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Diesel Exposure in Mines: Biomarkers in Urine and Realtime Air Monitoring

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Abstract

Elevated exposure to diesel exhaust (DE) is widespread and has been linked to a variety of adverse health outcomes. IARC recently classified DE as carcinogenic to humans. However, limitations in the specificity and reliability of metrics of DE exposure hamper quantitative evaluation of links between DE exposure and adverse health outcomes. Therefore, studies which improve our ability to reliably assess personal exposure to DE are critically required to understand the relationship between DE exposures and disease, and to support the setting of science-based exposure standards that adequately protect the health of workers. Underground miners experience amongst the highest exposures to DE of any occupation. Thus, miners are at high risk for suffering adverse health effects associated with DE exposure.

The overall goal of the current project was to evaluate novel approaches for assessing the exposure of underground miners to DE. We recruited a cohort of 20 mine workers at a large underground metal mine employing about 1,300 employees. Area samples, personal air sampling and collection of urine samples pre-and post shift was performed over four work weeks during March, June, August and October in 2014.

We evaluated associations between TC measured using an MSHA-approved DPM impactor and a variety of integrated and continuously monitored parameters. TC was strongly associated with EC in personal and area air samples. EC concentrations were substantially lower than those measured in the same mine in 2003, indicating that control strategies implemented by the mine to comply with the MSHA PEL have been effective. We found that NO_2 and PM_{10} , acquired in real-time, were positively correlated with TC – however the association was only moderate.

Continuous-reading low costs sensors deployed at fixed locations throughout the mine yielded air contaminant measurements that reflected density of diesel vehicles in the proximity of the sensor.

Regression analysis of 1-nitropyrene 1-NP vs. EC yields a 8.2% increase in geometric mean (GM) 1-NP for each 10% increase in GM EC ($p < 0.001$), demonstrating that 1-NP concentration in air is a viable surrogate measure of diesel exhaust exposure. Associations between 1-NP and OC or TC were not found to be statistically significant, likely due to a large number of OC samples below the limit of detection. Location within the mine, fuel type, and cigarette use as examined in this study were not found to significantly affect the 1-NP/EC association.

Of the measured urinary 1-NP metabolites 6-OHNP and 8-OHNP were detected at the highest levels (6-OHNP: GM = 0.13 pg/mg creatinine, GSD = 2.9 pg/mg creatinine; 8-OHNP: GM = 0.006 pg/mg creatinine, GSD = 2.8 pg/mg creatinine). A significant trend for increasing metabolite levels with day of work week was observed indicating that the timecourse of uptake and elimination of 1-NP is longer than the within-day variability in occupational exposure. Urinary concentrations of 6-OHNP and 8-OHNP were not associated with exposure measured on the day urine sample collection, but were associated with exposures aggregated over the four days prior to sample collection.

Section One

Significant findings

Study aim 1

In underground metal/non-metal mines the MSHA PEL for DE is defined in terms of workshift TWA exposure to total carbon (TC). We evaluated associations between TC measured using an MSHA-approved DPM impactor and a variety of integrated and continuously monitored parameters. Our significant findings were as follows:

1. DPM concentrations acquired from area and personal monitoring were well below the MSHA PEL, and much improved over those observed during a similar study at this same mine in 2003. This demonstrates that control methods (i.e. ventilation, diesel particulate filters retrofitted to vehicles, use of biodiesel) aimed at reducing DPM exposures in the underground mining environment have been very effective.
2. Using the MSHA approved DPM impactor, EC concentrations in personal and area air samples were consistently and positively associated with TC air concentrations.
3. The mine operators informally use real time measurements of NO_2 as a proxy indicators for high personal exposures to DE. We found that NO_2 and PM_{10} , acquired in real-time, were positively correlated with TC, but this correlation was moderate at best.

Study Aim 2

A small network of fixed-location low-cost multi-pollutant sensors with wireless communication capability was deployed at four locations throughout the mine. In addition, two wireless personal multipollutant monitors were worn by study personnel through the project. Our significant findings were as follows:

1. The fixed-location low cost sensors proved effective in acquiring data on multiple contaminants (CO , CO_2 particle count). Relative contaminant concentrations at the different locations measured by these devices were consistent with our *a priori* expectations: locations impacted by high volumes of diesel vehicles had higher contaminant levels than less impacted locations.
2. Although the sensor elements appeared to work effectively in the wireless personal multipollutant monitors, the devices themselves were not sufficiently robust for the mine environment, and the form factor of these personal sensor boxes interfered with worker activities. These limitations can be overcome by redesigning the enclosures for the wireless personal multipollutant monitors.

Study Aim 3

1-nitropyrene (1-NP) is enriched in DE relative to other sources of particulate matter, and has long been proposed as a potential marker for exposure to DE. We investigated the hypothesis that 1-NP concentration in air is a viable surrogate measure of diesel exhaust exposure, as compared with industry-standard elemental carbon (EC) and total carbon (TC) measurements. Further, we investigated the hypothesis that urinary metabolites of 1-NP could serve as a potential biomarker of exposure to diesel exhaust. Our significant findings were as follows:

1. Greater than 90% of 1-NP is present in particles less than $0.5 \mu\text{m}$ in diameter. This is consistent with prior literature describing the size distribution of diesel exhaust particles.
2. We observed a robust association between 1-NP and EC. Regression analysis of 1-NP vs. EC yields an 8.2% increase in geometric mean (GM) 1-NP for each 10% increase in GM EC ($p < 0.001$). Location within the mine, fuel type, and cigarette use as examined in this study were not found to significantly affect the 1-NP/EC association.
3. Associations between 1-NP and OC or TC were not found to be statistically significant, likely due to a large number of OC samples below the limit of detection.
4. Information on primary job tasks can be used to predict personal air concentrations of 1-NP.
5. Of the measured urinary 1-NP metabolites 6-OHNP and 8-OHNP were detected at the highest levels (6-OHNP: GM = 0.13 pg/mg creatinine, GSD = 2.9 pg/mg creatinine; 8-OHNP: GM = 0.006 pg/mg creatinine, GSD = 2.8 pg/mg creatinine).

6. A significant trend for increasing metabolite levels with day of work week was observed indicating that the timecourse of uptake and elimination of 1-NP is longer than the within-day variability in occupational exposure.
7. Urinary concentrations of 6-OHNP and 8-OHNP were not associated with exposure measured on the day urine sample collection, but were associated with exposures aggregated over the four days prior to sample collection.
8. Post-shift urinary concentrations of 6-OHNP and 8-OHNP could be predicted by workshift characteristics including job location, time exposed to diesel exhaust, respirator use, time since previous void, and day of week.

Translation of key findings

In this study in a contemporary mine where emission control devices have been installed on the diesel engines, we observed a robust association between EC and TC even at relatively low air concentrations. This indicates that the PEL could be written in terms of exposure to EC (as was the case historically), which would eliminate the need for testing of area samples to correct for non-diesel sources of OC. A network of low cost sensors with continuous measurement capability offers the potential to provide mine operators immediate feedback when air contaminant levels become elevated, however additional development is required to build personal monitors that are rugged, but not cumbersome, and capable of being networked in a mine environment.

Our observation of a robust association between 1-NP and EC in this underground metal mine where non non-diesel sources of EC are not expected to be present, indicates that 1-NP is a viable marker of exposure to DE. Furthermore, our observations that urinary metabolites of 1-NP are associated with exposures to DE provides the clearest indication to date that these compounds may be useful as biomarkers of exposure to DE. Biological monitoring of exposure offers several key advantages compared to collection of environmental samples, including the fact that biomarkers reflect the dose of DE that has been absorbed into the body, and corrects for subject-specific differences in DE uptake associated with increased exertion due to specific job tasks. Biological monitoring also permits evaluation of the effectiveness of PPE. This is of particular importance to mine workers, as respirator use is mandated as an exposure control measure for specific activities in which DE exposure is expected to be especially high (e.g. driving load-haul-dump equipment that is not outfitted with enclosed cabs).

Outcomes/Impact

Several potential outcomes of this project that impact worker health are outlined below:

1. Expressing the MSHA PEL for DE in terms of EC exposures is feasible, and would reduce costs associated with area monitoring required to correct for non-diesel sources of organic carbon (OC).
2. Emissions control strategies undertaken by mine operators to meet the current DE PEL have substantially reduced DE concentrations within the compared to levels 10 years prior.
3. Inexpensive continuous-reading sensors could provide mine operators helpful data on DE concentrations within the mine in real time. Continued development to optimize these systems for the mine environment is required.
4. Measurements of 1-NP and its metabolites may be particularly valuable in settings such as coal mines or the ambient environment, where EC and TC are confounded by non-diesel sources. Currently, MSHA regulations do not require monitoring of personal exposures to DE in coal mines, because it is not possible to reliably differentiate DE-derived EC/TC from coal dust derived EC/TC.
5. In addition, literature on the epidemiology of exposure to ambient traffic related air pollution, including DE clearly shows an association with chronic cardiovascular disease. However, this association has not yet been well studied in an occupational setting. These improved measures of DE exposure and dose based on 1-NP and its metabolites would be useful in occupational epidemiology studies to evaluate the risk of chronic cardiovascular disease arising from occupational exposure to DE.

Section Two

Project Background

Elevated exposure to diesel exhaust (DE) is widespread and has been linked to adverse health outcomes including respiratory irritation, cardiovascular disease, immune dysfunction and lung cancer. IARC recently classified DE as carcinogenic to humans (Group 1), based on sufficient evidence that exposure is associated with an increased risk for lung cancer. However, limitations in the specificity and reliability of metrics of DE exposure hamper quantitative evaluation of links between DE exposure and adverse health outcomes. Therefore, studies which improve our ability to reliably assess personal exposure to DE are critically required to understand the relationship between DE exposures and disease, and to support the setting of science-based exposure standards that adequately protect the health of workers. Underground miners experience amongst the highest exposures to DE of any occupation. Thus, miners are at high risk for suffering adverse health effects associated with DE exposure.

