predict $PM_{2.5}$ dose, LAC, and LAC dose. (Table 20).

Estimating the dose of PAHs is especially important as many PAHs are carcinogenic, and having a more accurate estimate of how much is taken in and retained in the body gives a better idea of the true risk the wildland freighters are exposed subject to.

Recommendations for future work:

For a future study, it would be ideal to have a larger sample size. This was a small study with only 12 subjects and limited repeat measures on each subject. This small sample size is especially a problem when performing an analysis for particular tasks such as holding and lighting, since they only have approximately 16 and 30 observations respectively. Therefore, some statistical analyses may be lacking the appropriate power to identify a significant result as well as having the ability to identify confounding when present.

Another limitation was the possible confounding due to past exposures to PAHs since a small number of subjects worked previous burn days. Subjects who worked a previous burn day should be considered for exclusion form this study. To determine the actual contribution of LAC and PAHs from the drip torch, a study needs to be made in which subjects are using a drip torch in the same way and duration that it would be used in the field, but not lighting anything on fire. This can be a way to confirm that the large LAC absorbance values seen for those who were lighting were due to the drip torch and not another source.

Conclusions:

Urinary OH-PAH metabolites may not be effective biomarkers for exposure to wood smoke in wildland firefighters conducting controlled burns. Despite subjects who performed holding being exposed to a 2.6-fold higher levoglucosan:$PM_{2.5}$ ratio than those who were lighting, which indicates a substantial higher exposure to wood smoke, the increases in GM cross-shift ratio for all six metabolites for workers performing holding was generally modest, and was not meaningfully elevated.

In contrast, all six metabolites exhibited a significant increase in GM cross-shift ratio for subjects who performed lighting, who also had a 4.2-fold higher GM LAC absorbance than subjects who performed holding. This observation suggests that OH-PAH metabolites may be effective biomarkers for exposure to emissions from the diesel/gas mixture the drip torch uses.

Of all the metabolites studied, 1-OHP showed the most promise as a biomarker for exposure to wood smoke in this setting, as it had a nearly significant result (p-value 0.080) for subjects that performed holding (Table 19).

Subjects that performed lighting may be at higher risk of cancer and other health morbidities as they are exposed to higher levels of PAHs, as shown by their significant metabolite level increases, and high LAC absorbance measurements. We found that 3/30 of the samples from lighters were over the ACGIH BEI of 2500 pg/mL and 10/30 were over 1000 pg/mL. Since 10% of samples were over the BEI, and many more were close to this exposure index, interventions may need to be developed to mitigate exposure during this task.

LAC had the strongest association with the OH-PAH metabolites, indicating that LAC contains high levels of PAHs, or that PAHs are present in high levels when LAC is present. LAC could be used a surrogate of

exposure to PAHs. We anticipated that the accelerometry data should give a better estimate of the actual risk by providing a dose estimate for the monitored air contaminants. Contrary to expectations, measures of association between exposure and biomarkers were not consistently improved when using accelerometry data to convert measured exposures into dose estimates.

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Appendix I – Supplemental Tables, Figures, and Equations:

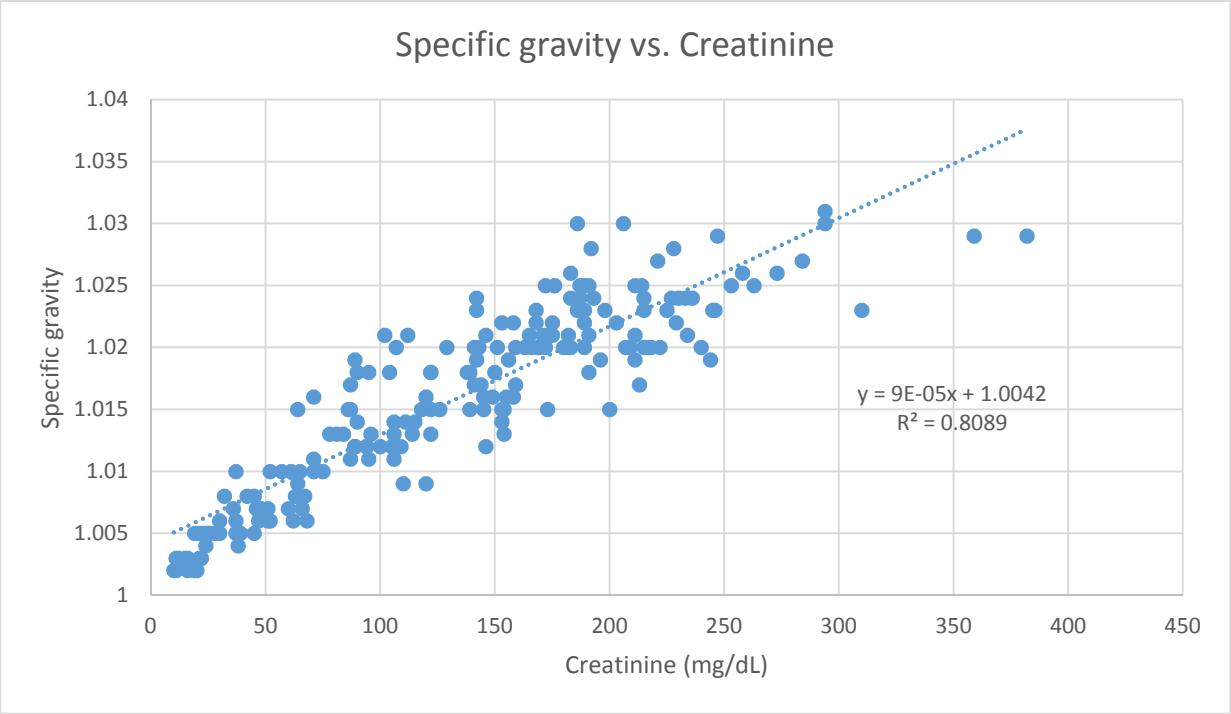


Figure 4: Comparison of Specific Gravity and Creatinine in Urine Samples

Table 21: Results for Mixed Effect Models for Natural Log-transformed Cross-shift Ratio (Post-shift/Pre-shift) Urine Metabolite Concentrations. ln(pg/mL). Burn day and Non-burn Day Comparison. (Specific Gravity Corrected) (Non-burn day coded 0, burn day coded 1)

	β (SE)	p	95% Conf. Interval	Subject-Specific Random Effect Est.(SE) (95% CI)	Residual Random Effect Est. (SE) (95% CI)	Number of Observations
Model 1: Cross-shift 2-nap						
				0.55 (0.25) (0.23, 1.32)	1.03 (0.10) (0.85, 1.24)	67
Day type	1.04 (0.28)	0.000	(0.49, 1.58)			
Intercept	-0.04 (0.29)	0.880	(-0.62, 0.53)			
Model 2: Cross-shift 1-nap						
				0.49 (0.43) (0.09, 2.74)	1.89 (0.19) (1.57, 2.29)	64
Day type	0.40 (0.52)	0.443	(-0.62, 1.42)			
Intercept	0.27 (0.47)	0.558	(-0.64, 1.19)			
Model 3: Cross-shift 2-flu + 9-flu						
				1.6e-8 (1.2e-7) (7.9e-15, 0.03)	1.29 (.112) (1.09, 1.54)	68
Day type	0.93 (0.35)	0.007	(0.25, 1.60)			
Intercept	0.03 (0.29)	0.915	(-0.54, 0.59)			
Model 4: Cross-shift 2-hydroxyphenanthrene						
				2.5e-9 (1.4e-8) (2.5e-14, 2e-4)	2.07 (0.18) (1.75, 2.46)	68
Day type	1.39 (0.55)	0.012	(0.30, 2.47)			
Intercept	-0.63 (0.46)	0.176	(-1.54, 0.28)			
Model 5: Cross-shift 3-hydroxyphenanthrene						
				4.0e-13 (4.5e-9) (0, .)	1.74 (0.15) (1.46, 2.07)	66
Day type	1.22 (0.47)	0.009	(0.31, 2.13)			
Intercept	-0.06 (0.39)	0.887	(-0.81, 0.71)			
Model 6: Cross-shift 1-hydroxypyrene (1-hydroxypyrene gluconronide d-9 adjusted)						
				0.30 (0.12) (0.14, 0.65)	0.59 (0.06) (0.49, 0.71)	68
Day type	0.63 (0.16)	0.000	(0.31, 0.94)			
Intercept	-0.08 (0.17)	0.632	(-0.41, 0.25)			

Table 22: Results for Mixed Effect Models for Natural Log-transformed Cross-shift Ratio (post-shift/pre-shift) Urine Metabolite Concentrations (pg/mL). Burn day and Non-burn Day Comparison. (Creatinine Corrected) (Non-burn day coded 0, burn day coded 1)

	β (SE)	p	95% Conf. Interval	Subject-Specific Random Effect Est.(SE) (95% CI)	Residual Random Effect Est. (SE) (95% CI)	Number of Observations
Model 1: Cross-shift 2-nap						
				0.35 (0.20) (0.11, 1.10)	0.94 (0.09) (0.78, 1.14)	67
Day type	0.96 (0.25)	0.000	(0.46, 1.45)			
Intercept	-0.11 (0.24)	0.624	(-0.59, 0.36)			
Model 2: Cross-shift 1-nap						
				0.60 (0.40) (0.17, 2.20)	1.86 (0.18) (1.53, 2.25)	64
Day type	0.26 (0.51)	0.605	(-0.74, 1.27)			
Intercept	0.24 (0.47)	0.614	(-0.69, 1.17)			
Model 3: Cross-shift 2-flu + 9-flu						
				1.6e-11 (1.4e-7) (0, .)	1.2 (0.10) (1.00, 1.41)	68
Day type	0.80 (0.32)	0.011	(0.19, 1.42)			
Intercept	-0.006 (0.27)	0.983	(-0.53, 0.51)			
Model 4: Cross-shift 2-hydroxyphenanthrene						
				1.6e-10 (1.1e-9) (3.8e-16, 7e-5)	2.01 (0.18) (1.70, 2.40)	68
Day type	1.27 (0.54)	0.019	(0.21, 2.32)			
Intercept	-0.67 (0.45)	0.14	(-1.55, 0.22)			
Model 5: Cross-shift 3-hydroxyphenanthrene						
				4.5e-13 (5.5e-0) (0, .)	1.66 (0.15) (1.40, 1.98)	66
Day type	1.10 (0.45)	0.013	(0.23, 1.97)			
Intercept	-0.09 (0.37)	0.804	(-0.82, 0.64)			
Model 6: Cross-shift 1-hydroxypyrene (1-hydroxypyrene gluconronide d-9 adjusted)						
				0.24 (0.10) (0.10, 0.55)	0.58 (0.05) (0.48, 0.69)	68
Day type	0.53 (0.16)	0.001	(0.22, 0.84)			
Intercept	-0.16 (0.15)	0.303	(-0.46, 0.69)			

Table 23: Burn Day Output (Linear Combination of Day Type + Intercept) (Creatinine Adjusted)

	β (SE)	p	95% Conf. Interval
2-nap	0.84 (0.18)	0.000	(0.49, 1.18)
1-nap	0.50 (0.39)	0.136	(-0.16, 1.17)
2+9-flu	0.80 (0.17)	0.000	(0.46, 1.14)
2-phen	0.60 (0.29)	0.040	(0.03, 1.17)
3-phen	1.00 (0.24)	0.000	(0.53, 1.49
1-OHP	0.37 (0.11)	0.001	(0.15, 0.59)

Table 24: Results for Mixed Effect Models for Natural Log-transformed Post-shift (Burn Day vs. Non-burn Day) Urine Metabolite Concentrations ln(pg/mL). (Specific Gravity Corrected) (Non-burn day coded 0, burn day coded 1)

	β (SE)	p	95% Conf. Interval	Subject-Specific Random Effect Est.(SE) (95% CI)	Residual Random Effect Est. (SE) (95% CI)	Number of Observations
Model 1: Post-shift 2-nap burn day vs. non-bun day						
				0.44 (0.18) (0.20, 0.97)	0.97 (0.09) (0.81, 1.16)	70
Day type	0.92 (0.26)	0.000	(0.41, 1.43)			
Intercept	7.91 (0.26)	0.000	(7.39, 8.42)			
Model 2: Post-shift 1-nap burn day vs. non-bun day						
				0.49 (0.32) (0.13, 1.78)	1.41 (0.13) (1.17, 1.70)	69
Day type	-0.24 (0.38)	0.520	(-0.98, 0.50)			
Intercept	10.69 (0.36)	0.000	(9.99, 11.39)			
Model 3: Post-shift 2-flu + 9-flu burn day vs. non-bun day						
				0.38 (0.14) (0.19, 0.77)	0.81 (0.07) (0.67, 0.96)	70
Day type	0.84 (0.22)	0.000	(0.41, 1.26)			
Intercept	6.085 (0.222)	0.000	(5.650, 6.520)			
Model 4: Post-shift 2-phen burn day vs. non-bun day						
				0.43 (0.27) (0.19, 1.45)	1.54 (0.14) (1.29, 1.84)	70
Day type	1.49 (0.41)	0.000	(0.69, 2.29)			
Intercept	3.84 (0.38)	0.000	(3.10, 4.57)			
Model 5: Post-shift 3-phen burn day vs. non-bun day						
				2.8e-7 (0.001) (0, .)	1.1 (0.10) (0.95, 1.33)	70
Day type	1.23 (0.30)	0.000	(0.65, 1.82)			
Intercept	4.29 (0.25)	0.000	(3.78, 4.78)			
Model 6: Post-shift 1-OHP burn day vs. non-bun day (1-hydroxypyrene gluconronide d-9 adjusted)						
				0.58 (0.16) (0.34, 1.00)	0.68 (0.06) (0.57, 0.82)	70
Day type	0.55 (0.18)	0.003	(0.19, 0.91)			
Intercept	5.47 (0.24)	0.000	(5.00, 5.94)			

Table 25: Results for Mixed Effect Models for Natural Log-transformed (Morning After/Pre-shift) Ratio Urine Metabolite Concentrations ln(pg/mL). Burn Days Only. (Specific Gravity Corrected)