The overall goal of the current project is to evaluate novel approaches for assessing the exposure of underground miners to DE. The specific aims are: (1) Determine the association between the MSHA-approved DPM impactor and realtime measurements of particle mass concentration, particle number concentration and CO; (2) Determine the reliability of a newly designed network of personal and fixed-location wireless remote sensors to provide realtime measurements of surrogates for DE exposure including particle number concentration; and (3) Determine whether urinary metabolites of the DE-specific chemical 1-nitropyrene (1-NP) are a reliable measure of individual workers exposure to DE.

Our proposed research addresses the mining sector, and NIOSH's cross-sector programs in exposure assessment and respiratory diseases. The outputs from this project are new exposure monitoring approaches (urinary biomarkers, real-time measures of DE, wireless sensor arrays for exposure monitoring) that will provide improved estimation of miner's exposure to DE – likely at significantly lower cost than the MSHA-approved DPM impactor technology. The improved exposure assessment methods will in turn facilitate evaluation of the burden of disease associated with these exposures, and the effectiveness of recent interventions to reduce miner's exposures to DE (e.g. introduction of biodiesel and engine emission control devices) (the project outcomes).

Research to practice: These studies evaluated the reliability, accuracy and ease of use of the novel exposure monitoring tools in the field under real-world conditions. Data collected regarding DE concentrations in the mine were with mine management and health and safety staff, and can be used to identify any locations or activities where DE exposures may approach hazardous levels.

Specific Aims

Exposure to DE in underground mines is potentially an important risk factor for a number of debilitating diseases amongst miners, including respiratory disease, cardiovascular disease, and lung cancer.

However, epidemiological studies that reported associations between DE exposure and adverse health outcomes have been criticized for using inaccurate or imprecise measures of DE exposure. In order to adequately protect the health of miners, studies that reliably measure miners' exposure to diesel exhaust are required, so that the link between diesel exposure and potential adverse health outcomes can be quantified. In the current study, we evaluated the suitability of several direct reading aerosol monitoring devices and a DE-specific urinary biomarker for monitoring exposure to DE in mines. The specific aims were:

- (1) Determine the association between the MSHA-approved DPM impactor and realtime measurements of particle mass and particle number concentration;**
- (2) Determine the reliability of a newly designed wireless network of personal and fixed-location remote sensing devices to provide reliable realtime estimates of DE exposure based on particle count;**
- (3) Using a cohort of underground miners, determine whether urinary metabolites of the DE-specific chemical 1-nitropyrene (1-NP) are a reliable measure of individual workers' exposure to DE.**

To address these aims we enrolled a cohort of underground miners, and undertook personal, environmental and biological monitoring of DE exposures.

Methodology

Study Setting and Subjects

We studied exposures to DE among workers at a large underground metal mine employing about 1,300 employees. Twenty mine workers of various job titles were recruited and followed for four work weeks during March, June, August and October in 2014. Subjects were assigned *a priori* to one of three exposure groups (low, medium and high) by mine health and safety staff, based on job titles and typical work location. At this mine site, workers typically work a schedule of four days on followed by four days off. Further, successive four-day work weeks alternate between day-shift and night-shift. To simplify study logistics, all study participants were members of "B crew" - one of four rotating shifts of workers at the mine, and sample collection only took place when B crew were on the day shift.

Intake Questionnaire and Surveys

At the commencement of the project we administered an intake questionnaire to study subjects to obtain demographic information about the study subjects including date of birth, gender, years worked in mining, and years worked at the mine. Study subjects also completed work-activity surveys at the

conclusion of each monitored work shift, to obtain information about specific tasks performed during the workshift, respirator use, time spent underground, time spent in proximity to DE, and tobacco use.

Air Sampling

Area Samples: Environmental area monitoring was conducted to evaluate contaminant air concentrations at 7 different locations in the mine (see table 1 below). Determination of area location was based on the degree of mining activity at a given location and the potential to capture the variability of environmental air contamination, with the intention of performing air monitoring at locations where low, moderate, and high contaminant concentrations were anticipated. The extent of area environmental monitoring was limited by instrument availability and the cost of sample analysis. Thus, over each four-day field campaign, locations one through three were monitored on days one and three and locations four through six were monitored on days two and four. Table 1 shows that the anticipated concentration levels (i.e. high, moderate, and low) for the group of area locations that were monitored on alternating days were matched in such a way that each group had an equal number in high and moderate category. Location seven was a conference room located in an operations building at the surface of the mine and served as the study’s control location. Monitoring at this location was performed daily over the entire four-day field campaign.

Table 1: Area monitoring locations

| Location # | Mine Location Coordinates | Anticipated Concentration Level | Monitoring Days |
|-------------------|----------------------------------|--|------------------------|
| 1 | 2000 East 300 East | High | |
| 2 | 2600 East 1600 West | High | 1 and 3 |
| 3 | 2900 Shop | Moderate | |
| 4 | 4400 West 13950 West | High | |
| 5 | 4100 West 13800 West | High | 2 and 4 |
| 6 | 5000 Shop | Moderate | |
| 7 | Surface Conference Room | Low | All Four Days |

Instrumentation placed at each monitoring location included an array of real-time and time-integrated sample collection devices. (see Table 2 below). Each of these sampling devices was co-located in a sampling basket so that results from each device would represent the same area being monitored. For monitoring locations 1, 2, 4, and 5, sampling baskets were hung in free space adjacent to the wall of a mine shaft. Monitoring locations 3 and 6 were underground vehicle maintenance and repairs shops and sampling baskets were strategically placed in an area of each shop that would allow sampling devices to provide results that were representative of typical daily exposures to airborne contaminants.

Time durations of area environmental monitoring varied and depended upon the time required to travel to the underground area location. This variability was a function of the time required to adequately prep for the day's sampling event at the mine surface. Even so, time durations for all area monitoring performed over all four field campaigns averaged approximately ten hours per day.

Table 2. Instrumentation at each area monitoring site

| Instrument Type | Data Collection Type | Acquired Measurement |
|--------------------------------|-----------------------------|--|
| SKC® DPM impactor | Time-Integrated | DPM (EC & OC), 1-NP |
| SKC® GS-3 Cyclone | Time-Integrated | PM ₄ Mass, 1-NP |
| TSI™ DustTrak 8520 | Real Time, Data Logging | PM ₁ Mass Concentration |
| TSI™ QTrak 8552 | Real Time, Data Logging | RH, Temp, CO, CO ₂ |
| TSI™ AeroTrak 9303 | Real Time, Data Logging | Particle Count (300nm to 10µm) |
| TSI™ PTrak 8525 | Real Time, Data Logging | Particle Count (200nm to 1µm) |
| Wireless Remote Sensing Device | Real Time, Data Logging | Particle Count, CO, CO ₂ |
| MX-4 4-Gas Monitor | Real Time, Data Logging | NO ₂ , CO, hydrocarbons (LEL), O ₂ |
| SKC® Sioutas Impactor | Time-Integrated | 8-stage Mass Fractionation |

Personal and area wireless monitors: We performed personal and area monitoring to evaluate the ability of the newly developed Boise State University (BSU) wireless Remote Sensing Device to aid in the characterization of DE exposures. Personal and area monitoring using these devices occurred on all four days of every field campaign. . The particle size range for particle count data is 300nm to 10µm. The larger portable remote sensing device was included in a sampling basket associated with four environmental monitoring areas and the methods to perform area monitoring with this device are in accordance with those described in the section above. The Personal Remote Sensing Device was used to monitor personal exposures received by two individuals on our research team. Each monitoring day two members of the research team attached the smaller personal monitoring device to their work belt and it accompanied them underground where they spent the work day traveling between area locations checking that the instruments housed in the sampling baskets were functioning properly. Time durations for the operation of the personal Remote Sensing Devices was approximately ten hours each day.

Personal Filter Samples: Data collection consisted of full shift personal sampling for four days per monitoring period. Due to equipment limitations, personal air samples were collected on the first ten subjects on days one and three, and the other ten were monitored on days two and four. Both sets of ten subjects were approximately balanced with respect to *a priori* exposure category. Personal air sampling consisted of fitting an MSHA compliant SKC DPM impactor downstream of a GS-1 cyclone pre-filter to the collar of each worker. Air was drawn through the DPM impactor sampling trains using SKC AirChek and

PXCR personal sampling pumps at a nominal flow rate of 1.7 liters/minute to provide a nominal cutpoint of 0.8 μm at the DPM impactor. The SKC GS-1 Respirable Cyclone is used with the SKC DPM Cassette as a prefilter to remove large particles that might otherwise foul the inlet to the DPM sub-micron impactor. Particles less than 0.8 μm are collected on the first quartz filter inside the impactor. About 80-90% of DPM is less than 1 μm by mass, so these samplers meet NIOSH Method 5040 specifications for DPM collection and analysis through quantification of EC, OC, and TC. Additionally, we conducted a supplementary analysis to examine the size distribution of 1-NP in area samples collected in the mine using a Sioutas cascade impactor, and determined that overall greater than 90% of the 1-NP is found in particles $<0.5 \mu\text{m}$. (see Figure 1 below). Thus, the SKC DPM impactor is expected to capture the majority of 1-NP-containing particles present in the mine. Air flow through the sampling train was calibrated prior to the work shift using a drycal calibrator (SKC Inc., Eighty-Four PA). At the end of each subject's workshift, flow through the sampling train was re-measured with the loaded sample filter intact. Air volume for each sample was calculated as the average of the pre and post flow, multiplied by the sample time.

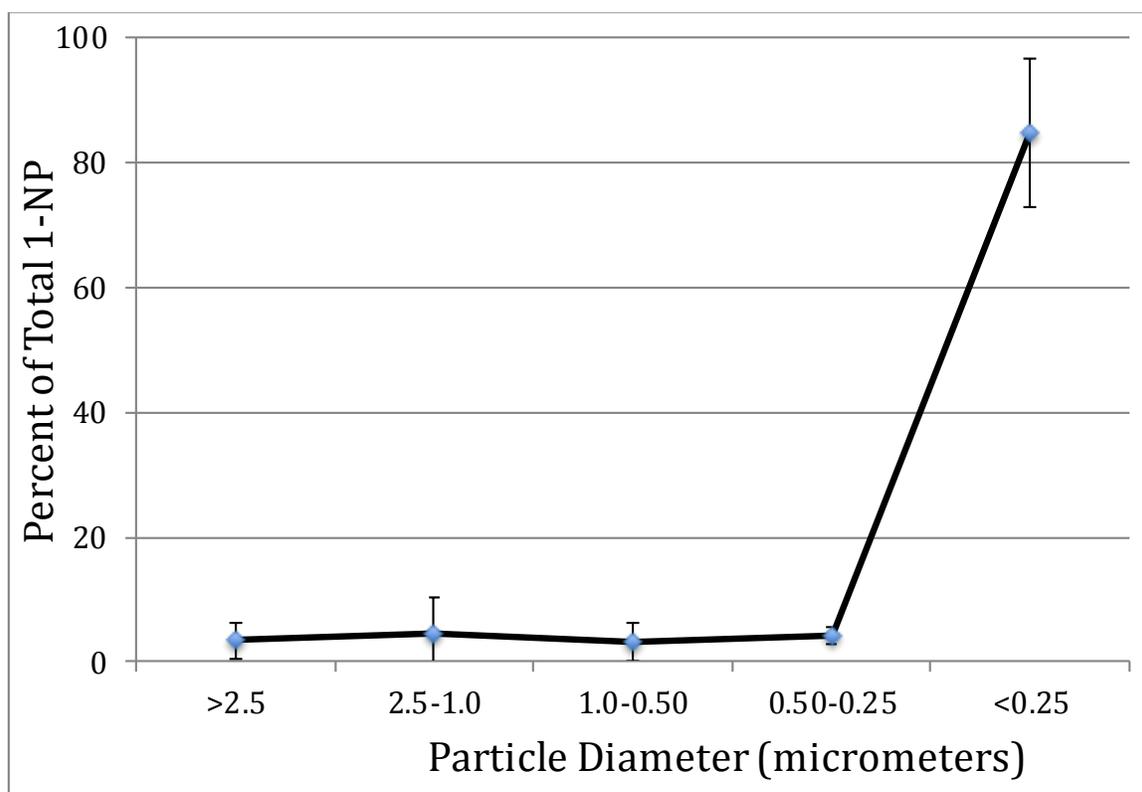


Figure 1: Size distribution of 1-nitropyrene

Filter Analysis

EC, OC, and TC were determined from the DPM quartz filters by ALS Environmental (Salt Lake city, UT) using the NIOSH 5040 method. EC and OC concentrations were reported in units of $\mu\text{g}/\text{sample}$ and added together to calculate TC. Average field blank OC mass ($17.8 \mu\text{g}/\text{sample}$) was subtracted from each reported sample OC mass, and for QC purposes the limit of detection (LOD) for OC was set as three times the standard deviation of the blank samples ($20.2 \mu\text{g}/\text{sample}$). ALS Environmental provided a reporting

limit of 1.7 µg/sample for EC, and censored all EC values less than 1.7 µg/sample. EC, OC and TC values were converted to air concentrations (µg/m³) using air volume data from pump calibration field logs. Four field blanks were included in each of the four sample batches (one for each study campaign) for analysis.

1-NP concentrations were measured as described by Miller-Schulze [Miller-Schulze, 2007]. 1-NP determination including filter extraction and LC/MS/MS analysis occurred in five analytical batches. In brief, filter samples were spiked with internal standard (d⁹-1-nitropyrene) and extracted by ultrasonication in methylene chloride. Extracts were reduced to dryness under a stream of nitrogen, resuspended in 3:1 ethanol: sodium acetate buffer (20mM, pH 5.5), filtered, then analyzed using two-dimensional high performance liquid chromatography tandem mass spectrometry (2D-HPLC-MS/MS). The instrument LOD for 1-NP extract concentration was calculated by taking the average plus three times the standard deviation of 1-NP extract concentrations from field and process blank filters. Instrumental LODs (0.2-2.7 pg/sample) varied by sample batch, and were converted to concentration-equivalent LODs (units of fg/m³) by dividing by the sampled air volume for each sample. The accuracy and precision of the analysis was 87±7% of the expected values as determined by measuring concentrations of 1-NP in spiked (fortified) filters.

Urine sample collection and analysis

Urine samples were collected from each worker pre and post shift for each of the 16 workshifts that were monitored. Time of current void was recorded for each sample, and workers self-reported the time of the previous urine void. Void volume and specific gravity were measured in the field, then aliquots of 110mL were retained for each sample, frozen on dry ice, and shipped to the University of Washington for subsequent analysis. A total of 535 urine samples were collected, each subject gave an average of 32 samples (range: 8 – 39). Due to resource limitations, only 170 of the 535 unique samples were selected for measurement of 1-NP metabolite levels.

Urine samples were analyzed for 1-NP metabolites using liquid chromatography-tandem mass spectrometry (LC/MS/MS), using the method of Toriba *et al.* [2007]. Briefly, samples were filtered and the pH stabilized using a 4M acetate buffer (pH 5) then the metabolites were deconjugated using β-Glucuronidase/aryl sulfatase. The deconjugated metabolites were extracted using a blue rayon sorbent, eluted from the sorbent, then evaporated to near dryness and reconstituted. The reconstituted samples were cleaned using Alumina Sep-Paks then evaporated to near dryness, the sample volume was then brought to roughly 300µL in methanol and filtered into HPLC vial inserts. Prior to analysis the samples were evaporated to approximately 50µL then mixed with 150µL water. Samples were analyzed in the order they were prepared using HPLC/MS/MS (Agilent 1100 HPLC with a 6410 tandem mass

spectrometer). The analysis method was optimized for sample volumes of 100mL, so any samples less than 100mL were brought to volume using DI water after filtering. Aliquots of 1mL were taken prior to each sample being filtered for measurement of creatinine.

The limit of detection (LOD) for 1-NP metabolites was calculated by taking the average concentration detected in method blank samples and adding three times the standard deviation across these samples. The resulting LOD values were 0.004pg/ μ L for 6-OHNP and 0.006pg/ μ L for 8-OHNP.

Statistical Analysis

Job location groupings: We classified study subjects into twelve different job titles, multiple of which experience similar exposures to DE, and some of which individually experience a wide range of exposure to DE that depends on shift-specific tasks and task location. In a previous mine study, Coble and associates assigned DE exposure groups based on location in the mine [Coble et al, 2010]. We adapted this approach and assigned each work-shift into one of three exposure categories or “location groups”: “Face” represents work at the ends of mine tunnels such as drilling and haulage operation; “Shop” represents work in well-ventilated underground shops; and “Surface” represents work done outside the mine or near the surface directly adjacent to fresh-air ventilation. If a worker’s time spent underground was reported as 1.5 hours or less (the 10th percentile of the time underground variable values) for any given shift, we assigned the value of their location group variable as “surface” for that shift, regardless of their job title.

Job task groupings: We hypothesized that specific tasks reported by subjects in daily surveys could be predictive of the 1-NP and EC exposures, or might modify the association between 1-NP and EC/OC/TC. We used information on each task’s reported duration per shift to isolate the single primary task a worker conducted for a majority of the shift. Upon tabulation of 1-NP measurements per reported job task, we deemed that it was feasible to assess Ore Channeling (n=25), Jack leg drill operation (n=19), Load-Haul-Dump operation (n=31), and Diesel Engine Repair (n=13) individually as refinements of the “face” and “shop” location groupings. We assigned geologists to the group “face geology” for certain shifts if their time underground was greater than 1.5 hours. We gave other tasks generally located at any of the three location groups designations of “other face task”, “other shop task”, or “other surface task”. Thus, each shift was assigned to one of the eight primary task groupings.

Regression analyses for aim 1: We examined the association between: 1) 226 measurements of EC and TC, 2) 67 measurements of TC and NO₂, and 30 44 measurements of PM₁ and TC. Examination of the relationship between EC and TC included data obtained from both personal and area samples. The relationship between NO₂ and TC was examined using side-by-side monitoring results taken at area

sampling locations. We found all variables to be lognormally distributed based on histograms and Shapiro-Wilk tests.

Bivariate correlation analysis was performed using Pearson's Correlation Coefficient to estimate the association between: 1) ln-transformed EC and ln-transformed TC, 2) ln-transformed NO₂ and ln-transformed TC, and 3) ln-transformed PM₁ and ln-transformed TC. During examination of the association between EC and TC prediction intervals were determined using PredInt.xls version 7.7 to estimate the range of predicted TC values using a single EC measure. During the examination of the association between NO₂ and TC the majority of the NO₂ values were between zero and one. Thus, we added 1 ppm to all NO₂ values prior to ln-transformation. For EC data below the LOD, the LOD was replaced with the LOD divided by the square root of two (Hornung and Reed, 1990). During examination of the association between PM₁ and TC, PM₁ concentrations were acquired using the TSI™ DustTrak 8520, a real-time mass concentration aerosol monitor, fitted with a PM₁ impactor. Because a few of the acquired PM₁ results were between zero and one, 1 µg/m³ was added to all PM₁ measurements prior to Ln-transformation.

Regression analyses for aim 3: We examined the association between 103 measurements of 1-NP and EC, OC, and TC, in turn, when no predictor variables are considered. We found these variables were lognormally distributed based on histograms and Shapiro-Wilk tests.

We used mixed-model linear regression to estimate the association between ln-transformed 1-NP and ln-transformed EC and TC, in separate models. The mixed effects model accounted for correlation of the data, with the subject included as a random effect. We used restricted maximum likelihood (reml) and computed 95% confidence intervals computed using the Wald statistic. For our primary analyses, data below the LOD was included in original form, and no substitutions, replacements, or interaction terms were incorporated. As several of the 1-NP values were negative, we added 1 pg/m³ to the 1-NP data prior to ln-transformation. In a sensitivity analyses we also explored models in which data below the LOD was replaced with the LOD divided by the square root of two. Our findings were not materially different for this sensitivity analysis, compared to our primary analysis.