	β (SE)	p	95% Conf. Interval	Subject-Specific Random Effect Est.(SE) (95% CI)	Residual Random Effect Est. (SE) (95% CI)	Number of Observations
Model 1: Morning after / pre-shift 2-nap						
				7.2e-11 (4.6e-10) (2.5e-16, 2e-5)	1.04 (0.12) (0.84, 1.30)	41
Intercept	0.08 (0.16)	0.612	(-0.24, 0.40)			
Model 1: Morning after / pre-shift 1-nap						
				0.49 (0.71) (0.03, 8.30)	2.11 (0.28) (1.63, 2.73)	38
Intercept	0.26 (0.38)	0.487	(-0.48, 1.00)			
Model 1: Morning after / pre-shift 2-flu + 9-flu						
				1e-5 (0.02) (0, .)	1.63 (0.18) (1.31, 2.02)	42
Intercept	0.09 (0.25)	0.718	(-0.40, 0.58)			
Model 1: Morning after / pre-shift 2-phen						
				0.69 (0.57) (0.13, 3.53)	2.23 (0.27) (1.76, 2.84)	42
Intercept	-0.31 (0.41)	0.455	(-1.11, 0.50)			
Model 1: Morning after / pre-shift 3-phen						
				6.7e-8 (4.1e-7) (4.5e-13, 0.01)	1.67 (0.19) (1.34, 2.09)	40
Intercept	-0.09 (0.26)	0.722	(-0.61, 0.42)			
Model 6: Morning after / pre-shift 1-OHP (1-hydroxypyrene gluconronide d-9 adjusted)						
				0.4 (0.17) (0.17, 0.91)	0.69 (0.09) (0.54, 0.89)	42
Intercept	0.32 (0.16)	0.055	(-0.01, 0.64)			

Table 26: Results for Mixed Effect Models for Natural Log-transformed Cross-shift Ratio (Post-shift/Pre-shift) Urine Metabolite Concentrations and Task. Burn Days Only. ln(pg/mL) (Specific Gravity Corrected) (Holding coded 0, lighting coded 1)

	β (SE)	p	95% Conf. Interval	Subject-Specific Random Effect Est.(SE)	Residual Random Effect Est. (SE)	Number of Observations
Model 1: Cross-shift 2-nap						
				0.61 (0.29) (0.24, 1.55)	1.04 (0.13) (0.81, 1.33)	45
Task	1.02 (0.34)	0.003	(0.35, 1.70)			
Intercept	0.36 (0.33)	0.282	(-0.29, 1.00)			
Model 2: Cross-shift 1-nap						
				0.46 (0.71) (0.02, 9.79)	1.92 (0.25) (1.50, 2.47)	44
Task	0.90 (0.62)	0.146	(-0.31, 2.11)			
Intercept	0.16 (0.51)	0.750	(-0.84, 1.17)			
Model 3: Cross-shift 2-flu + 9-flu						
				1.2e-10 (7.4e-10) (7.8e-16, 2e-5)	1.28 (0.14) (1.04, 1.58)	46
Task	1.13 (0.39)	0.004	(0.36, 1.90)			
Intercept	0.30 (0.31)	0.331	(-0.31, 0.91)			
Model 4: Cross-shift 2-hydroxyphenanthrene						
				8.8e-13 (4.9e-12) (1.5e-17, 5.1e-8)	2.11 (0.23) (1.72, 2.61)	46
Task	0.85 (0.65)	0.189	(-0.42, 2.11)			
Intercept	0.24 (0.51)	0.642	(-0.76, 1.24)			
Model 5: Cross-shift 3-hydroxyphenanthrene						
				0.20 (0.58) (0.0008, 53.25)	1.47 (0.17) (1.16, 1.85)	44
Task	1.60 (0.46)	0.000	(0.70, 2.50)			
Intercept	0.25 (0.36)	0.499	(-0.47, 0.96)			
Model 6: Cross-shift 1-hydroxypyrene (1-hydroxypyrene gluconronide d-9 adjusted)						
				0.37 (0.13) (0.19, 0.73)	0.57 (0.07) (0.45, 0.72)	46
Task	0.33 (0.19)	0.080	(-0.04, 0.70)			
Intercept	0.36 (0.19)	0.054	(-0.01, 0.73)			

Table 27: Summary of p-values for Task Stratified Association Models for Air Contaminants and Metabolites

		2-nap	1-nap	2+9-flu	2-phen	3-phen	1-OHP
CO	Holding	0.467	0.931	0.793	0.808	0.383	0.902
	Lighting	0.017	0.952	0.638	0.435	0.146	0.731
Real-Time $PM_{2.5}$	Holding	0.693	0.475	0.430	0.792	0.619	0.101
	Lighting	0.008	0.563	0.727	0.809	0.072	0.509
Gravimetric $PM_{2.5}$	Holding	0.480	0.724	0.188	0.704	0.627	0.605
	Lighting	0.457	0.822	0.472	0.592	0.049	0.544
Levoglucosan	Holding	0.647	0.994	0.120	0.786	0.275	0.433
	Lighting	0.005	0.704	0.741	0.574	0.427	0.650
LAC	Holding	0.736	0.734	0.095	0.845	0.443	0.813
	Lighting	0.240	0.613	0.264	0.089	0.043	0.302
$PM_{2.5}$ Dose	Holding	0.536	0.996	0.180	0.781	0.537	0.447
	Lighting	0.422	0.879	0.495	0.815	0.102	0.129
LAC Dose	Holding	0.734	0.566	0.106	0.894	0.436	0.539
	Lighting	0.543	0.695	0.283	0.290	0.058	0.011
Levoglucosan Dose	Holding	0.661	0.867	0.125	0.822	0.318	0.380
	Lighting	0.014	0.730	0.832	0.535	0.419	0.324

Table 28: Basic and Full Models for Association of Air Contaminants and Metabolites

		2-nap	1-nap	2+9-flu	2-phen	3-phen	1-OHP
CO	Basic Model	0.135	0.981	0.954	0.408	0.926	0.828
	Full Model	0.507	0.681	0.840	0.693	0.847	0.888
Real-Time $PM_{2.5}$	Basic Model	0.587	0.563	0.449	0.965	0.280	0.245
	Full Model	0.816	0.552	0.532	0.564	0.270	0.445
Gravimetric $PM_{2.5}$	Basic Model	0.617	0.321	0.044	0.829	0.154	0.177
	Full Model	0.336	0.309	0.059	0.429	0.150	0.369
Levoglucosan	Basic Model	0.406	0.869	0.287	0.595	0.886	0.147
	Full Model	0.766	0.675	0.317	0.958	0.880	0.275
LAC	Basic Model	0.033	0.184	0.001	0.249	0.003	0.050
	Full Model	0.045	0.357	0.005	0.117	0.005	0.090
$PM_{2.5}$ Dose	Basic Model	0.435	0.390	0.024	0.789	0.061	0.038
	Full Model	0.053	0.178	0.008	0.471	0.024	0.045
LAC Dose	Basic Model	0.044	0.278	0.002	0.327	0.003	0.015
	Full Model	0.018	0.283	0.003	0.202	0.003	0.027
Levoglucosan Dose	Basic Model	0.663	0.831	0.165	0.673	0.574	0.052
	Full Model	0.555	0.408	0.093	0.924	0.422	0.063

Table 29: Table 30: NHANES Urinary OH-PAH Concentration In US Population

	GM (ng/L)	Median (ng/L)	75th percentile (ng/L)
2-Napthol	3550	3490	7810
1-Napthol	2050	1740	5190
2+9-hydroxyfluorene	495	464	994
2-hydroxyphenanthrene	64.1	64	114
3-hydroxyphenanthrene	71.7	71	130
1-hydroxypyrene	119	118	231

(NHANES 2017)

Table 30: NHANES Creatinine Adjusted OH-PAH Concentration In US Population

	GM (ng/g)	Median (ng/g)	75th percentile (ng/g)
2-Napthol	3710	3330	6690
1-Napthol	2140	1690	4680
2+9-hydroxyfluorene	516	435	834
2-hydroxyphenanthrene	66.9	62.2	97.7
3-hydroxyphenanthrene	74.9	68.4	114
1-hydroxypyrene	125	117	202

(NHANES 2017)

HPLC LOD: To obtain LOD values, I used the values from lowest standard in OHP CAL 12 since it was a recent standard, but all standards had nearly the same concentrations. The signal to noise ratio was approximately 3:20, so the urine concentration was multiplied by 3/20. This value was then divided by the square root of 2 to. This is what was inputted to replace values with a flag code of 6.

(OHP CAL 12 extract conc. pg/µL*220 µL extract vol)/(urine vol) = equivalent urine conc. pg/mL

Equivalent urine conc. pg/mL*3/20 signal to noise ratio = LOD

LOD/sqrt(2) = **Inputted LOD**

Table 31: HPLC and GC-MS Flags

Symbol	Meaning	Action	numeric 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 ^ 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

Levoglucosan data work up: The following equation was used to convert the extract concentration to an air concentration. The extract concentration (called “amount” in GC-MS) was found on the data

summary from the integration made on the GC-MS. The factor of 2 in the equation was to correct for a two fold excess of spiked internal standard.

Extract concentration (amount) (µg/mL) * 0.5 mL extract volume * 2 internal standard correction factor = µg levoglucosan

Levoglucosan (µg)/ air sample volume (m^3) = **µg/m^3 levoglucosan air concentration**

For samples with the "amount" lower than 0.1, a more accurate regression equation was used to calculate the amount to give a newly calculated “adjusted amount.” This regression equation was made for low concentration samples by using the calibration samples that were 0.1 and lower in concentration.

Table 32: CO Metabolite Association Output

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2-nap vs CO	ln(2-nap) vs ln(CO) *n=46*			ln(2-nap) vs ln(CO) + 3 covariates n=46		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln CO	-0.21 (0.14)	0.135	(-0.50, 0.07)	-0.10 (0.16)	0.507	(-0.41, 0.20)
Acres	-	-	-	-0.001 (0.001)	0.301	(-0.003, 0.0009)
Sample Time	-	-	-	0.003 (0.003)	0.402	(-0.004, 0.009)
Season	-	-	-	0.9 (0.46)	0.048	(0.009, 1.80)
Intercept	0.96 (0.24)	0	(0.49, 1.44)	0.27 (0.76)	0.727	(-1.22, 1.76)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.52 (0.32) (0.16, 1.71)			0.44 (0.33) (0.10, 1.90)		
Residual Random Effect Est. (SE) (95% CI)	1.13 (0.14) (0.89, 1.44)			1.11 (0.15) (0.87, 1.44)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 2: Cross-shift 1-nap vs CO	ln(1-nap) vs ln(CO) *n=45*			ln(1-nap) vs ln(CO) + 3 covariates n=45		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln CO	0.006 (0.25)	0.981	(-0.49, 0.50)	0.11 (0.28)	0.681	(-0.43, 0.66)
Acres	-	-	-	-0.002 (0.001)	0.145	(-0.006, 0.001)
Sample Time	-	-	-	0.005 (0.005)	0.363	(-0.01, 0.02)
Season	-	-	-	0.97 (0.81)	0.231	(-0.62, 2.56)
Intercept	0.69 (0.34)	0.044	(0.02, 1.35)	0.22 (1.27)	0.861	(-2.72, 2.27)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.50 (0.61) (0.04, 5.58)			0.23 (1.19) (1e-5, 5,066)		
Residual Random Effect Est. (SE) (95% CI)	1.97 (0.24) (1.55, 2.51)			2.00 (0.26) (1.55, 2.56)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 3: Cross-shift 2+9-flu vs CO	ln(2+9-flu) vs ln(CO) *n=47*	ln(2+9-flu) vs ln(CO) + 3 covariates n=47

	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln CO	-0.01 (0.17)	0.954	(-0.34, 0.32)	0.04 (0.19)	0.84	(-0.33, 0.41)
Acres	-	-	-	-0.001 (0.001)	0.42	(-0.003, 0.001)
Sample Time	-	-	-	-0.0002 (0.004)	0.968	(-0.008, 0.007)
Season	-	-	-	0.20 (0.56)	0.724	(-0.90, 1.29)
Intercept	0.95 (0.21)	0	(0.54, 1.37)	1.20 (0.90)	0.18	(-0.55, 2.96)
Subject-Specific Random Effect Est.(SE) (95% CI)	2.1e-13 (1.5e-12) (3.0e-19, 1.5e-7)			3.7e-12 (2.1e-11) (5.4e-17, 2.5e-7)		
Residual Random Effect Est. (SE) (95% CI)	1.41 (0.15) (1.15, 1.74)			1.41 (0.15) (1.14, 1.75)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 4: Cross-shift 2-phen vs CO	ln(2-phen) vs ln(CO) *n=47*			ln(2-phen) vs ln(CO) + 3 covariates n=47		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln CO	-0.21 (0.26)	0.408	(-0.72, 0.29)	-0.11 (0.29)	0.693	(-0.68, 0.45)
Acres	-	-	-	0.001 (0.002)	0.488	(-0.002, 0.005)
Sample Time	-	-	-	-0.001 (0.006)	0.924	(-0.01, 0.01)
Season	-	-	-	0.70 (0.85)	0.413	(-0.97, 2.36)
Intercept	0.69 (0.32)	0.028	(0.08, 1.31)	0.29 (1.36)	0.834	(-2.38, 2.95)
Subject-Specific Random Effect Est.(SE) (95% CI)	2.9e-12 (1.9e-11) (6.1e-18, 1.4e-6)			6.8e-12 (5.2e-11) (2.3e-18, 2e-5)		
Residual Random Effect Est. (SE) (95% CI)	2.11 (0.22) (1.72, 2.60)			2.15 (0.23) (1.74, 2.66)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 5: Cross-shift 3-phen vs CO	ln(3-phen) vs ln(CO) *n=45*			ln(3-phen) vs ln(CO) + 3 covariates n=45		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln CO	0.02 (0.20)	0.926	(-0.38, 0.42)	0.05 (0.23)	0.847	(-0.41, 0.50)
Acres	-	-	-	-0.001 (0.001)	0.647	(-0.003, 0.002)
Sample Time	-	-	-	0.0001 (0.004)	0.98	(-0.01, 0.01)

Season	-	-	-	0.12 (0.71)	0.864	(-1.26, 1.50)
Intercept	1.19 (0.25)	0	(0.69, 1.69)	1.31 (1.10)	0.234	(-0.85, 3.46)
Subject-Specific Random Effect Est.(SE) (95% CI)	3.7e-12 (2.3e-11) (2.3e-17, 6.0e-7)			6.2e-13 (4.8e-12) (1.7e-19, 2.23e-6)		
Residual Random Effect Est. (SE) (95% CI)	1.68 (0.18) (1.36, 2.07)			1.72 (0.19) (1.38, 2.14)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 6: Cross-shift 1-OHP vs CO	ln(1-OHP) vs ln(CO) *n=47*			ln(1-OHP) vs ln(CO) + 3 covariates n=47		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln CO	0.02 (0.08)	0.828	(-0.14, 0.17)	0.01 (0.09)	0.888	(-0.15, 0.18)
Acres	-	-	-	-0.001 (0.001)	0.046	(-0.002, 2e-5)
Sample Time	-	-	-	0.003 (0.002)	0.053	(4e-5, 0.01)
Season	-	-	-	0.28 (0.25)	0.265	(-0.21, 0.76)
Intercept	0.54 (0.16)	0	(0.24, 0.85)	-0.13 (0.42)	0.75	(-0.95, 0.69)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.40 (0.14) (0.20, 0.79)			0.41 (0.14) (0.21, 0.79)		
Residual Random Effect Est. (SE) (95% CI)	0.61 (0.07) (0.48, 0.77)			0.59 (0.07) (0.47, 0.75)		