We studied the potential modifiers of the 1-NP-EC associations by examining a subset of the variables obtained from the Intake Questionnaire and Survey data. Due to the high proportion of OC and TC values below LOD we only considered EC. We used the same mixed-model linear regression formulation to examine the association between ln-transformed 1-NP and ln-transformed EC with additional terms for interactions. The model equations take the following form:

$$\ln(1NP) = \beta_0 + \beta_1 \cdot \ln(EC) + \beta_2 \cdot \text{covariate} + \beta_3 \cdot \text{covariate} \cdot \ln(EC)$$

where covariate is a binary variable indicating whether an observation matches a certain criteria, such as “face” location group (yes/no) or “low” cigarettes group (yes/no). We assessed effect modification of the association between 1-NP and EC by evaluating the statistical significance of the β_3 coefficient at the 0.05 level of significance.

Mixed model linear-regression was also used to examine the extent to which 1-NP exposures could be predicted based on work characteristics including job location, job task, self-reported time spent exposed to diesel exhaust, and self-reported time spent underground.

Statistical analysis was conducted using Stata Version 13.

Results and Discussion – Aim 1

The cohort of miners examined for aim 3 consisted of 18 men and two women. Average age was 41 years (range 27-58), and average years worked in mining was 11 years (range 0.75-34). During the study two male subjects dropped out after the first week of monitoring because they moved to one of the other work crews. Data from these two subjects were not included in the analyses. On several other occasions individual study participants were not available for sampling, for example due to vacation or family/medical leave.

Table 3 summarizes the output for the regression models examining the association between EC and TC. Scatter plots illustrating this relationship are shown in Figure 2. The coefficient associated with the lnEC predictor variable for lnTC was statistically significant at the 0.001 level - for a 10% increase in EC concentration, TC is estimated to increase by approximately 4%. Figure 2 also provides prediction intervals for the association between EC and TC. This is the interval around the predicted lnTC value such that there is a 95% probability ($\alpha=0.05$) that the real value of lnTC is within this interval. Using a t-distribution critical value of 1.96 ($\alpha=0.05$, two-tailed, $df=226$) and a computed standard error of 0.29 for all predicted lnTC values, we find that there is a 95% probability that the real value of LnTC lies between its predicted value $\pm 0.57 \mu\text{g}/\text{m}^3$.

Table 3: Relationship between EC and TC (n=226)

| Model Version: | Values below LOD replaced with LOD/ $\sqrt{2}$ | | |
|-----------------------|--|-------|---------|
| Model Coefficients: | Value | SE | p-value |
| β_0 (intercept) | 2.606 | 0.045 | <0.001 |
| β_1 (lnEC) | 0.399 | 0.019 | <0.001 |

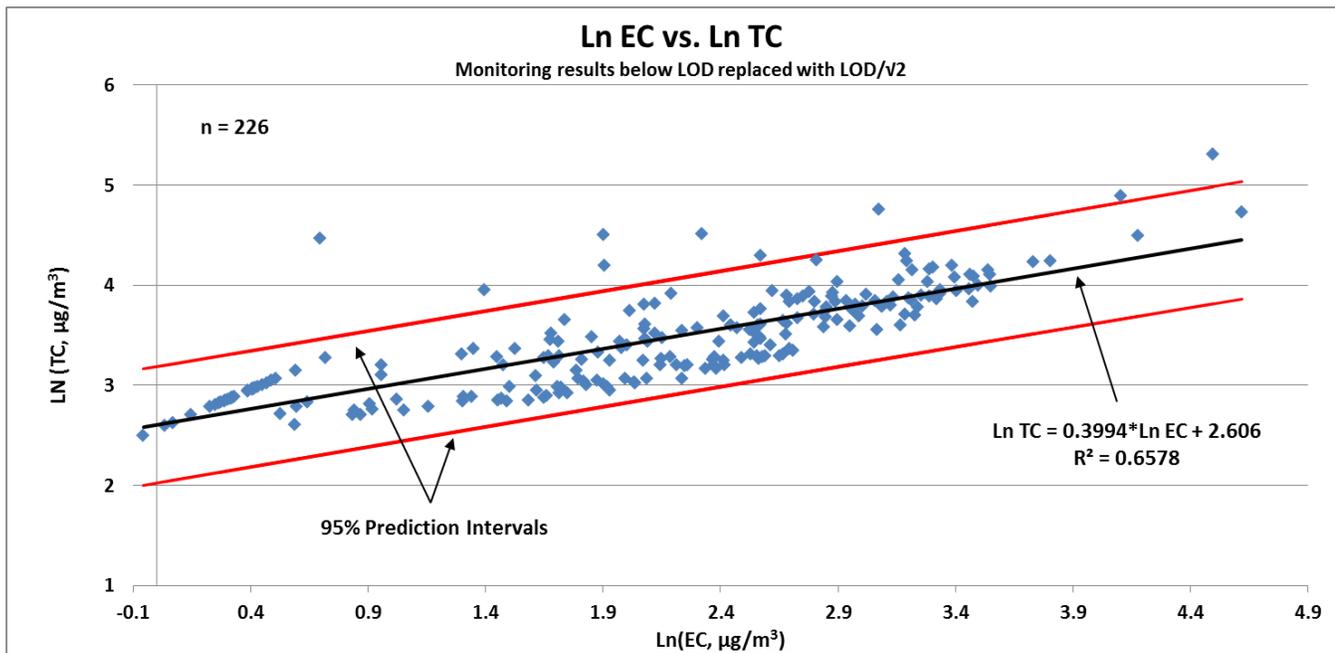


Figure 2: Scatter plot of Relationship between EC and TC

Table 4 summarizes the output for the regression models examining the association between NO_2 and TC. Scatter plots illustrating this relationship are shown in Figure 3. The coefficient associated with the lnNO_2 predictor variable for lnTC was statistically significant at the 0.05 level - for a 10% increase in NO_2 concentration, TC is estimated to increase by approximately 2.5%.

Table 4: Relationship between NO_2 and TC (n=56)

| Model Version: | Values below LOD replaced with LOD/v2 | | |
|-------------------------------|---------------------------------------|-------|---------|
| Model Coefficients: | Value | SE | p-value |
| β_0 (intercept) | 1.750 | 0.186 | <0.001 |
| β_1 (lnNO_2) | 2.445 | 0.968 | 0.015 |

Table 5 summarizes the output for the regression models examining the association between PM_{10} concentration and TC. Scatter plots illustrating this relationship are shown in Figure 4. The coefficient associated with the lnPM_{10} predictor variable for lnTC was statistically significant at the 0.001 - for a 10% increase in PM_{10} concentration, TC is estimated to increase by approximately 7%. It should be noted that while the constant (y-intercept) was not statistically significant, this provides little intrinsic meaning as each NO_2 value represents a work shift TWA concentration, which is unlikely to equal zero over an entire work shift.

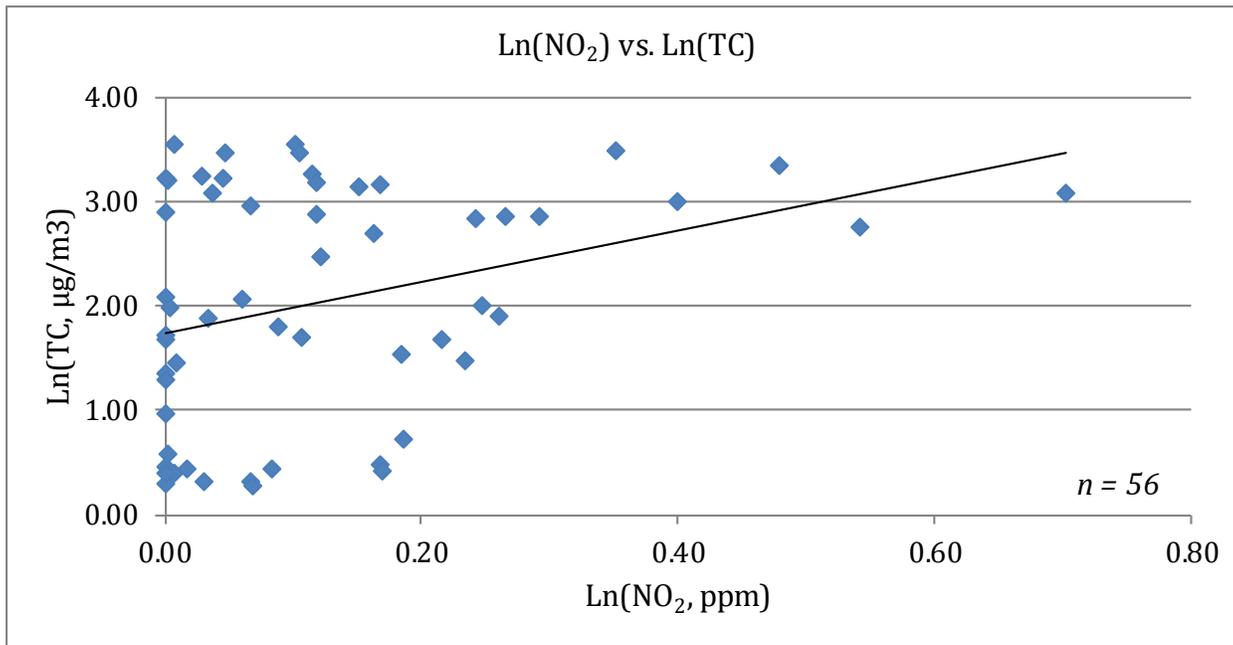


Figure 3: Scatter plot of Relationship between NO₂ and EC

Table 5: Relationship between PM1-Dustrak and TC (n=44)

| Model Coefficients: | Value | SE | p-value |
|--|-------|-------|---------|
| β ₀ (intercept) | 0,494 | 0.395 | 0.218 |
| β ₁ (ln µg/m ³ - DustTrak) | 0.716 | 0.113 | <0.001 |