Table 33: Real-Time $PM_{2.5}$ Metabolite Association Output

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2-nap vs Real-time PM2.5	ln(2-nap) vs ln(RT PM2.5) *n=43*			ln(2-nap) vs ln(RT PM2.5) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln RT PM2.5	-0.12 (0.22)	0.587	(-0.56, 0.32)	-0.05 (0.23)	0.816	(-0.51, 0.40)
Acres	-	-	-	-0.001 (0.001)	0.191	(-0.003, 0.001)
Sample Time	-	-	-	0.004 (0.003)	0.269	(-0.003, 0.01)
Season	-	-	-	1.09 (0.48)	0.023	0.15 (2.03)
Intercept	1.59 (1.16)	0.173	(-0.69, 3.86)	0.22 (1.42)	0.876	(-2.57, 3.01)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.37 (0.36) (0.05, 2.51)			0.44 (0.34) (0.10, 1.98)		
Residual Random Effect Est. (SE) (95% CI)	1.14 (0.15) (0.89, 1.48)			1.06 (0.15) (0.81, 1.40)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 2: Cross-shift 1-nap vs Real-time PM2.5	ln(1-nap) vs ln(RT PM2.5) *n=41*			ln(1-nap) vs ln(RT PM2.5) + 3 covariates n=41		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln RT PM2.5	0.23 (0.40)	0.563	(-0.55, 1.01)	0.25 (0.42)	0.552	(-0.57, 1.07)
Acres	-	-	-	-0.002 (0.002)	0.199	(-0.01, 0.001)
Sample Time	-	-	-	0.005 (0.006)	0.404	(-0.01, 0.02)
Season	-	-	-	1.02 (0.87)	0.241	(-0.69, 2.74
Intercept	-0.44 (2.06)	0.83	(-4.47, 3.59)	-1.56 (2.53)	0.536	(-6.52, 3.39)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.42 (0.73) (0.01, 12.12)			0.30 (1.00) (4e-4, 211.8)		
Residual Random Effect Est. (SE) (95% CI)	1.99 (0.26) (1.54, 2.56)			1.99 (0.27) (1.53, 2.60)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 3: Cross-shift 2+9-flu vs Real-time PM2.5	ln(2+9-flu) vs ln(RT PM2.5) *n=44*	ln(2+9-flu) vs ln(RT PM2.5) + 3 covariates n=44

	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln RT PM2.5	0.16 (0.21)	0.449	(-0.26, 0.58)	0.14 (0.23)	0.532	(-0.30, 0.58)
Acres	-	-	-	-0.001 (0.001)	0.292	(-0.003, 0.001)
Sample Time	-	-	-	0.001 (0.003)	0.968	(-0.006, 0.006)
Season	-	-	-	0.19 (0.47)	0.68	(-0.73, 1.11)
Intercept	0.09 (1.10)	0.937	(-2.05, 2.23)	0.36 (1.36)	0.79	(-2.30, 3.02)
Subject-Specific Random Effect Est.(SE) (95% CI)	8.5e-14 (8.4e-10) (0, .)			3.6e-13 (2.6e-12) (1.9e-19, 6.5e-7)		
Residual Random Effect Est. (SE) (95% CI)	1.19 (0.12) (0.90, 1.39)			1.10 (0.12) (0.88, 1.37)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 4: Cross-shift 2-phen vs Real-time PM2.5	ln(2-phen) vs ln(RT PM2.5) *n=44*			ln(2-phen) vs ln(RT PM2.5) + 3 covariates n=44		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln RT PM2.5	0.017 (0.38)	0.965	(-0.73, 0.76)	0.24 (0.41)	0.564	(-0.57, 1.05)
Acres	-	-	-	0.002 (0.002)	0.183	(-0.001, 0.006)
Sample Time	-	-	-	-0.005 (0.006)	0.429	(-0.02, 0.01)
Season	-	-	-	0.54 (0.85)	0.522	(-1.12, 2.21)
Intercept	0.74 (1.96)	0.705	(-3.10, 4.57)	0.03 (2.52)	0.991	(-4.92, 4.98)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.33 (0.80) (0.003, 36.07)			0.61 (0.54) (0.10, 3.52)		
Residual Random Effect Est. (SE) (95% CI)	1.98 (0.24) (1.55, 2.51)			1.94 (0.25) (1.50, 2.50)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 5: Cross-shift 3-phen vs Real-time PM2.5	ln(3-phen) vs ln(RT PM2.5) *n=42*			ln(3-phen) vs ln(RT PM2.5) + 3 covariates n=42		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln RT PM2.5	0.32 (0.30)	0.28	(-0.26, 0.91)	0.37 (0.33)	0.27	(-0.28, 1.01)

Acres	-	-	-	-0.001 (0.001)	0.624	(-0.003, 0.002)
Sample Time	-	-	-	0.002 (0.005)	0.712	(-0.007, 0.01)
Season	-	-	-	0.60 (0.72)	0.398	(-0.80, 2.00)
Intercept	-0.57 (1.53)	0.71	(-3.58, 2.44)	-1.27 (2.01)	0.529	(-5.20, 2.67)
Subject-Specific Random Effect Est.(SE) (95% CI)	2.6e-8 (1.5e-7) (2.9e-13, 0.002)			1.4e-10 (7.7e-10) (1.9e-15, 9.6e-6)		
Residual Random Effect Est. (SE) (95% CI)	1.57 (0.18) (1.26, 1.96)			1.6 (0.19) (1.28, 2.02)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 6: Cross-shift 1-OHP vs Real-time PM2.5	ln(1-OHP) vs ln(RT PM2.5) *n=44*			ln(1-OHP) vs ln(RT PM2.5) + 3 covariates n=44		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln RT PM2.5	0.14 (0.11)	0.245	(-0.09, 0.37)	0.10 (0.13)	0.445	(-0.16, 0.36)
Acres	-	-	-	-0.001 (0.0005)	0.098	(-0.002, 0.0001)
Sample Time	-	-	-	0.003 (0.002)	0.151	(-0.001, 0.0060
Season	-	-	-	0.18 (0.27)	0.501	(-0.34, 0.70)
Intercept	-0.17 (0.62)	0.783	(-1.39, 1.04)	-0.48 (0.81)	0.552	(-2.08, 1.11)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.31 (0.14) (0.13, 0.75)			0.34 (0.14) (0.15, 0.75)		
Residual Random Effect Est. (SE) (95% CI)	0.59 (0.07) (0.47, 76)			0.59 (0.08) (0.46, 0.76)		

Table 34: Gravimetric $PM_{2.5}$ Metabolite Association Output

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2-nap vs Gravimetric PM2.5	ln(2-nap) vs ln(grav. PM2.5) *n=42*			ln(2-nap) vs ln(grav. PM2.5) + 3 covariates n=42		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln grav. PM2.5	0.09 (0.19)	0.617	(-0.28, 0.47)	0.19 (0.20)	0.336	(-0.20, 0.58)
Acres	-	-	-	-0.001, (0.001)	0.361	(-0.003, 0.001)
Sample Time	-	-	-	0.004 (0.003)	0.248	(-0.003, 0.010)
Season	-	-	-	1.29 (0.47)	0.006	(0.37, 2.19)
Intercept	0.48 (1.04)	0.644	(-1.56, 2.53)	-1.26 (1.29)	0.329	(-3.79, 1.27)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.41 (0.34) (0.08, 2.04)			0.47 (0.31) (0.13, 1.72)		
Residual Random Effect Est. (SE) (95% CI)	1.12 (0.15) (0.87, 1.45)			1.03 (0.15) (0.78, 1.36)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 2: Cross-shift 1-nap vs Gravimetric PM2.5	ln(1-nap) vs ln(grav. PM2.5) *n=40*			ln(1-nap) vs ln(grav. PM2.5) + 3 covariates n=40		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln grav. PM2.5	0.35 (0.35)	0.321	(-0.34, 1.02)	0.38 (0.37)	0.309	(-0.35, 1.11)
Acres	-	-	-	-0.002 (0.002)	0.252	(-0.006, 0.001)
Sample Time	-	-	-	0.004 (0.006)	0.471	(-0.008, 0.02)
Season	-	-	-	1.10 (0.89)	0.218	(-0.64, 2.83)
Intercept	-1.11 (1.90)	0.56	(-4.83, 2.62)	-2.3 (2.40)	0.339	(-6.98, 2.40)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.48 (0.69) (0.03, 8.01)			0.34 (0.92) (0.002, 63.77)		
Residual Random Effect Est. (SE) (95% CI)	1.98 (0.256) (1.53, 2.57			1.99 (0.28) (1.52, 2.62)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

	ln(2+9-flu) vs ln(grav. PM2.5)	ln(2+9-flu) vs ln(grav. PM2.5) + 3 covariates

Model 3: Cross-shift 2+9-flu vs Gravimetric PM2.5	*n=43*			n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln grav. PM2.5	0.32 (0.16)	0.044	(0.01, 0.63)	0.33 (0.18)	0.059	(-0.01, 0.67)
Acres	-	-	-	-0.001 (0.001)	0.3982	(-0.002, 0.001)
Sample Time	-	-	-	0.002 (0.003)	0.54	(-0.004, 0.007)
Season	-	-	-	0.48 (0.42)	0.248	(-0.34, 1.30)
Intercept	-0.72 (0.86)	0.401	(-2.39, 0.96)	-1.21 (1.12)	0.282	(-3.42, 0.99)
Subject-Specific Random Effect Est.(SE) (95% CI)	1.3e-12 (8.5e-12) (3.0e-18, 5.6e-7)			3.1e-12 (2.0e-11) (1.5e-17, 6.4e-7)		
Residual Random Effect Est. (SE) (95% CI)	0.76 (0.11) (0.78, 1.20)			0.97 (0.11) (0.78, 1.21)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 4: Cross-shift 2-phen vs Gravimetric PM2.5	ln(2-phen) vs ln(grav. PM2.5) *n=43*			ln(2-phen) vs ln(grav. PM2.5) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln grav. PM2.5	0.07 (0.33)	0.829	(-0.58, 0.72)	0.29 (0.37)	0.429	(-0.42, 0.99)
Acres	-	-	-	0.002 (0.002)	0.167	(-0.001, 0.006)
Sample Time	-	-	-	-0.005 (0.006)	0.367	(-0.02, 0.01)
Season	-	-	-	0.51 (0.86)	0.553	(-1.18, 2.19)
Intercept	0.42 (1.80)	0.815	(-3.10, 3.99)	-0.10 (2.37)	0.966	(-4.74, 4.54)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.23 (1.13) (1e-5, 3490)			0.58 (0.56) (0.09, 3.91)		
Residual Random Effect Est. (SE) (95% CI)	2.00 (0.25) (1.57, 2.56)			1.96 (0.26) (1.51, 2.53)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 5: Cross-shift 3-phen vs Gravimetric PM2.5	ln(3-phen) vs ln(grav. PM2.5) *n=41*			ln(3-phen) vs ln(grav. PM2.5) + 3 covariates n=41		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln grav. PM2.5	0.37 (0.26)	0.154	(-0.14, 0.88)	0.42 (0.29)	0.15	(-0.15, 0.99)

Acres	-	-	-	-0.0005 (0.001)	0.742	(-0.003, 0.002)
Sample Time	-	-	-	0.002 (0.005)	0.742	(-0.008, 0.01)
Season	-	-	-	0.67 (0.72)	0.355	(-0.75, 2.09)
Intercept	-0.89 (1.40)	0.528	(-3.62, 1.86)	-1.67 (1.90)	0.377	(-5.39, 2.04)
Subject-Specific Random Effect Est.(SE) (95% CI)	4.0e-10 (2.5e-9) (2.1e-15, 7.5e-5)			5.1e-8 (3.7e-7) (3.4e-14, 0.08)		
Residual Random Effect Est. (SE) (95% CI)	1.58 (0.18) (1.26, 1.96)			1.61 (0.19) (1.28, 2.02)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 6: Cross-shift 1-OHP vs Gravimetric PM2.5	ln(1-OHP) vs ln(grav. PM2.5) *n=43*			ln(1-OHP) vs ln(grav. PM2.5) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln grav. PM2.5	0.14 (0.10)	0.177	(-0.06, 0.34)	0.10 (0.11)	0.369	(-0.12, 0.32)
Acres	-	-	-	-0.0008 (0.0005)	0.117	(-0.002, 0.0002)
Sample Time	-	-	-	0.003 (0.002)	0.115	(-0.001, 0.006)
Season	-	-	-	0.22 (0.26)	0.41	-0.30 (0.73)
Intercept	-0.19, (0.56)	0.737	(-1.29, 0.91)	-0.61 (0.75)	0.412	(-2.07, 0.85)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.32 (0.14) (0.14, 0.74)			0.34 (0.14) (0.16, 0.75)		
Residual Random Effect Est. (SE) (95% CI)	0.59 (0.07) (0.46, 0.75)			0.58 (0.07) (0.45, 0.75)		