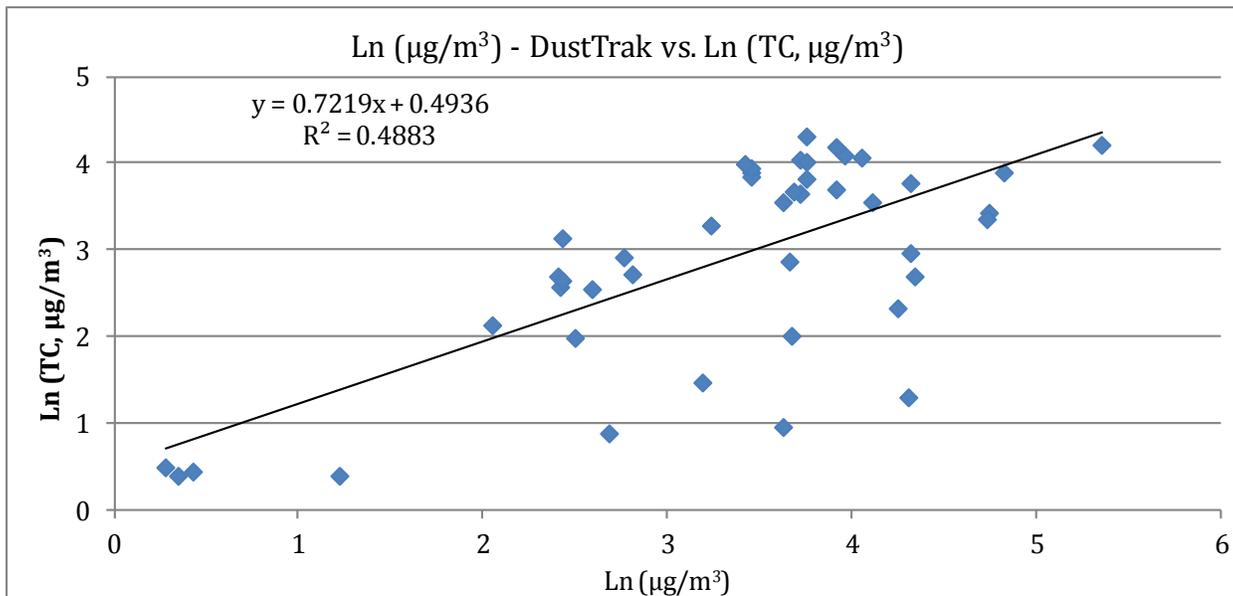


Figure 4: Scatter plot of relationship between real-time mass concentration (PM₁) and TC

Figure 5 and Figure 6 below document personal and area TWA concentrations for DPM-TC acquired over all four monitoring campaigns. On each figure visual context concerning compliance with occupational exposure limits is provided by noting MSHA's occupational exposure limit of 202 µg/m³ on the y-axis. In Figure 5 all personal monitoring results represent DPM-TC TWA concentrations measured over a full work shift - all but one of these concentration were less than 150 µg/m³. This is a marked improvement in air quality compared to an earlier study conducted at this facility in 2003 when DPM-TC

concentrations as high as 490 $\mu\text{g}/\text{m}^3$ where reported by [Stephenson ref Mining Eng. 2006]. Like the personal monitoring results, the area monitoring results shown in Figure 6 represent full shift DPM-TC TWA concentrations. In general, results of area monitoring show DPM-TC TWA concentrations less than 100 $\mu\text{g}/\text{m}^3$ and relatively low intra-location variability. Of note are the lower concentrations and variability observed at Area 7. This was expected as this area served as our control and was located in an operations conference room at the surface of the mine.

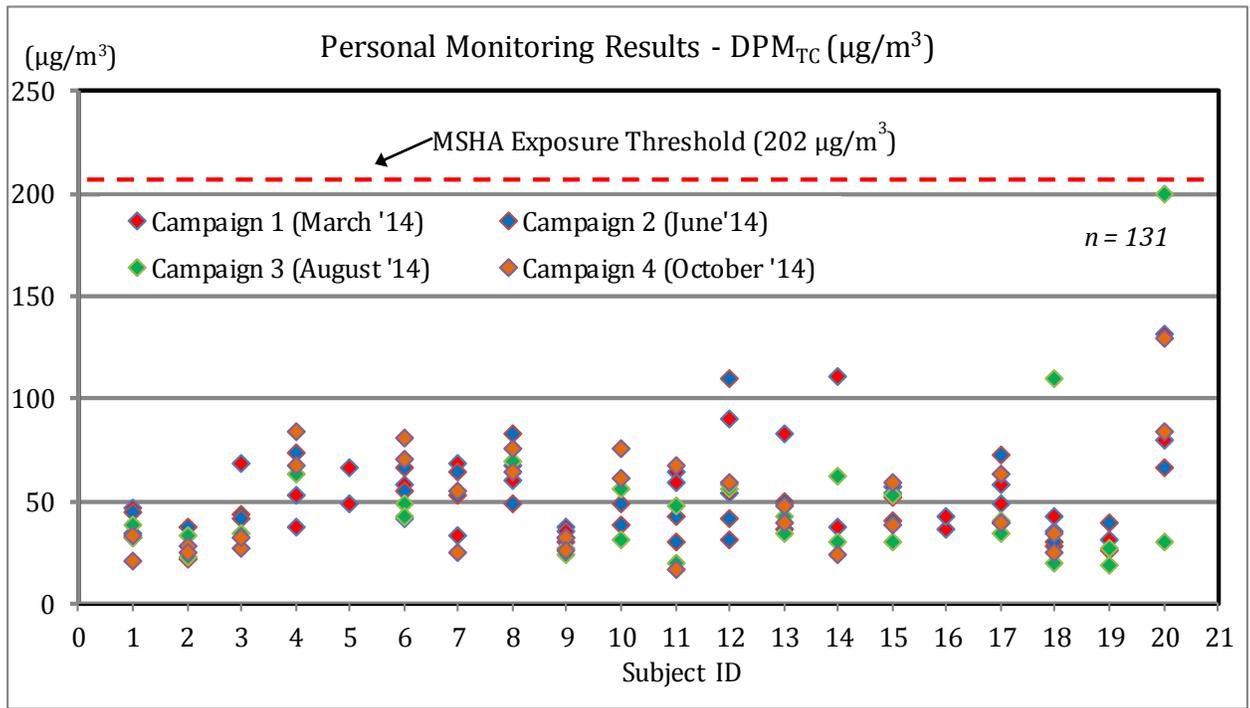


Figure 5: Personal monitoring results of subject exposure to DPM-TC

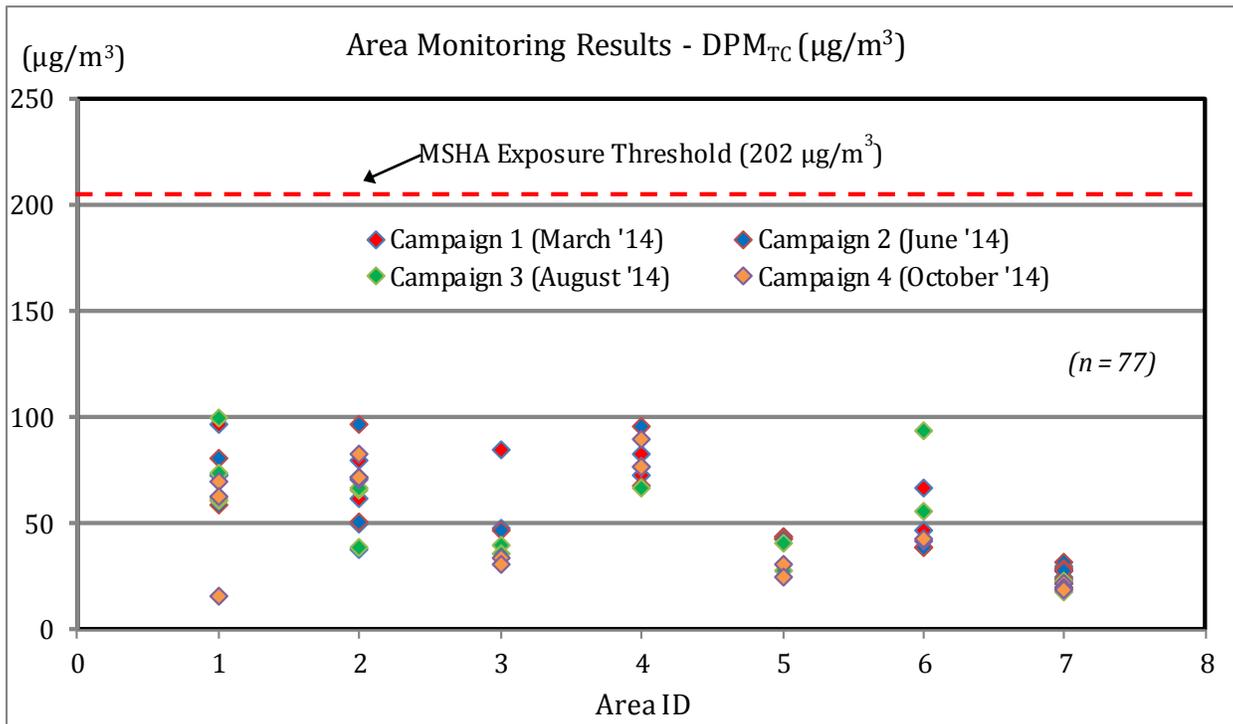


Figure 6: Area monitoring results of subject exposure to DPM-TC

Results and Discussion – Aim 2

Provided in Table 6 are the results of environmental monitoring using the portable remote wireless sensing device. The area monitoring locations are associated with those cited in Table 1 provided earlier in this report. Based on our *a priori* assessment, it was anticipated that environmental monitoring in Areas 2 and 5 would yield high contaminant concentration levels due to the presence of a high volume of diesel-powered vehicles passing this location. Area 6 was an underground vehicle maintenance and repair shop where it was anticipated that contaminant concentration levels would be moderate. Area 7 is our control location, which was anticipated to have relatively lower airborne contamination. Initial observation of the results provided Table 6 appear to support our contaminant level designations with Areas 2 and 5 having relatively higher concentration values and Area 7 yielding much lower concentration values.

It should be noted that the personal wireless sensing device was used to collect data during each field campaign. During our field campaigns we discovered that the use of this device was compromised by its lack of robustness. Because this is a personal sampling device it is meant to be worn near the breathing zone of an individual throughout the work day. When using this device we experienced complications with ensuring that it remained appropriately attached to a person's body and interferences with device operation due to a person's body movements during the time period it was being worn. At present we are in the process of interpreting the data acquired using this device to determine its meaning and usefulness. We also plan to re-engineer the device to increase its robustness, and make its form factor more convenient for workers.

Table 6: Area sampling results using the portable remote wireless sensing device

| | | ^a CO (ppm) | ^a CO ₂ (ppm) | ^a Particle Count (particles/cc) |
|--------|------------|--------------------------|---------------------------------------|---|
| Area 2 | Campaign 1 | 4.55 | 1290.70 | 4475.72 |
| | Campaign 2 | 4.09 | 1223.68 | 8062.48 |
| | Campaign 3 | 6.10 | 1547.19 | 5633.13 |
| | Campaign 4 | 4.54 | 1273.89 | 5289.59 |
| Area 5 | Campaign 1 | ----- | 1100.27 | 4100.16 |
| | Campaign 2 | 2.73 | 1302.57 | 4005.41 |
| | Campaign 3 | 1.16 | 891.40 | 4676.51 |
| | Campaign 4 | 3.05 | 1163.03 | 1827.75 |
| Area 6 | Campaign 1 | 1.54 | 733.03 | 1811.38 |
| | Campaign 2 | 1.16 | 598.53 | 1189.95 |
| | Campaign 3 | 1.75 | 637.03 | 2632.92 |
| | Campaign 4 | 1.29 | 624.40 | 1211.27 |
| Area 7 | Campaign 1 | 0.27 | 499.58 | 71.04 |
| | Campaign 2 | 0.25 | 418.94 | 99.33 |
| | Campaign 3 | 0.30 | 455.46 | 370.37 |
| | Campaign 4 | 0.26 | 484.83 | 38.35 |

^a Data represents the Geometric means averaged over the four days of each campaign

Results and Discussion – Aim 3

Analysis for 1-NP in air

Several additional samples were invalidated due to leakage of filter cassettes, pump failures, or analytical problems with the 1-NP or EC/OC assays. The final data set consisted of 103 personal air samples from 18 subjects, an average of 5.7 samples per worker (range 3-8).

Table 7 reports summary statistics for the 1-NP, EC, OC and TC. To calculate these statistics, values <LOD were substituted with $LOD/\sqrt{2}$. Although EC typically comprises a majority of the mass of DE, the mean concentration of OC ($19.0 \mu\text{g}/\text{m}^3$) in this dataset is higher than that of EC ($12.4 \mu\text{g}/\text{m}^3$). This is likely due to the blank correction for the OC values and the resulting high LOD for OC: 79 of the 103 samples had OC values less than the LOD (76.7%). With the LOD-replaced values, the set of OC personal air concentrations is artificially high, and thus drives the TC values to be artificially high.

Table 7: Summary Statistics for 1-NP, EC, OC, and TC (n = 103)

| Analyte | mean | SD | GM | GSD | % <LOD | min | max |
|---------------------------|------|------|------|------|--------|------|------|
| 1-NP (pg/m ³) | 77.0 | 88.8 | 39.4 | 4.00 | 8.7 | 0.53 | 497 |
| EC (µg/m ³) | 12.4 | 12.5 | 8.46 | 2.51 | 6.80 | 1.04 | 101 |
| OC (µg/m ³) | 19.0 | 12.3 | 17.1 | 1.49 | 76.7 | 12.1 | 95.0 |
| TC (µg/m ³) | 35.4 | 24.5 | 29.6 | 1.77 | 76.7 | 13.4 | 135 |

Figures 7 and 8 illustrate 1-NP and EC personal air concentration distributions. These plots demonstrate substantial within- and between-subject variability in exposures to DE components. Sample sizes ranged from three to eight usable air measurements per subject. In many cases the patterns of within and between subject variability shown figures 7 and 8 are interpretable in terms of subjects' work locations and activities. In general both 1-NP and EC exposures were highest at face locations, intermediate at shop locations and lowest at the surface (Table 8). For example, subjects 7 and 17 had high variability in both 1-NP and EC because they both have the job title "geologist", which involves a variety of work locations including surface office work (low DE exposures) and underground work at the face (high DE exposures). In contrast Subject 19 is a "sandplant operator" who spent every shift in a sandplant facility near the surface of the mine that had ventilation directly with the outdoors. There appears to be a clear stratification of 1-NP and EC concentration medians and ranges between different location groups, as shown in Table 8.

Table 8: 1-NP and EC Personal Air Concentrations by Location Group

| Job Group | n | GM 1-NP (95% CI), pg/m ³ | GM EC (95% CI), µg/m ³ |
|-----------|----|-------------------------------------|-----------------------------------|
| face | 67 | 64.2 (50.1-82.2) | 12.1 (10.3-14.2) |
| shop | 21 | 29.0 (18.3-46.0) | 5.30 (3.72-7.54) |
| surface | 15 | 6.90 (2.57-18.5) | 3.31(1.72-6.35) |