Table 35: Levoglucosan Metabolite Association Output

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2-nap vs Levoglucosan	ln(2-nap) vs ln(levo) *n=42*			ln(2-nap) vs ln(levo) + 3 covariates n=42		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln levo.	-0.08 (0.10)	0.406	(-0.27, 0.11)	-0.03 (0.10)	0.766	(-0.23, 0.17)
Acres	-	-	-	-0.001 (0.001)	0.183	(-0.003, 0.001)
Sample Time	-	-	-	0.005 (0.003)	0.156	(-0.002, 0.01)
Season	-	-	-	1.16 (0.47)	0.014	(0.24, 2.10)
Intercept	1.10 (0.28)	0	(0.56, 1.64)	-0.28 (0.83)	0.733	(-1.91, 1.35)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.53 (0.30) (0.17, 1.63)			0.55 (0.31) (0.18, 1.68)		
Residual Random Effect Est. (SE) (95% CI)	1.09 (0.14) (0.84, 1.41)			1.02 (0.15) (0.77, 1.35)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 2: Cross-shift 1-nap vs Levoglucosan	ln(1-nap) vs ln(levo) *n=40*			ln(1-nap) vs ln(levo) + 3 covariates n=40		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln levo.	0.03 (0.19)	0.869	(-0.34, 0.41)	0.09 (0.21)	0.675	(-0.32, 0.51)
Acres	-	-	-	-0.002 (0.002)	0.184	(-0.006, 0.001)
Sample Time	-	-	-	0.005 (0.006)	0.405	(-0.01, 0.02)
Season	-	-	-	1.05 (0.92)	0.254	(-0.75, 2.85)
Intercept	0.70 (0.47)	0.136	(-0.22, 1.61)	-0.48 (1.52)	0.755	(-3.46, 2.51)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.48 (0.69) (0.03, 7.96)			0.31 (1.00) (0.001, 168.9)		
Residual Random Effect Est. (SE) (95% CI)	2.01 (0.26) (1.56, 2.60)			2.03 (0.28) (1.55, 2.65)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 3: Cross-shift 2+9-flu vs Levoglucosan	ln(2+9-flu) vs ln(levo) *n=43*			ln(2+9-flu) vs ln(levo) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval

ln levo.	0.09 (0.08)	0.287	(-0.07, 0.25)	0.09 (0.09)	0.317	(-0.09, 0.28)
Acres	-	-	-	-0.001, (0.001)	0.253	(-0.003, 0.001)
Sample Time	-	-	-	0.002 (0.003)	0.426	(-0.003, 0.008)
Season	-	-	-	0.46 (0.44)	0.293	(-0.40, 1.32)
Intercept	0.85 (0.20)	0	(0.47, 1.23)	0.32 (0.74)	0.666	(-1.13, 1.77)
Subject-Specific Random Effect Est.(SE) (95% CI)	2.9e-8 (2.1e-7) (1.7e-14, 0.05)			7.1e-12 (4.4e-11) (3.5e-17, 1.4e-6)		
Residual Random Effect Est. (SE) (95% CI)	0.99 (0.11) (0.80, 1.24)			0.99 (0.11) (0.79, 1.25)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 4: Cross-shift 2-phen vs Levoglucosan	ln(2-phen) vs ln(levo) *n=43*			ln(2-phen) vs ln(levo) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln levo.	-0.09 (0.17)	0.595	(-0.42, 0.24)	0.01 (0.19)	0.958	(-0.36, 0.38)
Acres	-	-	-	0.002 (0.002)	0.237	(-0.001, 0.005)
Sample Time	-	-	-	-0.005 (0.005)	0.431	(-0.02, 0.01)
Season	-	-	-	0.39(0.89)	0.66	(-1.34, 2.12)
Intercept	0.94 (0.41)	0.023	(0.13, 1.75)	1.37 (1.51)	0.364	(-1.59, 4.33)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.40 (0.70) (0.01, 12.56)			0.61 (0.56) (0.10, 3.65)		
Residual Random Effect Est. (SE) (95% CI)	1.99 (0.24) (1.55, 2.52)			1.97 (0.26) (2.54)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 5: Cross-shift 3-phen vs Levoglucosan	ln(3-phen) vs ln(levo) *n=41*			ln(3-phen) vs ln(levo) + 3 covariates n=41		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln levo.	0.02 (0.13)	0.886	(-0.24, 0.28)	0.02 (0.15)	0.88	(-0.28, 0.33)
Acres	-	-	-	-0.001, (0.001)	0.471	(-0.004, 0.002)
Sample Time	-	-	-	0.003 (0.005)	0.572	(-0.007, 0.012)
Season	-	-	-	0.51 (0.76)	0.501	(-0.98, 1.99)

Intercept	1.05 (0.32)	0.001	(0.42, 1.68)	0.41 (1.25)	0.743	(-2.04, 2.86)
Subject-Specific Random Effect Est.(SE) (95% CI)	2.8e-9 (1.8e-8) (9.3e-15, 0.001)			9.6e-8 (5.2e-7) (6.7e-13, -0.01)		
Residual Random Effect Est. (SE) (95% CI)	1.61 (0.18) (1.29, 2.01)			1.65 (0.19) (1.31, 2.08)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 6: Cross-shift 1-OHP vs Levoglucosan	ln(1-OHP) vs ln(levo) *n=43*			ln(1-OHP) vs ln(levo) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln levo.	0.07 (0.05)	0.147	(-0.03, 0.18)	0.06 (0.06)	0.275	(-0.05, 0.18)
Acres	-	-	-	-0.001 (0.001)	0.117	(-0.002, 0.0002)
Sample Time	-	-	-	0.003 (0.002)	0.107	(-0.001, 0.01)
Season	-	-	-	0.26 (0.27)	0.336	(-0.27, 0.78)
Intercept	0.44 (0.16)	0.005	(0.13, 0.74)	-0.19 (0.47)	0.687	(-1.11, 0.73)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.33 (0.14) (0.14, 0.75)			0.35 (0.14) (0.16, 0.76)		
Residual Random Effect Est. (SE) (95% CI)	0.58 (0.07) (0.46, 0.75)			0.58 (0.08) (0.45, 0.75)		

Table 36: LAC Metabolite Association Output

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2-nap vs LAC	ln(2-nap) vs ln(LAC) *n=42*			ln(2-nap) vs ln(LAC) + 3 covariates n=42		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC	0.32 (0.15)	0.033	(0.03, 0.61)	0.31 (0.15)	0.045	(0.007, 0.61)
Acres	-	-	-	-0.001 (0.001)	0.504	(-0.003, 0.001)
Sample Time	-	-	-	0.004 (0.003)	0.208	(-0.002, 0.01)
Season	-	-	-	1.19 (0.44)	0.007	(0.33, 2.04)
Intercept	-0.19 (0.59)	0.743	(-1.36, 0.97)	-1.45 (0.97)	0.135	(-3.35, 0.45)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.42 (0.29) (0.11, 1.63)			0.48 (0.27) (0.16, 1.45)		
Residual Random Effect Est. (SE) (95% CI)	1.06 (0.14) (0.82, 1.37)			0.99 (0.14) (0.75, 1.29)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 2: Cross-shift 1-nap vs LAC	ln(1-nap) vs ln(LAC) *n=40*			ln(1-nap) vs ln(LAC) + 3 covariates n=40		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC	0.39 (0.29)	0.184	(-0.18, 0.96)	0.29 (0.31)	0.357	(-0.32, 0.89)
Acres	-	-	-	-0.002 (0.002)	0.251	(-0.005, 0.001)
Sample Time	-	-	-	0.005 (0.006)	0.409	(0.007, 0.017)
Season	-	-	-	0.94 (0.88)	0.283	(-0.78, 2.66)
Intercept	-0.73 (1.16)	0.531	(-3.01, 1.55)	-1.42 (1.87)	0.448	(-5.09, 2.24)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.44 (0.80) (0.12, 15.46)			0.33 (1.02) (0.001, 146.4)		
Residual Random Effect Est. (SE) (95% CI)	1.97 (0.27) (1.51, 2.56)			2.00 (0.28) (1.52, 2.64)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 3: Cross-shift 2+9-flu vs LAC	ln(2+9-flu) vs ln(LAC) *n=43*			ln(2+9-flu) vs ln(LAC) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC	0.39 (0.12)	0.001	(0.16, 0.62)	0.38 (0.13)	0.005	(0.12, 0.64)

Acres	-	-	-	-0.0005 (0.0008)	0.547	(-0.002, 0.001)
Sample Time	-	-	-	0.002 (0.003)	0.462	(-0.003, 0.007)
Season	-	-	-	0.33 (0.39)	0.406	(-0.44, 1.09)
Intercept	-0.49 (0.47)	0.296	(-1.39, 0.42)	-0.93 (0.83)	0.264	(-2.56, 0.70)
Subject-Specific Random Effect Est.(SE) (95% CI)	1.5e-9 (8.9e-9) (9.9e-15, 2e-4)			1.3e-10 (9.9e-10) (2.6e-16, 6.5e-5)		
Residual Random Effect Est. (SE) (95% CI)	0.89 (0.10) (0.72, 1.11)			0.92 (0.11) (0.74, 1.15)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 4: Cross-shift 2-phen vs LAC	ln(2-phen) vs ln(LAC) *n=43*			ln(2-phen) vs ln(LAC) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC	0.30 (0.26)	0.249	(-0.21, 0.82)	0.45 (0.29)	0.117	(-0.11, 1.01)
Acres	-	-	-	0.003 (0.002)	0.112	(-0.0006, 0.006)
Sample Time	-	-	-	-0.005 (0.006)	0.384	(-0.016, 0.006)
Season	-	-	-	0.37 (0.83)	0.659	(-1.26, 1.99)
Intercept	-0.34 (1.03)	0.744	(-2.36, 1.68)	-0.38 (1.80)	0.834	(-3.90, 3.15)
Subject-Specific Random Effect Est.(SE) (95% CI)	1.6e-7 (1.1e-6) (4.1e-13, 0.06)			0.46 (0.59) (0.04, 5.74)		
Residual Random Effect Est. (SE) (95% CI)	1.99 (0.22) (1.60, 2.27)			1.94 (0.25) (1.50, 2.50)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 5: Cross-shift 3-phen vs LAC	ln(3-phen) vs ln(LAC) *n=41*			ln(3-phen) vs ln(LAC) + 3 covariates n=41		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC	0.58 (0.19)	0.003	(0.19, 0.96)	0.62 (0.22)	0.005	(0.19, 1.06)
Acres	-	-	-	0.0002 (0.001)	0.895	(-0.002, 0.003)
Sample Time	-	-	-	0.002 (0.004)	0.726	(-0.007, 0.010)
Season	-	-	-	0.49 (0.66)	0.453	(-0.80, 1.79)
Intercept	-1.07 (0.76)	0.156	(-2.56, 0.41)	-1.85 (1.38)	0.18	(-4.56, 0.85)

Subject-Specific Random Effect Est.(SE) (95% CI)	6.4e-10 (4.9e-9) (2.2e-16, 0.002)	1.1e-9 (6.5e-9) (1.1e-14, 1.1e-4)
Residual Random Effect Est. (SE) (95% CI)	1.45 (0.16) (1.16, 1.81)	1.5 (0.18) (1.19, 1.89)

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 6: Cross-shift 1-OHP vs LAC	ln(1-OHP) vs ln(LAC) *n=43*			ln(1-OHP) vs ln(LAC) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC	0.16 (0.08)	0.05	(-0.0001, 0.32)	0.15 (0.09)	0.09	(-0.02, 0.33)
Acres	-	-	-	-0.0007 (0.0005)	0.176	(-0.002, 0.0003)
Sample Time	-	-	-	0.003 (0.002)	0.094	(-0.0005, 0.006)
Season	-	-	-	0.17 (0.25)	0.509	(-0.33, 0.66)
Intercept	-0.05 (0.33)	0.878	(-0.70, 0.60)	-0.67 (0.57)	0.237	(-1.79, 0.44)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.30 (0.13) (0.12, 0.72)			0.33 (0.13) (0.15, 0.72)		
Residual Random Effect Est. (SE) (95% CI)	0.58 (0.072) (0.45, 0.74)			0.57 (0.07) (0.44, 0.74)		

Table 37: $PM_{2.5}$ Inhaled Dose Metabolite Association Output

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2-nap vs PM2.5 Dose	ln(2-nap) vs ln(PM2.5 Dose) *n=42*			ln(2-nap) vs ln(PM2.5 Dose) + 3 covariates n=42		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln PM2.5 Dose	0.12 (0.15)	0.435	(-0.17, 0.41)	0.29 (0.15)	0.053	(-0.004, 0.577)
Acres	-	-	-	8e-6 (0.0006)	0.989	(-0.001, 0.001)
Season	-	-	-	1.11 (0.38)	0.004	(0.36, 1.86)
Intercept	0.70 (0.44)	0.116	(-0.17, 1.56)	-0.14 (0.54)	0.791	(-1.21, 0.92)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.42 (0.33) (0.09, 1.95)			0.44 (0.28) (0.12, 1.56)		
Residual Random Effect Est. (SE) (95% CI)	1.11 (0.15) (0.86, 1.45)			1.01 (0.14) (0.77, 1.31)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 1-nap vs PM2.5 Dose	ln(1-nap) vs ln(PM2.5 Dose) *n=40*			ln(1-nap) vs ln(PM2.5 Dose) + 3 covariates n=40		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln PM2.5 Dose	0.23 (0.27)	0.39	(-0.29, 0.75)	0.39 (0.29)	0.178	(-0.18, 0.95)
Acres	-	-	-	-0.001 (0.001)	0.266	(-0.003, 0.001)
Season	-	-	-	0.86 (0.75)	0.25	(-0.60, 2.33)
Intercept	0.14 (0.79)	0.859	(-1.41, 1.69)	-0.26 (1.02)	0.796	(-2.26, 1.73)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.52 (0.68) (0.04, 6.69)			0.26 (1.19) (3.6e-5, 1900)		
Residual Random Effect Est. (SE) (95% CI)	1.99 (0.26) (1.53, 2.57)			1.98 (0.27) (1.51, 2.60)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2+9-flu vs PM2.5 Dose	ln(2+9-flu) vs ln(PM2.5 Dose) *n=43*	ln(2+9-flu) vs ln(PM2.5 Dose) + 3 covariates n=43

	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln PM2.5 Dose	0.28 (0.12)	0.024	(0.04, 0.51)	0.35 (0.13)	0.008	(0.09, 0.60)
Acres	-	-	-	-0.0004 (0.004)	0.381	(-0.001, 0.0005)
Season	-	-	-	0.46 (0.34)	0.175	(-0.21, 1.13)
Intercept	0.27 (0.35)	0.446	(-0.42, 0.95)	0.03 (0.47)	0.947	(-0.88, 0.94)
Subject-Specific Random Effect Est.(SE) (95% CI)	3.2e-13 (1.9e-12) (3.3e-18, 3.1e-8)			8.1e-12 (5.7e-11) (8.0e-18, 8.1e-6)		
Residual Random Effect Est. (SE) (95% CI)	0.95 (0.11) (0.77, 1.18)			0.93 (0.10) (0.75, 1.16)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2-phen vs PM2.5 Dose	ln(2-phen) vs ln(PM2.5 Dose) *n=43*			ln(2-phen) vs ln(PM2.5 Dose) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln PM2.5 Dose	0.07 (0.26)	0.789	(-0.44, 0.57)	0.20 (0.28)	0.471	(-0.35, 0.75)
Acres	-	-	-	0.001 (0.001)	0.311	(-0.001, 0.003)
Season	-	-	-	0.96 (0.74)	0.192	(-0.48, 2.40)
Intercept	0.62 (0.74)	0.403	(-0.83, 2.07)	-0.32 (1.01)	0.753	(-2.30, 1.66)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.22 (1.17) (5.3e-6, 8871)			0.43 (0.65) (0.02, 8.55)		
Residual Random Effect Est. (SE) (95% CI)	2.0 (0.25) (1.58, 2.56)			1.98 (0.25) (1.54, 2.54)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 3-phen vs PM2.5 Dose	ln(3-phen) vs ln(PM2.5 Dose) *n=41*			ln(3-phen) vs ln(PM2.5 Dose) + 3 covariates n=41		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln PM2.5 Dose	0.37 (0.20)	0.061	(-0.02, 0.76)	0.49 (0.22)	0.024	(0.06, 0.91)
Acres	-	-	-	-0.0002 (0.00080	0.833	(-0.002, 0.001)
Season	-	-	-	0.77 (0.59)	0.193	(-0.39, 1.94)

Intercept	0.12 (0.57)	0.827	(-0.98, 1.23)	-0.39 (0.79)	0.611	(-1.94, 1.14)
Subject-Specific Random Effect Est.(SE) (95% CI)	5.2e-9 (3.4e-8) (1.6e-14, 1.6e-3)			1.1e-8 (4e-5) (0, .)		
Residual Random Effect Est. (SE) (95% CI)	1.54 (0.17) (1.24, 1.93)			1.54 (0.18) (1.23, 1.94)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 1-OHP vs PM2.5 Dose	ln(1-OHP) vs ln(PM2.5 Dose) *n=43*			ln(1-OHP) vs ln(PM2.5 Dose) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln PM2.5 Dose	0.16 (0.08)	0.038	(0.01, 0.31)	0.17 (0.09)	0.045	(0.004, 0.344)
Acres	-	-	-	-0.0001 (0.0003)	0.56	(-0.0008, 0.0004)
Season	-	-	-	0.07 (0.22)	0.743	(-0.06, 0.51)
Intercept	0.13 (0.24)	0.593	(-0.34, 0.60)	0.11 (0.32)	0.728	(-0.51, 0.74)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.30 (0.14) (0.12, 0.72)			0.29 (0.14) (0.11, 0.73)		
Residual Random Effect Est. (SE) (95% CI)	0.58 (0.07) (0.45, 0.74)			0.59 (0.08) (0.46, 0.76)		

Table 38: LAC Inhaled Dose Metabolite Association

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2-nap vs LAC Dose	ln(2-nap) vs ln(LAC Dose) *n=42*			ln(2-nap) vs ln(LAC Dose) + 3 covariates n=42		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC Dose	0.234 (0.117)	0.044	(0.006, 0.463)	0.26 (0.11)	0.018	(0.05, 0.49)
Acres	-	-	-	0.0002 (0.006)	0.764	(-0.001, 0.001)
Season	-	-	-	0.93 (0.34)	0.007	(0.26, 1.59)
Intercept				0.35 (0.33)	0.285	(-0.295, 1.00)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.46 (0.29) (0.13, 1.61)			0.48 (0.26) (0.17, 1.40)		
Residual Random Effect Est. (SE) (95% CI)	1.06 (0.14) (0.82, 1.37)			0.98 (0.13) (0.75, 1.27)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 2: Cross-shift 1-nap vs LAC Dose	ln(1-nap) vs ln(LAC) *n=40*			ln(1-nap) vs ln(LAC) + 3 covariates n=40		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC Dose	0.24 (0.22)	0.278	(-0.20, 0.68)	0.24 (0.22)	0.283	(-0.20, 0.68)
Acres	-	-	-	-0.001 (0.001)	0.324	(-0.003, 0.001)
Season	-	-	-	0.60 (0.71)	0.394	(-0.79, 1.99)
Intercept				0.56 (0.62)	0.372	(-0.66, 1.78)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.53 (0.72) (0.04, 7.61)			0.19 (1.66) (7.8e-9, 4661278)		
Residual Random Effect Est. (SE) (95% CI)	1.97 (0.27) (1.51, 2.57)			2.0 (0.28) (1.53, 2.63)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 3: Cross-shift 2+9-flu vs LAC Dose	ln(2+9-flu) vs ln(LAC) *n=43*			ln(2+9-flu) vs ln(LAC) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC Dose	0.29 (0.09)	0.002	(0.11, 0.48)	0.29 (0.10)	0.003	(0.098, 0.482)
Acres	-	-	-	-0.0002 (0.0005)	0.62	(-0.001, 0.001)
Season	-	-	-	0.24 (0.31)	0.433	(-0.37, 0.86)
Intercept				0.66 (0.27)	0.016	(0.12, 1.20)
Subject-Specific Random Effect Est.(SE) (95% CI)	2.0e-10 (1.3e-9) (9.6e-16, 4.4e-5)			9.0e-13 (5.6e-12) (4.7e-18, 1.7e-7)		

Residual Random Effect Est. (SE) (95% CI)	0.91 (0.10) (0.73, 1.12)	0.91 (0.10) (0.73, 1.14)

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 4: Cross-shift 2-phen vs LAC Dose	ln(2-phen) vs ln(LAC) *n=43*			ln(2-phen) vs ln(LAC) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC Dose	0.20 (0.21)	0.327	(-0.20, 0.61)	0.27 (0.21)	0.202	(-0.15, 0.69)
Acres	-	-	-	0.001 (0.001)	0.225	(-0.001, 0.003)
Season	-	-	-	0.87 (0.67)	0.198	(-0.45, 2.19)
Intercept	0.59 (0.37)	0.112	(-0.14, 1.31)	-0.095 (0.60)	0.874	(-1.28, 1.09)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.09 (2.58) (5.9e-27, 1.3e24)			0.38 (0.69) (0.01, 12.14)		
Residual Random Effect Est. (SE) (95% CI)	1.99 (0.24) (1.57, 2.53)			1.96 (0.25) (1.53, 2.51)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 5: Cross-shift 3-phen vs LAC Dose	ln(3-phen) vs ln(LAC) *n=41*			ln(3-phen) vs ln(LAC) + 3 covariates n=41		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC Dose	0.46 (0.15)	0.003	(0.16, 0.76)	0.48 (0.16)	0.003	(0.16, 0.79)
Acres	-	-	-	0.0002 (0.0080	0.814	(-0.001, 0.002)
Season	-	-	-	0.52 (0.53)	0.327	(-0.52, 1.55)
Intercept	0.64 (0.27)	0.018	(0.11, 0.17)	0.39 (0.46)	0.389	(-0.50, 1.29)
Subject-Specific Random Effect Est.(SE) (95% CI)	7.7e-9 (4.4e-8) (1.1e-13, 0.0005)			2.5e-7(1.9e-6) (1.3e-13, 0.48)		
Residual Random Effect Est. (SE) (95% CI)	1.45 (0.16) (1.17, 1.82)			1.47 (0.17) (1.17, 1.85)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 6: Cross-shift 1-OHP vs LAC Dose	ln(1-OHP) vs ln(LAC) *n=43*			ln(1-OHP) vs ln(LAC) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln LAC Dose	0.15 (0.06)	0.015	(0.03, 0.28)	0.15 (0.07)	0.027	(0.017, 0.28)
Acres	-	-	-	8.6e-5 (0.0003)	0.785	(-0.0007, 0.0005)
Season	-	-	-	-0.046 (0.204)	0.821	(-0.45, 0.35)
Intercept	0.39 (0.14)	0.005	(0.12, 0.66)	0.43 (0.20)	0.028	(0.05, 0.82)

Subject-Specific Random Effect Est.(SE) (95% CI)	0.28 (0.13) (0.11, 0.71)	0.27 (0.14) (0.10, 0.73)
Residual Random Effect Est. (SE) (95% CI)	0.57 (0.07) (0.44, 0.73)	0.59 (0.08) (0.46, 0.75)

Table 39: Levoglucosan Inhaled Dose Metabolite Association

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 1: Cross-shift 2-nap vs Levo Dose	ln(2-nap) vs ln(Levo Dose) *n=42*			ln(2-nap) vs ln(Levo Dose) + 3 covariates n=42		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln Levo Dose	-0.04(0.09)	0.633	(-0.22, 0.13)	0.06 (0.09)	0.555	(-0.13, 0.25)
Acres	-	-	-	8.8e-5 (0.0006)	0.884	(-0.001, 0.001)
Season	-	-	-	0.92 (0.41)	0.026	(0.10, 1.73)
Intercept	0.94 (0.26)	0	(0.44, 1.44)	0.76 (0.30)	0.013	(0.16, 1.35)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.50 (0.31) (0.14, 1.71)			0.46 (0.31) (0.12, 1.73)		
Residual Random Effect Est. (SE) (95% CI)	1.10 (0.15) (0.85, 1.43)			1.06 (0.15) (0.80, 1.38)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 2: Cross-shift 1-nap vs Levo Dose	ln(1-nap) vs ln(Levo) *n=40*			ln(1-nap) vs ln(Levo) + 3 covariates n=40		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln Levo Dose	0.037 (0.17)	0.831	(-0.30, 0.38)	0.16 (0.20)	0.408	(-0.22, 0.55)
Acres	-	-	-	-0.001 (0.001)	0.255	(-0.003, 0.0009)
Season	-	-	-	0.79 (0.80)	0.322	(-0.77, 2.36)
Intercept	0.79 (0.40)	0.05	(-0.001, 1.581)	0.98 (0.54)	0.066	(-0.07, 2.03)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.47 (0.69) (0.03, 8.21)			8.8e-7 (5.6e-6) (3.8e-12, 0.21)		
Residual Random Effect Est. (SE) (95% CI)	2.01 (0.26) (1.55, 2.60)			2.03 (0.24) (1.61, 2.56)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 3: Cross-shift 2+9-flu vs Levo Dose	ln(2+9-flu) vs ln(Levo) *n=43*			ln(2+9-flu) vs ln(Levo) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln Levo Dose	0.11 (0.08)	0.165	(-0.04, 0.25)	0.14 (0.08)	0.093	(-0.023, 0.309)

Acres	-	-	-	-0.004 (0.005)	0.404	(-0.001, 0.0006)
Season	-	-	-	0.39 (0.37)	0.296	(-0.34, 1.11)
Intercept	1.12 (0.18)	0	(0.76, 1.46)	1.13 (0.28)	0	(0.65, 1.62)
Subject-Specific Random Effect Est.(SE) (95% CI)	1.9e-12 (1.4e-8) (0, .)			1.5e-11 (1.2e-10) (4.2e-18, 5.5e-5)		
Residual Random Effect Est. (SE) (95% CI)	0.99 (0.11) (0.80, 1.22)			0.98 (0.11) (0.78, 1.22)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 4: Cross-shift 2-phen vs Levo Dose	ln(2-phen) vs ln(Levo) *n=43*			ln(2-phen) vs ln(Levo) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln Levo Dose	-0.07 (0.16)	0.673	(-0.37, 0.24)	0.017 (0.17)	0.924	(-0.33, 0.36)
Acres	-	-	-	0.001 (0.001)	0.353	(-0.001, 0.003)
Season	-	-	-	0.79 (0.76)	0.304	(-0.71, 2.28)
Intercept	0.72 (0.38)	0.055	(-0.02, 1.46)	0.31 (0.52)	0.556	(-0.72, 1.34)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.37 (0.76) (0.006, 21.04)			0.47 (0.64) (0.03, 6.82)		
Residual Random Effect Est. (SE) (95% CI)	1.99 (0.25) (1.56, 2.54)			1.99 (0.26) (1.55, 2.55)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 5: Cross-shift 3-phen vs Levo Dose	ln(3-phen) vs ln(Levo) *n=41*			ln(3-phen) vs ln(Levo) + 3 covariates n=41		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln Levo Dose	0.07 (0.12)	0.574	(-0.17, 0.31)	0.11 (0.14)	0.422	(-0.16, 0.39)
Acres	-	-	-	-0.0003 (0.0009)	0.724	(-0.002, 0.001)
Season	-	-	-	0.46 (0.65)	0.476	(-0.81, 1.73)
Intercept	1.17 (0.30)	0	(0.59, 1.75)	1.15 (0.42)	0.007	(0.32, 1.98)
Subject-Specific Random Effect Est.(SE) (95% CI)	4.3e-7 (2.5e-6) (4.5e-12, 0.04)			5.3e-7 (3.9e-6) (2.6e-13, 1.10)		
Residual Random Effect Est. (SE) (95% CI)	1.6 (0.18) (1.29, 2.00)			1.63 (0.190 (1.30, 2.04)		

Results for mixed effect models for log-transformed urine metabolite data and its association with air contaminant data. Burn day only. (pg/mL) (specific gravity corrected)

Model 6: Cross-shift 1-OHP vs Levo Dose	ln(1-OHP) vs ln(Levo) *n=43*			ln(1-OHP) vs ln(Levo) + 3 covariates n=43		
	β (SE)	p	95% Conf. Interval	β (SE)	p	95% Conf. Interval
ln Levo Dose	0.09 (0.05)	0.052	(-0.0008, 0.18)	0.10 (0.05)	0.063	(-0.005, 0.21)
Acres	-	-	-	-0.0001 (0.0003)	0.652	(-0.0007, 0.0005)
Season	-	-	-	0.10 (0.23)	0.669	(-0.36, 0.55)
Intercept	0.66 (0.14)	0	(0.38, 0.94)	0.67 (0.18)	0	(0.33, 1.02)
Subject-Specific Random Effect Est.(SE) (95% CI)	0.32 (0.14) (0.14, 0.74)			0.32 (0.14) (0.14, 0.75)		
Residual Random Effect Est. (SE) (95% CI)	0.57 (0.07) (0.45, 0.74)			0.59 (0.08) (0.45, 0.76)		

Table 40: Change in Precision (Effect of Benchmark Adjustment) (pg/mL)

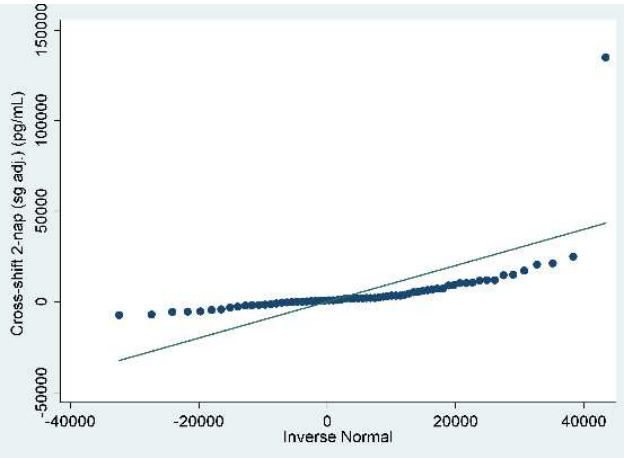
	High Miner Benchmark					
	Unadjusted			d9-adjusted		
	Mean	SD	%CV	Mean	SD	%CV
2-Nap	2266.06	170.03	7.5%	3671.37	384.76	10.5%
1-Nap	12778.45	4830.55	37.8%	20951.09	8804.20	42.0%
2+9-Flu	79.67	47.50	59.6%	132.99	84.64	63.6%
2-Phen	19.23	1.63	8.5%	31.18	3.66	11.7%
3-Phen	7.51	0.59	7.8%	12.15	1.12	9.2%
d9-OHP	15.87	1.02	6.4%	N/A	N/A	N/A
OHP	19.62	2.69	13.7%	31.68	4.15	13.1%
	Low Miner Benchmark					
	Unadjusted			d9-adjusted		
	Mean	SD	%CV	Mean	SD	%CV
2-Nap	55.35	25.52	46.1%	67.76	26.52	39.1%
1-Nap	1847.30	2184.19	118.2%	327.92	51.71	15.8%
2+9-Flu	4.65	2.35	50.6%	5.58	2.72	48.8%
2-Phen	2.12	0.81	38.0%	2.66	1.11	41.6%
3-Phen	0.85	0.54	63.5%	0.99	0.57	57.3%
d9-OHP	20.91	2.72	13.0%	N/A	N/A	N/A
OHP	2.73	0.88	32.1%	3.31	0.78	23.4%
	5+16 Benchmark					
	Unadjusted			d9-adjusted		
	Mean	SD	%CV	Mean	SD	%CV
2-Nap	472.06	98.34	20.8%	623.40	195.83	31.4%
1-Nap	3440.57	2537.99	73.8%	4613.08	3738.28	81.0%
2+9-Flu	61.39	16.07	26.2%	78.76	15.69	19.9%
2-Phen	22.74	7.80	34.3%	29.37	10.21	34.8%
3-Phen	5.23	0.90	17.1%	6.84	1.68	24.5%
d9-OHP	19.43	3.88	20.0%	N/A	N/A	N/A
OHP	7.07	2.54	35.9%	9.02	2.72	30.2%