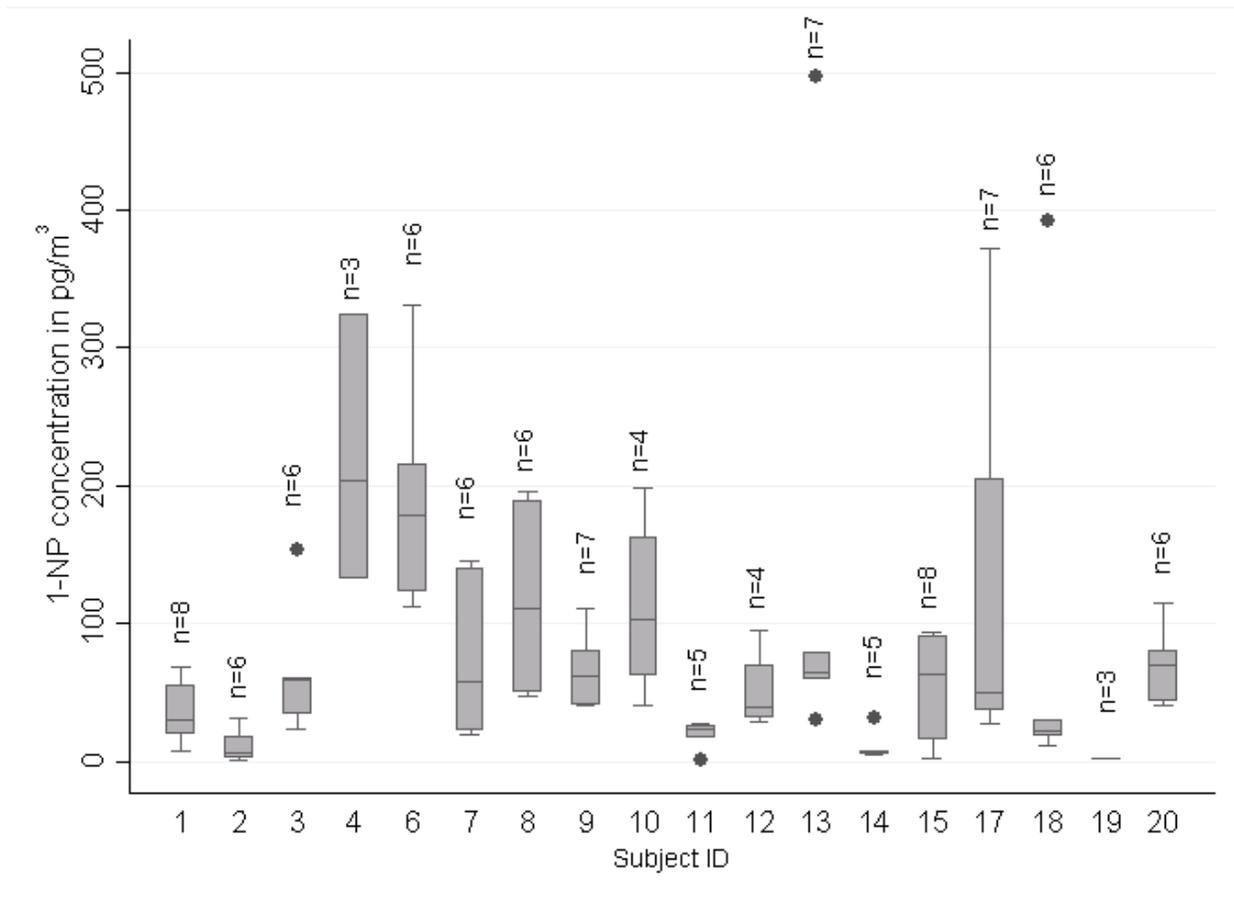


Figure 7: Boxplot of 1-NP Personal Air Concentrations by Subject

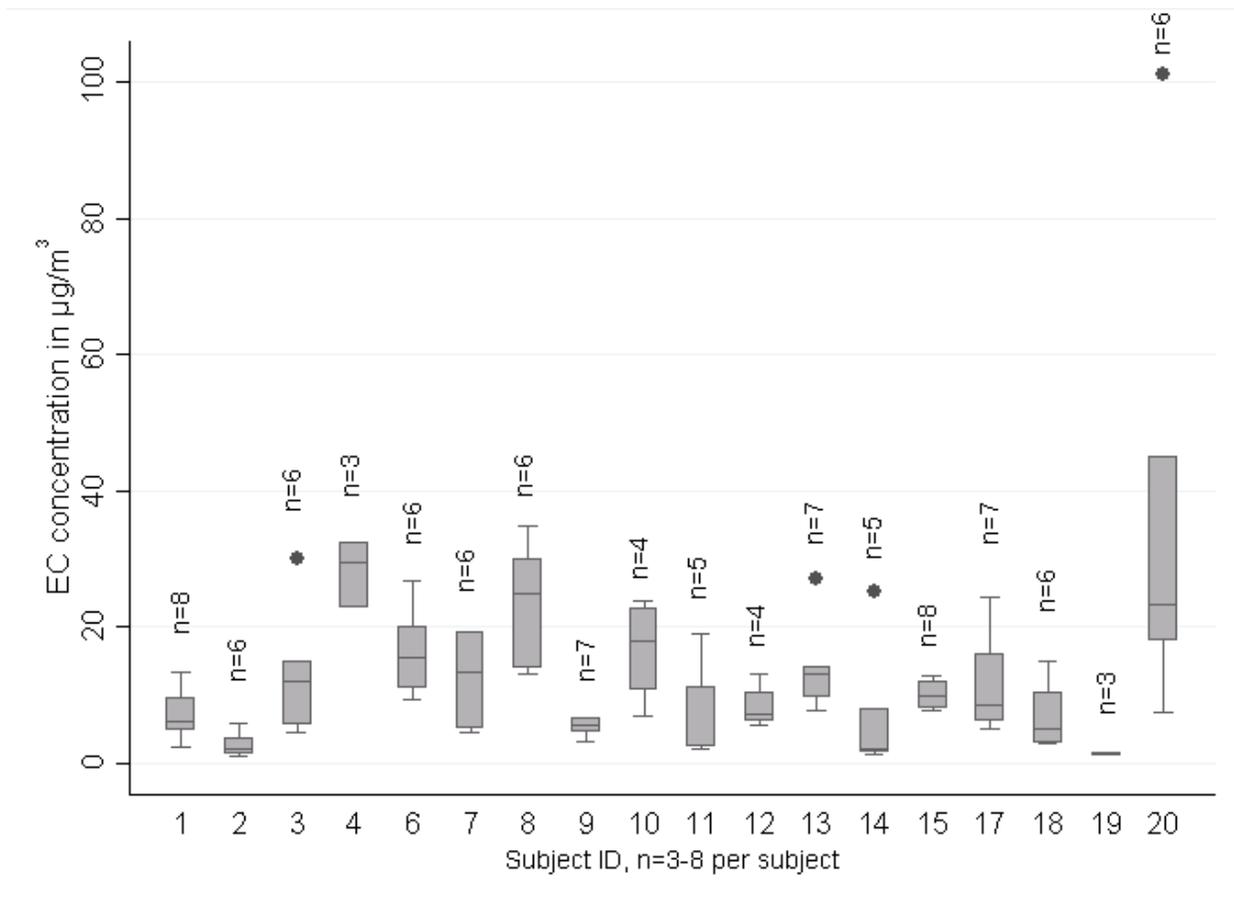


Figure 8: Boxplot of EC Personal Air Concentration by Subject

Table 9 summarizes the output for the regression models examining the association between 1-NP and EC. Scatter plots illustrating this relationship are shown in Figure 9. The coefficient associated with the lnEC predictor variable for ln(1-NP) was statistically significant - for a 10% increase in EC concentration, 1-NP is estimated to increase by 8.2%. The EC-NP relationship was relatively insensitive to how data <LOD were handled, with the β_1 coefficient being statistically significant, and similar if data <LOD was used as is, or replaced with $LOD/\sqrt{2}$.

Similar models examining the relationship between 1-NP and OC and TC were developed. However, a high proportion of OC and TC data were <LOD. Consequently the β_1 coefficients for the 1-NP-TC and 1-NP-OC relationships had wide confidence limits and were very sensitive to how data <LOD were handled.

Table 9: Relationship between 1-NP and EC (n=103)

| Model Version: Model Coefficients: | Values below LOD replaced with $LOD/\sqrt{2}$ | | | Values below LOD kept "as is" | | |
|---------------------------------------|---|-------|---------|-------------------------------|-------|---------|
| | Value | SE | p-value | Value | SE | p-value |
| β_0 (intercept) | -11.926 | 0.312 | <0.001 | -11.854 | 0.313 | <0.001 |
| β_1 (lnEC) | 0.827 | 0.128 | <0.001 | 0.809 | 0.129 | <0.001 |

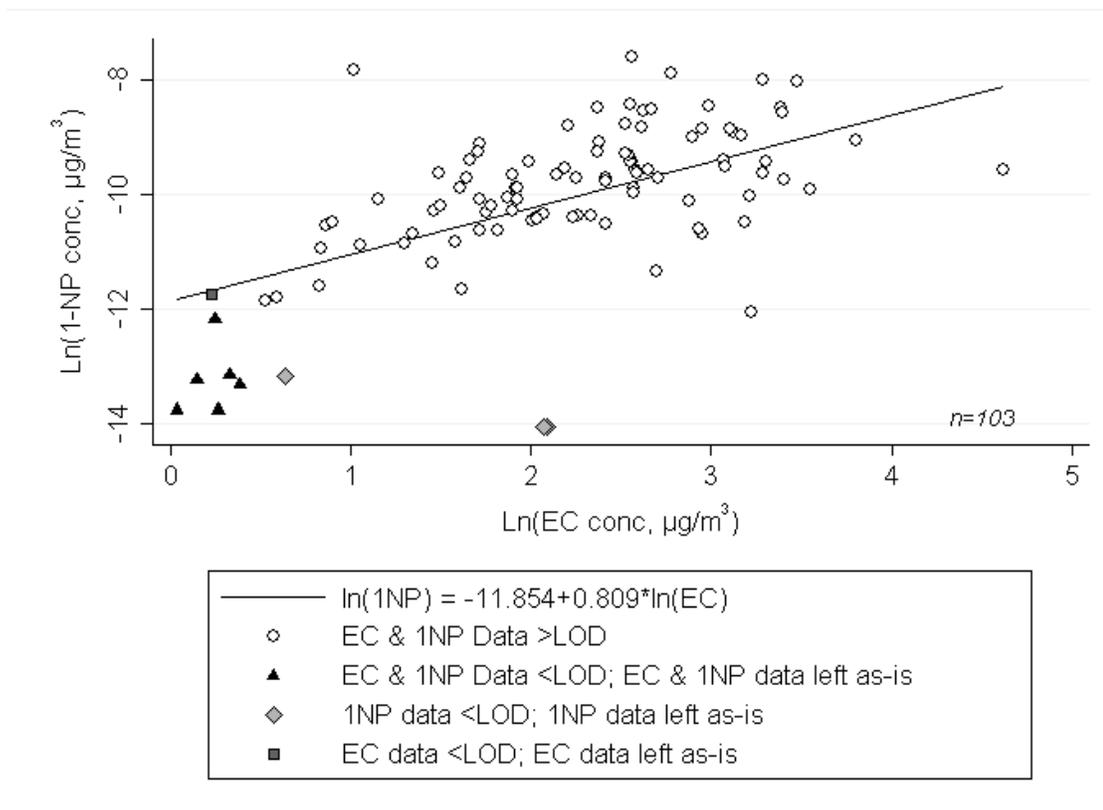


Figure 9 Scatter plot of Relationship between 1-NP and EC

In a secondary analysis, we examined possible effect modification of the 1-NP-EC association by fuel type, location group, or tobacco use. None of covariates tested were found to significantly modify the relationship between 1-NP and EC.

Finally, we examined the extent to which 1-NP exposure could be predicted based on a variety of work-shift characteristics; model assessment is summarized in Table 10 for the best performing models. Overall, models based on the eight primary task groups performed slightly better than models based on the three location groups, as judged by the cross-validated R². Refinements to these basic models by inclusion of additional predictors afforded only marginal improvement in model performance.

Table 10: Prediction Models Assessment

| Model | Covariates ¹ | In-Sample Predictions | | Cross-Validated Predictions | |
|-------|--|-----------------------|--------------------------|-----------------------------|--------------------------|
| | | RMSE | MSE-based R ² | RMSE | MSE-based R ² |
| A | location group (1-3) | 1.154 | 0.301 | 1.203 | 0.240 |
| B | primary task group (1-8) | 1.100 | 0.365 | 1.177 | 0.272 |
| C | primary task group (1-8), t.DE | 1.091 | 0.375 | 1.170 | 0.282 |
| D | primary task group (1-8), fuel type, t.DE | 1.079 | 0.389 | 1.176 | 0.274 |
| E | primary task group (1-8), fuel type, DOW (1-4), t.DE | 1.074 | 0.395 | 1.201 | 0.243 |

¹: t.DE = self-reported time per shift exposed to diesel exhaust in minutes; fuel type = summer or winter diesel fuel; DOW = day of week (1-4).

Of all valid samples examined in this study, no samples yielded a TC air concentration value in excess of the MSHA DPM PEL of 160 µg/m³. In contrast, an earlier study conducted at this facility in 2003 reported TC concentrations as high as 490 µg/m³ [Stephenson ref Mining Eng. 2006]. Since that time the mine operators have instituted multiple strategies to reduce DE concentrations including upgrading the mine ventilation system, installing diesel particulate filters on their fleet of diesel powered equipment and switching to a B70 blend biodiesel fuel.

1-NP personal air measurements from this study have an arithmetic mean of 77 pg/m³ and a range of 0.53-497 pg/m³ (GM 39 pg/m³). Our data are towards the lower end of values reported in the literature for underground mines. Other indoor workplace DPM investigations found 1-NP values with GMs ranging from 197-2483 pg/m³ (personal samples from underground workers), and GMs from 2.8-1800 pg/m³ (general indoor workplace samples) [Scheepers et al, 2003; IARC 2013]. It is likely that the various measures instituted by the mine to reduce TC concentrations below the MSHA standard have also substantially reduced 1-NP concentrations in comparison with historical levels. Nevertheless, 1-NP concentrations underground within the mine remain elevated compared to ambient air concentrations at the surface, especially at locations where active ore extraction tasks are taking place and ventilation is limited.

We observed a clear association between personal exposures to 1-NP and EC, with 1-NP predicted to increase 8.2% for every 10% increase in EC. This association was not significantly different in models that accounted for location in the mine, fuel type used, or number of cigarettes smoked. Together with the relatively tight confidence intervals on the effect estimate, our findings suggest that the EC:1-NP relationship was relatively insensitive to the different engines and operating conditions encountered in this mine, and further, that 1-NP can be reliably used as a surrogate measure for DPM. Nevertheless, the variables listed above could plausibly affect the EC:1-NP relationship by altering the composition of the collected particulate matter, and the relatively small sample size for the current study no doubt limited our ability to detect small changes in the 1:NP-EC relationship associated with these other factors.