With each batch of samples, benchmark urine samples were run along with the study samples. These were urine samples that were used to monitor performance and consistency of the assay and instrument. "Hi Miner" was used for batches 6-8, "Lo Miner" for batches 9-13, and "5+16" for batches 14-20. These benchmark samples were used to evaluate assay precision. As described in the methods section, d^9-hydroxypyrene-1- glucuronide was spiked into all samples as an internal standard. We

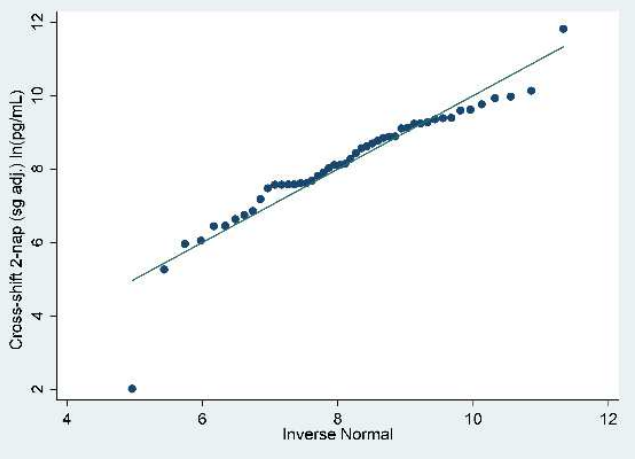
anticipated that by ratio the measured concentration of each metabolite to the d^9-1OHP internal standard we could correct for sample-to sample differences in recovery and hydrolysis efficiency.

Table 40 compares the precision of the measurements of the OHPAH metabolites in the benchmark samples, with and without the d9-OHP adjustment. For all benchmark types, the %CV was lower for the d9-adjusted 1-OHP concentrations, indicating that the d9-1-OHP adjustment improved precision for this metabolite. However, for the other metabolites precision generally was worse after the d9-1-OHP adjustment, indicating the adjustment created more variability in the data. Based in these findings, only 1-OHP was d9-adjusted.

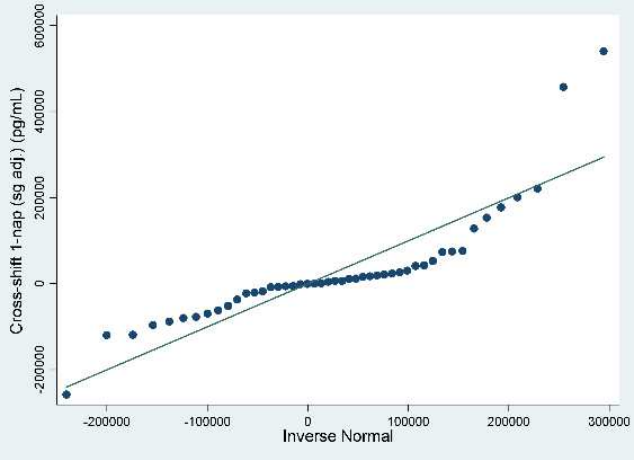
Figure 5: QQ plots for Native Scale and Natural Log Transformation of Variables:



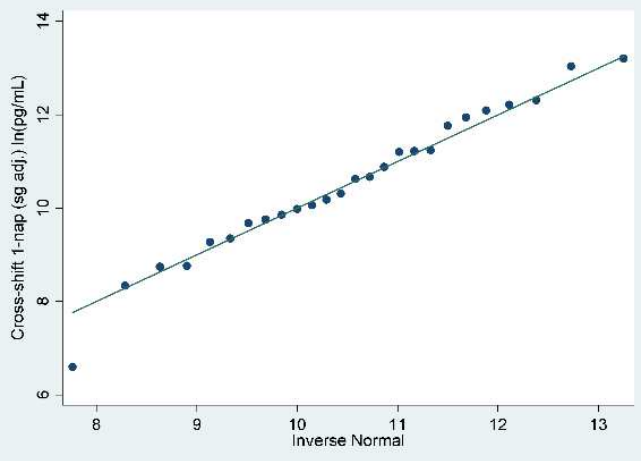
2-nap native scale



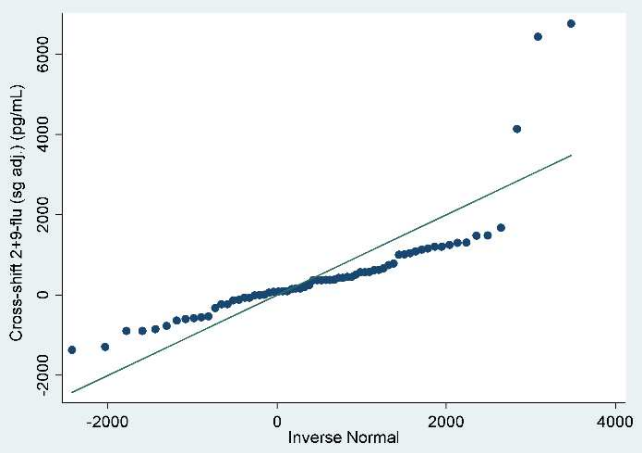
2-nap natural log scale



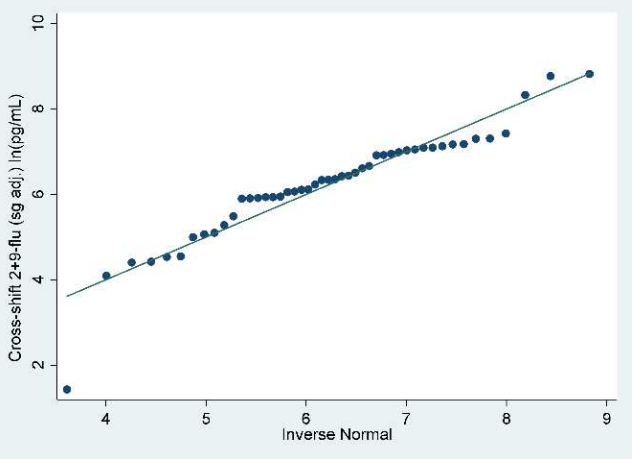
1-nap native scale



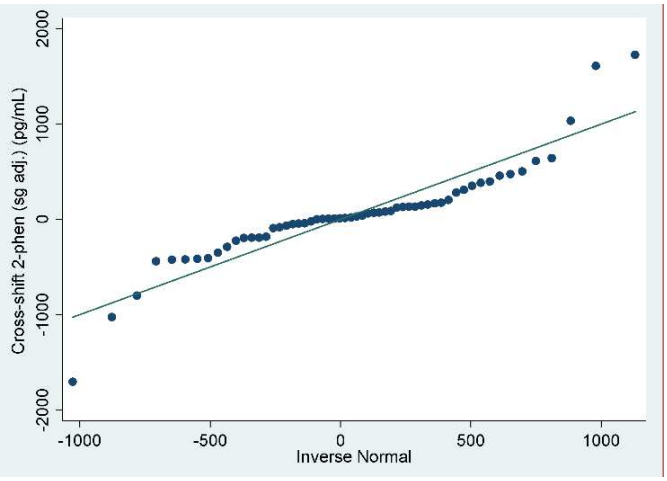
1-nap natural log scale



2+9-flu native scale



2+9-flu natural log scale



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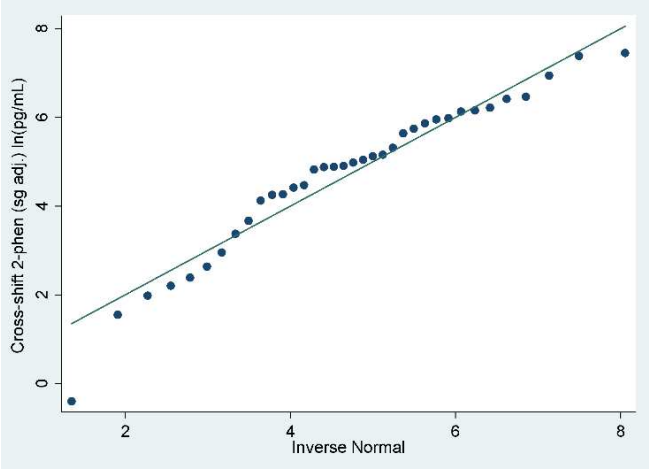

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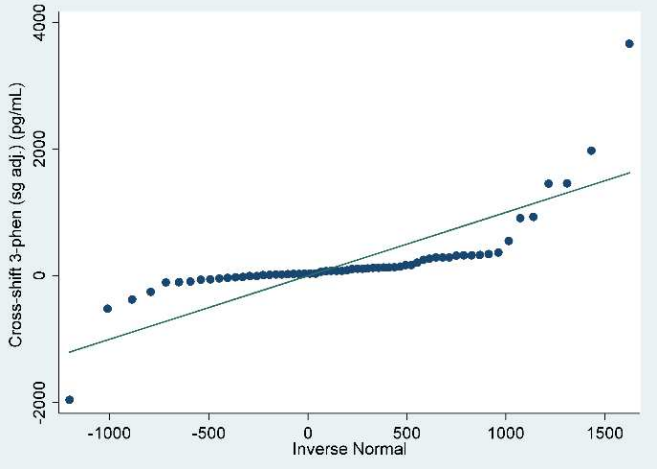
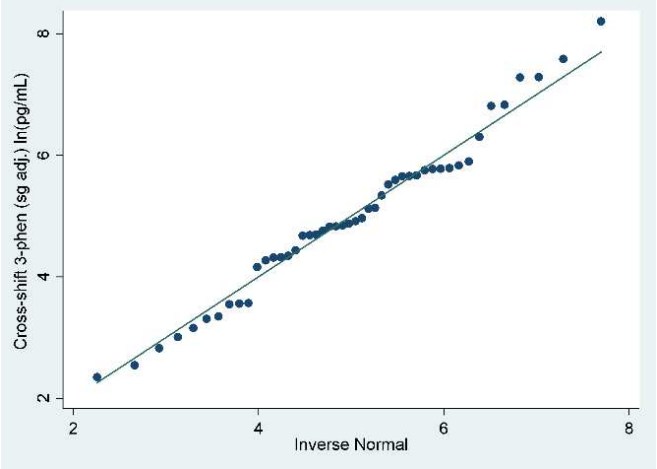
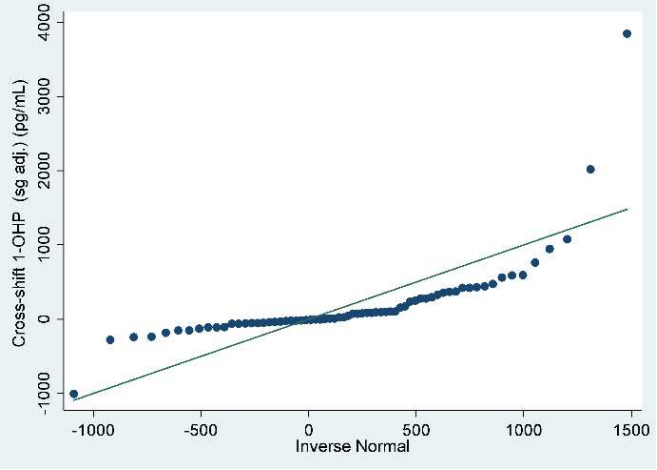


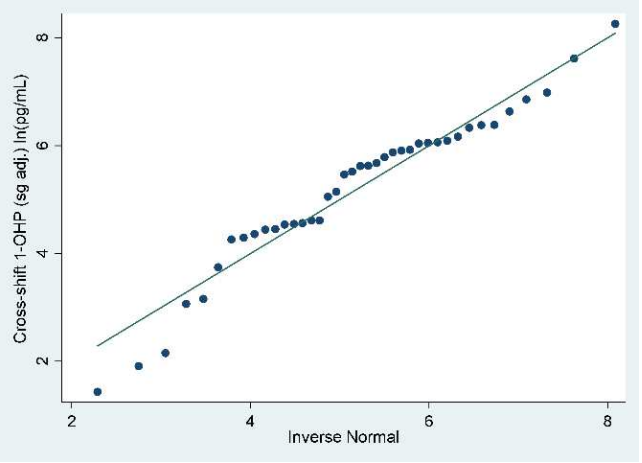
3-phen native scale



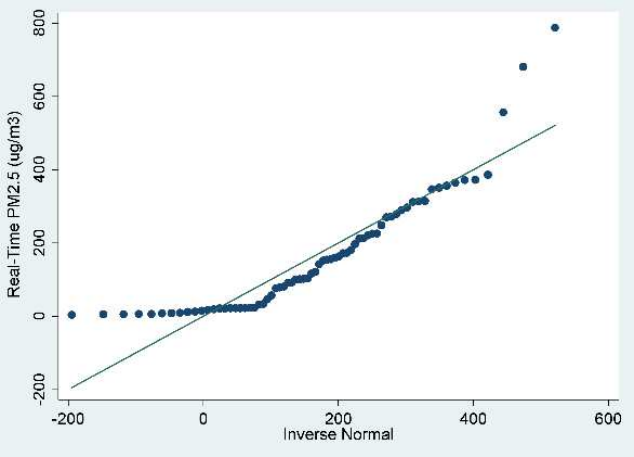
3-phen natural log scale



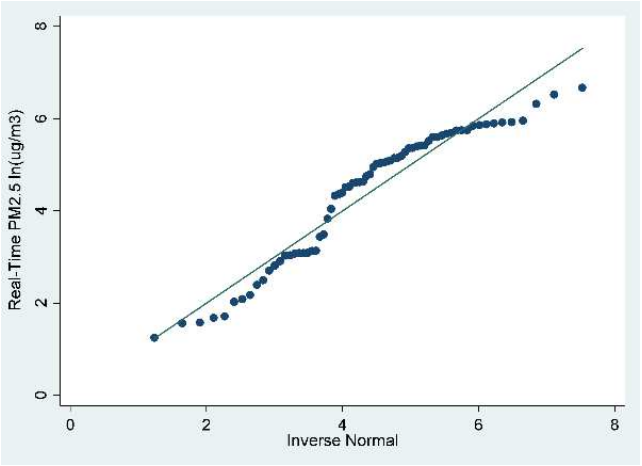
1-OHP native scale



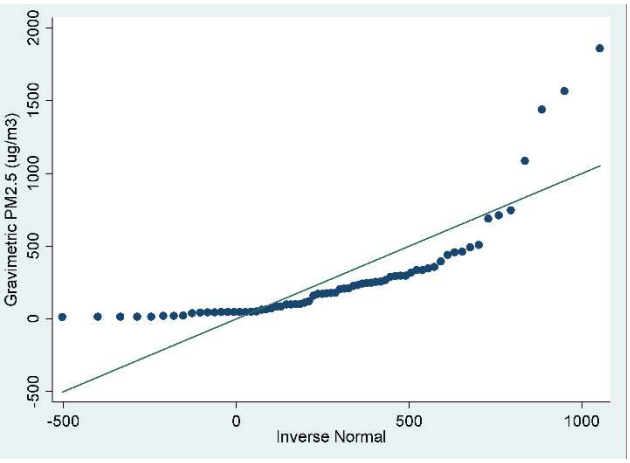
1-OHP natural log scale



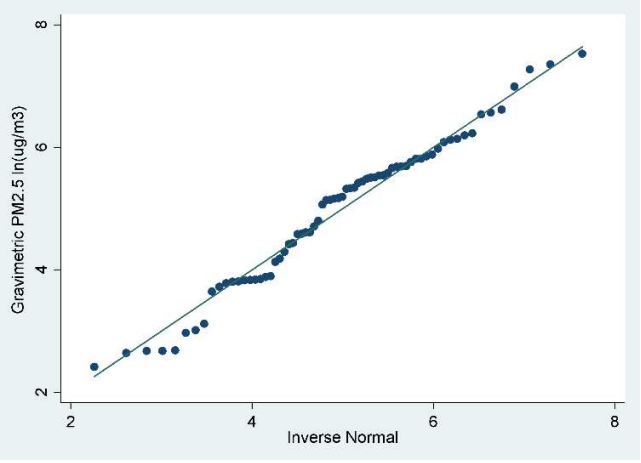
Real-Time $PM_{2.5}$ native scale



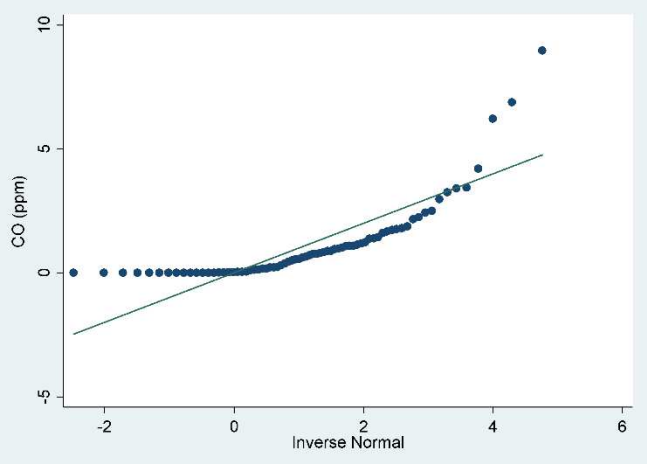
Real-Time $PM_{2.5}$ natural log scale



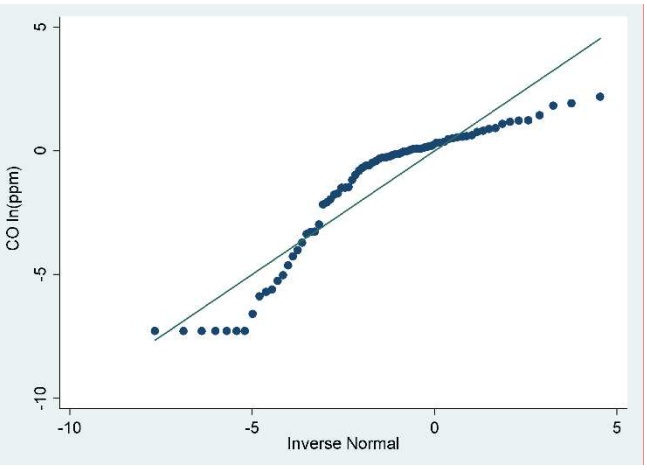
Gravimetric $PM_{2.5}$ native scale



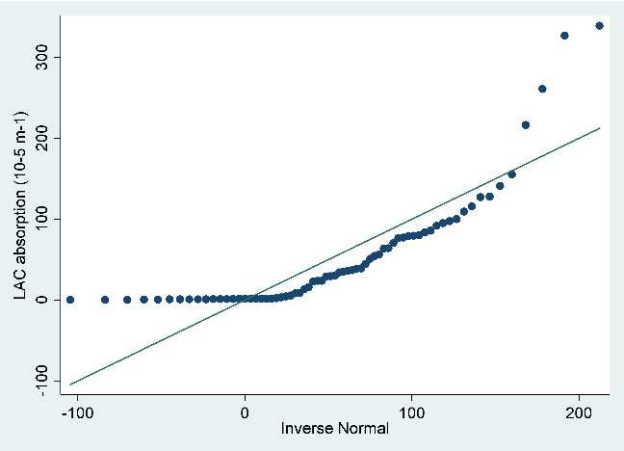
Gravimetric $PM_{2.5}$ natural log scale



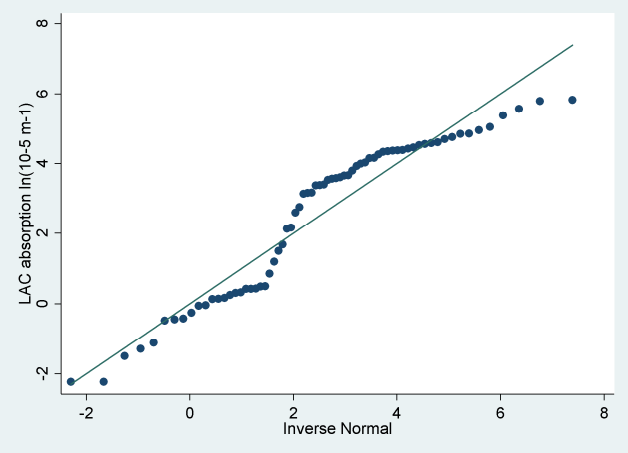
CO native scale



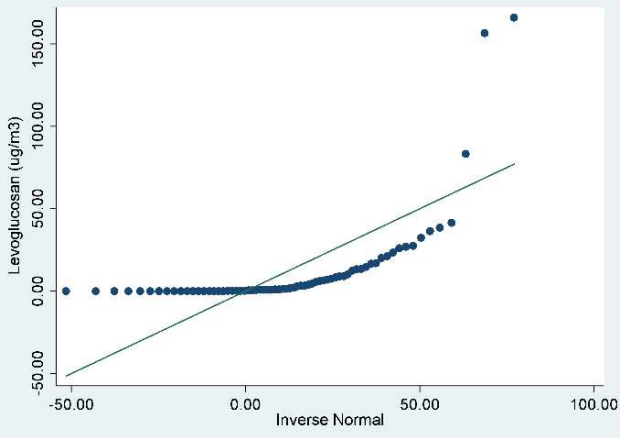
CO natural log scale



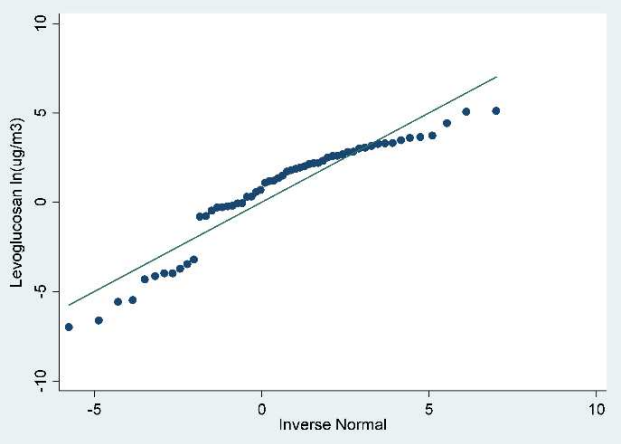
LAC native scale



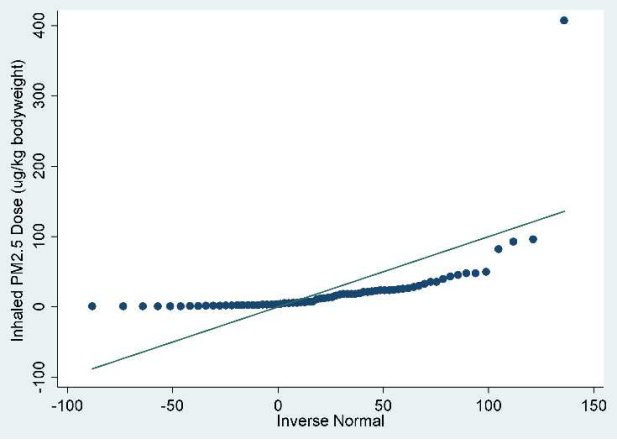
LAC natural log scale



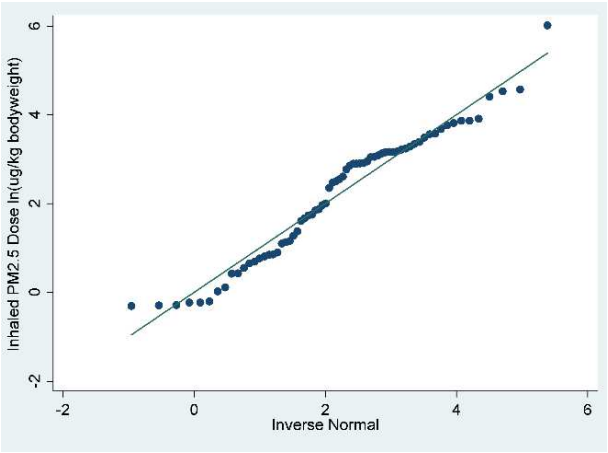
Levoglucosan native scale



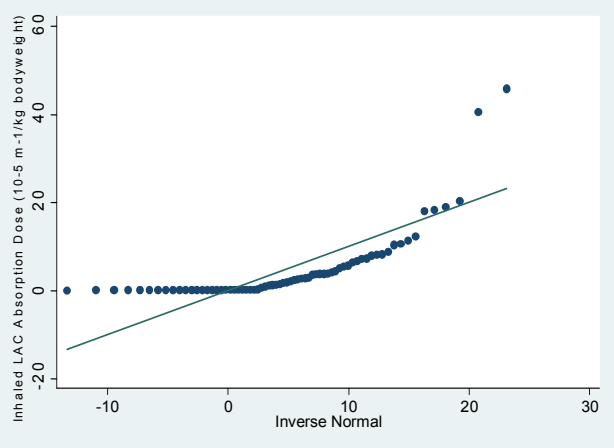
Levoglucosan natural log scale



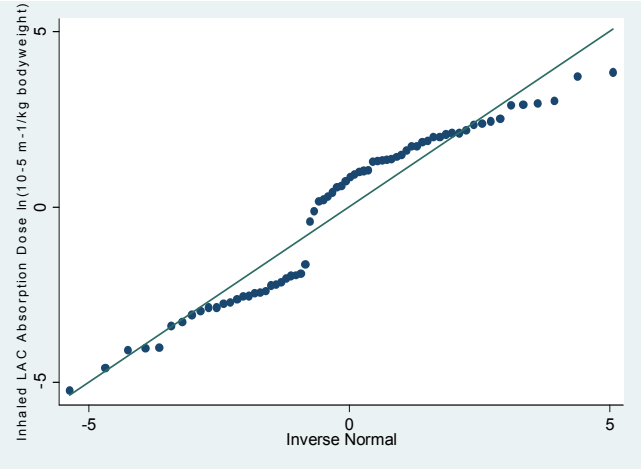
Inhaled $PM_{2.5}$ dose native scale



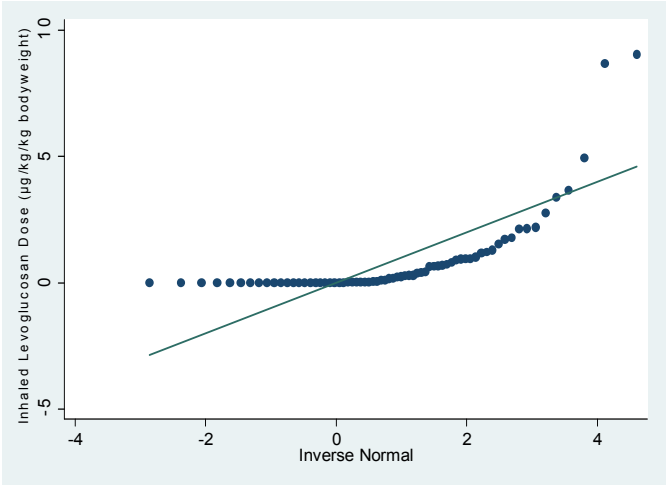
Inhaled $PM_{2.5}$ dose natural log scale



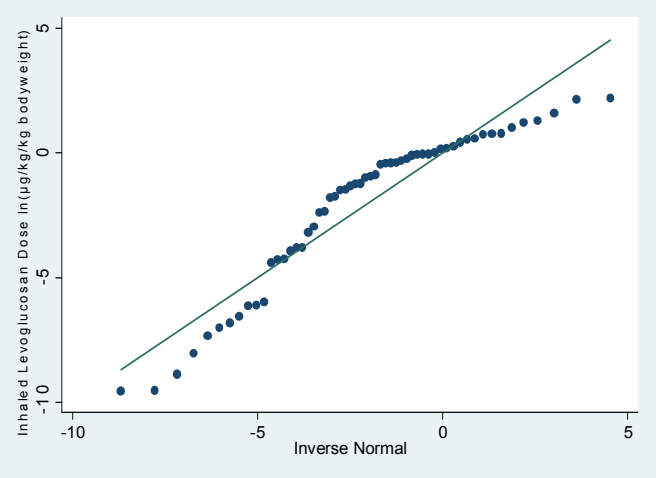
Inhaled LAC dose native scale



Inhaled LAC dose natural log scale



Inhaled Levo Dose native scale



Inhaled Levo Dose natural log scale

Appendix II: 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:	

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List of Tables

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 41: 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 not 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 42 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 43: 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 44: 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 45: 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

Appendix III: SOP for Levoglucosan Extraction and Derivatization

STANDARD OPERATING PROCEDURE (SOP)

FOR EXTRACTION OF LG

Prepared by:		Date:	
Revised by:	Jacqui Ahmad	Date:	8/22/03
Revised by:	Michael Paulsen	Date:	10/10/03
Revised by:	Mike Matles	Date:	12/6/07
Revised by:	Gretchen Onstad	Date:	6/13/08
Revised by:	Andy Leung	Date:	12/17/09
Revised by:	Gretchen Onstad	Date:	9/3/10
Revised by:	Mike Paulsen	Date:	2/1/13
Revised by:	Mike Paulsen	Date:	1/30/17
Revised by:	Mike Paulsen	Date:	2/1/17
Revised by:		Date:	
Reviewed by:	Jacqui Ahmad Research Scientist	Date:	
Approved by:	Chris Simpson Assistant Professor	Date:	

Modifications on 2/1/17:
Use the procedure for combined LG and NPAH extraction except don't split the extract. Rationale for change was to allow the entire extraction process to be completed in the Simpson lab and to increase efficiency by using the 50-sample small volume Turbovap rather than the 6-sample Turbovaps in EHL.

Standard operating procedures for the extraction of Levoglucosan on Teflon filters

This document describes the standard operating procedure for the extraction of anhydro-sugars (levoglucosan) in atmospheric fine particulate collected on Teflon filters. During method development, we have identified potential problems related to analyte losses to unsilanized glassware and volatilization losses of standard compounds. Be aware of these concerns during sample preparation, and endeavor to minimize problems by using silanized glassware. Minimize volatilization losses by storing samples, standards and extracts in the freezer when they are not in use and ensure that all containers (GC vial, headspace vials) are properly sealed.

Note also that expected levels of many of these analytes are less than 10 ng/sample. To prevent contamination, all equipment and supplies used in the assay must be thoroughly cleaned, and should not be mixed with general lab supplies, remembering that lab glassware has been used to synthesize gram quantities of the analytes of interest.

Supplies:

1. Silanized glassware: 8 ml screw top test tubes, Pasteur pipettes, 1.5 ml amber GC vials and GC vial micro inserts.
2. Various sized micro dispenser pipettes and corresponding silanized tubes.
3. Silanized disposable glass Pasteur pipettes (5 3/4 & 9 in).
4. Test tube caps, PTFE-lined.
5. 13 mm, PTFE syringe filters, pore size 0.2 μm.
6. Plastic, disposable, 1cc, luer-lok tip syringes.

Preparation:

1. All glassware which is in contact with the sample or sample extract must be silanized prior to use. See the silanization SOP for specific instructions on glassware preparation. Glassware may be used three times or less depending on cleanliness before resilanization should take place.
2. All glassware must be triple-rinsed with Ethyl acetate (EtOAc) and dry before each use.
3. **Extraction solvent:** Methylene chloride fitted with a 50 ml dispenser.
4. Record each unique reagent container id and lot number onto the extraction worksheet. This will aid with troubleshooting if problems arise.

Standard solutions required:

1. Levoglucosan spike, ~100 ng/µL in EtOAc (current stock is WS01-28-12; note for SRS 2015 filters, the older stock was used for preparing the H-LG spike solution)
2. d_7-Levoglucosan spike (ISTD), ~100 ng/µL in EtOAc
3. Anhydroheptulose (sugar deriviatization control), ~25 ng/µL in EtOAc
4. ~~Internal standard, ISTD, (60 ng/µL d8-Acenaphthalene) in EtOAc~~ (Removed from procedure and began using d7-LG as the internal standard rather than as a recovery standard)

Procedure:

1. Obtain clean, silanized 8 ml test tubes; label them with ToughTags (prepare with laser printer). Each individual sample id must be unique.

Extraction Batch Naming Convention Table

Sample id	# Samples	Sample type
BMMDDYY-XX	2	Method blank
DMMDDYY-XX	2	Deuterated spike
FMMDDYY-XX	2	Fortified spike
Environmental samples*	Up to 40	Sample
CMMDDYY-XX	2	100% Control

XX: sequential numerical order (e.g. 01-99).

MMDDYY: month day year of extraction date (e.g. 012403).

* *Most of our data analysis involves within-person time-series comparisons. Therefore, to prevent introduction of systematic bias into the data due to changes in instrument and assay performance over time, all samples for a single person of a specific type (e.g. outdoor PM10), should be extracted and analyzed in the same batch. Furthermore using numerical methods (i.e. we don't want to always be running samples in the order day 1 to day 10) should randomize the order in which the samples are extracted and analyzed.*

2. Holding one end of filter by the support ring with tweezers (EtOAc-rinsed), clip the support ring at opposite ends of the filter. Then use the other tweezer to fold the filter. Use one tweezer to stuff folded filter down into test tube.

3. Add the appropriate amount of spiking compounds to the appropriate sample id as directed in the spike table below. The standards should be spiked directly onto the filter surface (backside of filter). Cap the test tubes and allow to age for 30 min.
4. Record each unique standard id onto the extraction worksheet. This will aid with troubleshooting if problems arise.

Spike Table

Sample Id	Levoglucosan (H LG spike)	d_7-Levoglucosan (D LG spike)	EtOAc/TEA/ Anhydroheptulose (sample reconstitution solution)
Method Blanks			
Deuterated		20 µL	
Fortified	20 µL	20 µL	
Environmental Samples		20 µL	
100%Control ξ	20 µL	20 µL	460 µL

ξ 100% Control standard preparation described below.

5. Decant the first several draws of methylene chloride to waste container to eliminate bubbles from dispenser arm.
6. Add 7 ml of methylene chloride with dispenser.
7. Sonicate the sample extracts for 1 hour. The water level in the sonic bath should submerge the extraction solvent level in the test tubes. Verify the filter is contained within the extraction fluid. Preheat the TurboVap forty-five minutes during sonication.
8. TurboVap bath temp = 45 °C. Make sure the water level in the bath is near the top of the rack.
9. Remove the filters from the extract prior to evaporation. Pasteur pipettes can be used along with tweezers.
10. Reduce volume to ~1 ml, then cap and vortex the samples for 1 minute.
11. Return samples to the turbovap and evaporate just to dryness.
12. Reconstitute samples in LG reconstitution solution (0.5 ml per sample):
    a. 3.6 mM triethylamine (20 µl into 40 ml dry EtOAc)
    b. 1000 ng/ml AH (1600 µl of 25 ng/µl into 40 ml)
13. Vortex 10 minutes.
14. Use silanized glass Pasteur pipette (triple rinsed with EtOAc, do not need to wait for these to dry) to transfer filter extract to a 1 ml PP syringe with a 0.2 µm PTFE/PP filter into a labeled silanized 1.5 mL amber glass autosampler vial.

- *Make sure that the syringe is over the GC vial before putting the plunger back in, because samples come out using little or no pressure.*
- *Take particular care that the sample ids do not get confused when transferring to the new vessels.*

15. Store extracts in freezer until day of derivatization. Make sure all vials are capped tightly to avoid contamination by water.

Preparation of 100% Controls:

1. To each of 2 silanized amber GC vials add the aliquots indicated for each standard and EtOAc as described in the spike table above (Note that anhydroheptulose will be 92% of the concentration of the all environmental samples and calibrants).
2. Cap GC vials and mix for 30 seconds by vortex.

STANDARD OPERATING PROCEDURE (SOP)

FOR DERIVATIZATION AND ANALYSIS OF LEVOGLUCOSAN ON TEFLON FILTERS

Prepared by:	Jacqui Ahmad Research Scientist	Date:	8/22/03
Revised by:	Mike Paulsen	Date:	5/16/05
Revised by:	Gretchen Onstad	Date:	6/13/08
Revised by:	Andy Leung	Date:	12/17/09
Revised by:	Gretchen Onstad	Date:	9/3/10
Revised by:	Mike Paulsen	Date:	2/1/13
Revised by:	Mike Paulsen	Date:	7/14/16
Reviewed by:	Mike Paulsen Research Scientist	Date:	
Approved by:	Chris Simpson Assistant Professor	Date:	

Standard operating procedure for the derivatization and analysis of Levoglucosan on Teflon filters

This document describes the standard operating procedure for the derivatization and analysis of levoglucosan in atmospheric fine particulate collected on Teflon filters. During method development we have identified potential problems related to analyte losses to unsilanized glassware, volatilization losses of standard compounds, and chemical degradation of selected analytes. Be aware of these concerns during sample preparation, and endeavor to minimize problems by using silanized glassware and protecting samples, standards and extracts from light as much as possible. Minimize volatilization losses by storing samples, standards and extracts in the freezer in F047 when they are not in use and ensure that all sealed containers (GC vial, headspace vials) are properly sealed.

Note also that expected levels of many of these analytes are less than 10 ng/sample. To prevent contamination, all equipment and supplies used in the assay must be thoroughly cleaned, and should not be mixed with general lab supplies, remembering that lab glassware has been used to synthesize gram quantities of the analytes of interest.

Supplies:

1. Silanized glassware: GC vial micro inserts.
2. Crimp top amber GC vials
3. Various sized PDP pipettes and corresponding silanized capillaries.
4. Aluminum foil (used to line work surfaces)
5. Ethyl acetate dried with anhydrous sodium sulfate
6. MSTFA + 1% TCMS, Pierce Chemical Co. #48915
7. Pyridine, 99.9%, HPLC grade, Aldrich #27,040-7

Derivatization:

1. Locate the extracts prepared according to the SOP Filter Extraction. Sample and QC extracts are stored in F-047, freezer FZ-S4.
2. Derivatize a new calibration curve with each sample batch to be analyzed by GC/MS. Filter calibration standards are stored in freezer FZ-S2 located in F-047.
3. Allow extracts and calibration standards to come to room temperature.
4. Place a silanized glass insert into an unsilanized amber GC vial.
5. Label each GC vial with LG (indicating Levoglucosan preparation), the unique sample id or calibration standard id and the date.
6. Add 10 µl pyridine.
7. Add 50 µl MSTFA + 1% TCMS
8. Transfer a 50 µl aliquot of each sample and mix with the PDP pipet. Change capillary for each sample.
9. Cap vial and mix well with vortex mixer (10 seconds of vortex at highest setting is adequate). Make sure all the vials are tightly capped to avoid contamination by moisture.
10. Allow extracts and calibrants to derivatize overnight in the dark at room temperature (6 hour minimum).

Calibration Diluent

1. 4000 µl AH spike solution (25 µg/ml)
2. 2000 µl d7-LG spike solution (100 µg/ml)
3. 50 µl TEA
4. Dry EtOAc to a final volume of 100 ml in a volumetric flask

Table 1. Calibrant Stock and Standard 100 Preparation

Chemical	Receipt or Syn. Date	Chemical Purity	Cat. # or Isotopic Purity	Stock Solution	Stock Conc. (µg/ml)	Corr. Stock Conc. (µg/ml)	Corr. Std. 100 Conc. (µg/ml)	Vol. (µl) into 4 ml
Levoglucosan					876	876	100	456.6
EtOAc/TEA/ AH/d7-LG								3543.4

Table VI-II. Preparation of Calibrants

Conc. (µg/ml)	Source	Volume used of source (µl)	Volume EtOAc/TEA/ AH/d7-LG (ml)	Final Volume (ml)
100	See above			
25	100	1000	3	4
10	100	400	3.6	4
2.5	100	100	3.9	4
1.0	100	40	3.96	4
0.25	10	100	3.9	4
0.1	10	40	3.96	4
0.025	2.5	20	1.98	4
0.01	1	20	1.98	4
0.005	1	10	1.98	4
0	Cal Diluent	0	4	4

Instrumental Analysis:

1. Analyze by GC/MS using internal standard calibration. Typically, the 5973 MSD is used for this analysis. LEV1_300.M is current method.
2. Gas chromatographic conditions:
    a. Inlet
        i. Septa: Thermolite, 11 mm, Restek #20364.
        ii. Liner: Splitless with fused-silica wool, 4.0 mm i.d., Restek #22401
        iii. O-ring: Viton, Restek #20377
        iv. Seal: gold-plated, 0.8 mm, Restek #21318
        v. Carrier: He
        vi. Pressure program: Constant Flow at 0.77 ml/min (10 psi at 150 C)
        vii. Temperature: 250°C
    b. Column: RTX-5sil ms, 30 m, 0.25 mm i.d., 0.25 µm film thickness, Restek #12723
    c. Temperature program:
        i. Initial temperature, 100°C
        ii. Initial time, 2 min
        iii. Rate, 30 deg/min
        iv. Final temperature,300°C
        v. Final time, 1 min.
        vi. Run time 13 min.
        vii. Oven equilibration time, 0.5 min.
        viii. Transfer line, 280°C
3. Mass spectrometer conditions:
    a. Mode: SIM
    b. Solvent delay: 4.5 min
    c. Resolution High for all groups
4. Data Analysis Parameters:
    a. Mode: SIM
    b. Solvent delay: 5 min
    c. Integration: See Table VI-IV
    d. Resolution High for all groups
    a. Group 1
        i. Start time: 5.00
        ii. Plot 1 189, Plot 2 189
        iii. Dwell, 20 for all masses
        iv. Masses 189
    b. Group 2
        i. Start time: 8.50
        ii. Plot 204, Plot 204
        iii. Dwell, 20 for all masses
    e. Masses 204,206,333,339
    f. Deuser Macro: See Appendix I

1. Calibration
    a. Mode: Internal Standard using d7-LG
    b. Calibrants.
        i. See Table VI-I. Individual stock solution is prepared in ethyl acetate at approximately 876 μg/ml for levoglucosan. This is diluted in the Calibration Diluent solution to give Standard 100 (approximately 100 μg/ml).
        ii. Prepare calibrants by dilution of Standard 100 with Calibration Diluent solution according to Table 2.
        iii. Store in -20°C.
        iv. Aliquot and derivatize as described above in the "Derivatization" section.
2. Sequences
    a. The naming convention for a sequence is IIMMDDYY.s, where II is the two-character initial of the analyst, MM is two digit month, DD is two digit day and YY is two digit year.
    b. A new directory must be created to store the data. Use the same naming convention for the sequence (IIMMDDYY).
    c. A solvent blank (ethyl acetate) is analyzed at the beginning of the sequence to insure the system is clean.
    d. Calibrants are analyzed from low to high concentration, starting with the zero standard. Note that zero calibrant is usually not included in the calibration curve.
    e. A midlevel concentration standard is reanalyzed after every fifteen injections followed by a solvent blank. Usually Cal 2.5 is reanalyzed.
    f. One solvent blank and one instrument blank are analyzed after the highest concentration calibrant is analyzed.
    g. Samples are then analyzed, randomized with respect to collection time. All of an individual subject's samples should be run within a single sequence.
    h. Reanalyze instrument blank to eliminate carry-over in the GC injector.
    i. Blank QC samples are analyzed.
    j. Other QC samples (deuterated, fortified, 100% control) are analyzed last.
    k. Recap all the autosampler vials and store them in freezer 5.

Method Performance:

Note: these parameters will vary depending on current stock concentrations of standards.

Table VI-IV. Integration parameters

Chemical	Type	Ret. Time (min)	Target ion (m/z)	Confir-mation ion (m/z)	Integration Events File
~~d8-Acenaphthalene~~	~~ISTD~~	~~6.6~~	~~160~~		~~events.e~~
d_7-Levoglucosan	ISTD	8.06	339	206	events.e
Levoglucosan	A	8.09	204	333	events.e
Anhydroheptulose	RS	9.4	204	333	events.e

A, Analyte; RS, Recovery standard; ISTD, Internal standard