In general, a robust association was not observed between 1-NP and OC or TC. These associations were heavily influenced by the large number of samples with OC concentrations below the LOD (76.7% below LOD for both OC and TC), which in turn was a consequence of high OC content on field blank filters, relative to the OC concentrations in the samples. The MSHA DPM regulation for underground M/NM mines relies on measurements of TC (which includes OC), and this analytical limitation presents a problem for measuring low concentrations of DE-derived TC. However for full-shift personal samples the OC LOD in our study was around 50 $\mu\text{g}/\text{m}^3$, which is well below the MSHA PEL of 160 $\mu\text{g}/\text{m}^3$. Therefore the high OC content of field blanks for the DPM cassettes does not interfere with their use for determining compliance with the MSHA PEL.

In order to identify specific and easily measurable workshift characteristics that are risk factors for high DPM exposure, a variety of workshift-related predictor variables were tested in regression models for their ability to predict 1-NP exposures. In general, models using primary task group performed better than models based on location group, with only minor improvements when additional variables such as time spent underground or time exposed to diesel exhaust were included. However overall predictive performance for all these models was relatively poor (out-of-sample $r^2 < 0.3$).

This was a pilot study, with small sample sizes and resulting limited ability to assess the effect of location and specific job tasks upon the association between EC and 1-NP. Our findings should be replicated in a larger study, with cohort members selected to provide sufficient representation of multiple job locations and tasks in order to better assess potential effect modification by various shift conditions upon the EC:1-NP relationship, and upon the ability to predict 1-NP exposure from workshift characteristics. One potential advantage of 1-NP compared to EC as a surrogate measure of DPM, is the absence of confounding sources of 1-NP in a typical mine environment. Therefore, future work should include sampling in facilities such as a coal mine in which non-diesel sources of EC and TC are present, and EC or TC may not be suitable surrogate measures of exposure to DE. In addition, the specificity of 1-NP as a

surrogate for DPM would prove useful in a non-occupational setting such as examining community exposures to DPM.

1-nitropyrene metabolites in urine

Both 6-OHNP and 8-OHNP appeared to be log-normally distributed, and levels of 6-OHNP in urine tended to be higher than levels of 8-OHNP. The 6-OHNP and 8-OHNP metabolite concentrations showed variability between and within subjects (Table 11, Figure 10). For 6-OHNP the between subject standard deviation was estimated as 0.014 pg/mg creatinine, 23% of the total variance, and the within subject standard deviation was estimated as 0.026 pg/mg creatinine, 77% of total variance. For 8-OHNP the between subject standard deviation was estimated as 0.007 pg/mg creatinine, 23% of the total variance, and the within subject standard deviation was estimated as 0.013 pg/mg creatinine, 77% of the total variance. A consistent trend for higher metabolite levels by shift location was not observed, however, the metabolite levels were highly variable in all locations (Table 11).

Table 11: Summary statistics for post-shift metabolite levels by shift location

| | All Locations (n = 142) | Face (n = 96) | Shop (n = 27) | Surface (n = 19) |
|---|----------------------------|------------------|------------------|---------------------|
| <i>Post-shift 6-OHNP Concentration (pg/mg creatinine)</i> | | | | |
| Range | 0.0004 - 0.23 | 0.0007 - 0.16 | 0.0058 - 0.23 | 0.0004 - 0.095 |
| AM(ASD) | 0.024 (0.031) | 0.022 (0.024) | 0.031 (0.048) | 0.023 (0.030) |
| GM(GSD) | 0.014 (2.9) | 0.014 (2.6) | 0.017 (2.7) | 0.009 (4.7) |
| <i>Post-shift 8-OHNP Concentration (pg/mg creatinine)</i> | | | | |
| Range | 0.0006 - 0.11 | 0.0006 - 0.092 | 0.0014 - 0.11 | 0.0006 - 0.072 |
| AM(ASD) | 0.012 (0.016) | 0.011 (0.012) | 0.014 (0.023) | 0.015 (0.020) |
| GM(GSD) | 0.007 (2.8) | 0.007 (2.4) | 0.006 (3.4) | 0.006 (4.4) |

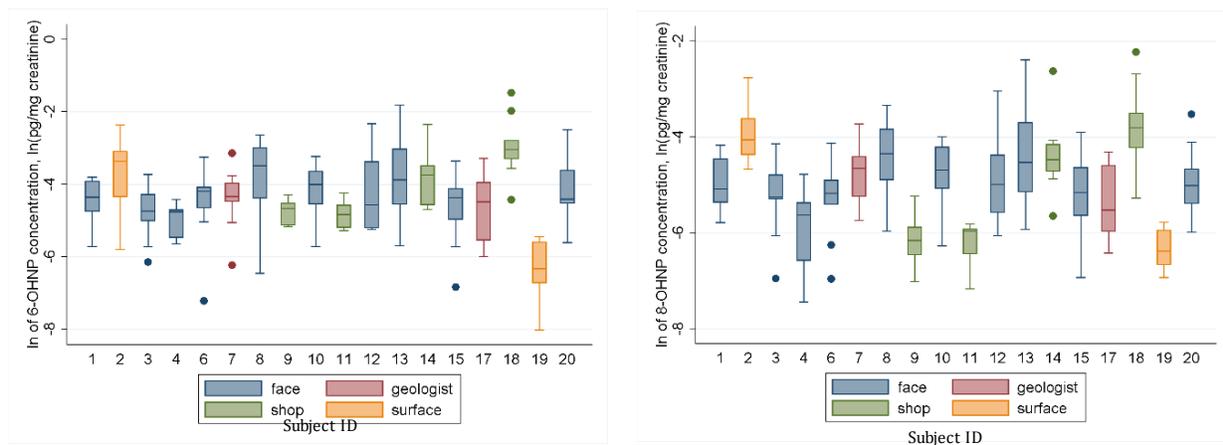


Figure 10: Distribution of log-transformed 6-OHNP and 8-OHNP by subject ID and job location

Metabolite levels tended to increase over the work week with an average increase in 6-OHNP of 0.007 pg/mg creatinine per day and an increase in 8-OHNP of 0.003 pg/mg creatinine per day (Table 12).

Table 12: Metabolite levels, stratified by day of week and primary job location

| | All Samples | Pre-Shift Day 1 | Post-Shift Day 1 | Post-Shift Day 2 | Post-Shift Day 3 | Post-Shift Day 4 | Trend for Increase |
|----------------------|---------------|--------------------|---------------------|---------------------|---------------------|---------------------|-----------------------|
| <i>All Locations</i> | | | | | | | |
| | (n = 170) | (n = 28) | (n = 41) | (n = 40) | (n = 31) | (n = 30) | |
| 6-OHNP | 0.022 (0.029) | 0.014 (0.012) | 0.014 (0.012) | 0.023 (0.029) | 0.025 (0.024) | 0.036 (0.049) | 0.007 (0.002)* |
| 8-OHNP | 0.011 (0.015) | 0.006 (0.005) | 0.008 (0.006) | 0.013 (0.018) | 0.013 (0.013) | 0.017 (0.023) | 0.003 (0.001)* |
| <i>Face</i> | | | | | | | |
| | (n = 116) | (n = 20) | (n = 28) | (n = 27) | (n = 22) | (n = 19) | |
| 6-OHNP | 0.020 (0.023) | 0.012 (0.011) | 0.014 (0.010) | 0.022 (0.030) | 0.024 (0.023) | 0.030 (0.029) | 0.006 (0.002)* |
| 8-OHNP | 0.010 (0.011) | 0.006 (0.005) | 0.007 (0.004) | 0.012 (0.017) | 0.012 (0.010) | 0.015 (0.013) | 0.003 (0.001)* |
| <i>Shop</i> | | | | | | | |
| | (n = 32) | (n = 5) | (n = 8) | (n = 9) | (n = 5) | (n = 5) | |
| 6-OHNP | 0.029 (0.045) | 0.015 (0.012) | 0.012 (0.012) | 0.025 (0.021) | 0.013 (0.006) | 0.091 (0.091) | 0.020 (0.006)* |
| 8-OHNP | 0.013 (0.022) | 0.005 (0.004) | 0.007 (0.010) | 0.011 (0.010) | 0.005 (0.004) | 0.042 (0.045) | 0.009 (0.003)* |
| <i>Surface</i> | | | | | | | |
| | (n = 22) | (n = 3) | (n = 5) | (n = 4) | (n = 4) | (n = 6) | |
| 6-OHNP | 0.023 (0.028) | 0.023 (0.019) | 0.019 (0.024) | 0.028 (0.045) | 0.045 (0.035) | 0.007 (0.007) | -0.003 (0.005) |
| 8-OHNP | 0.014 (0.019) | 0.010 (0.007) | 0.012 (0.010) | 0.024 (0.032) | 0.029 (0.024) | 0.002 (0.001) | -0.002 (0.003) |

Values are mean (SD). Concentration units are pg/mg creatinine. Units for trend are pg/mg creatinine per day

* significant at 0.05 level

Association between Urinary Metabolites and Predicted 1-NP in Air

All non-occupational exposures were assumed to be low relative to occupational exposures and were assigned the LOD of 1-NP in air divided by 5. No association was detected between either 1-NP metabolite in post-shift urine with 1-NP in air for air measurements taken on the same day as urine sample collection (Model 1) (Table 13, Table 14) Inclusion of pre-shift samples (Model 2) did not substantially improve the association between predicted air exposures and measured biomarker levels. (Table 13, Table 14)

These observations are consistent with the data shown in Table 12 that demonstrates a trend for metabolite levels to increase over the work week. Thus metabolite levels are not derived from a single work shift exposure, but represent integrated exposure to DE over time periods longer than a single workshift.

Consequently, we evaluated associations between biomarker levels and exposure accumulated over 2, 3 or 4 days (Models 3-5, Table 13, Table 14) The effect estimates (β) for these three models were all around 0.10, and the p values progressively decreased as the accumulation period increased, achieving statistical significant for the model that included exposure accumulated over four days (model 5, Figure 11).

Table 13: Results for mixed effects models for association between log-transformed 6-OHNP in ln(pg/mg creatinine) and log-transformed 1-NP in air in ln(pg/m³)

| | β (SE) | p | 95% Conf. Interval | Number of Observations | Subject- Specific Random Effect Est.(SE) | Residual Random Effect Est (SE) |
|---|--------------|--------|-----------------------|---------------------------|---|--|
| <i>Model 1: Post-Shift Urine with Predicted 1-NP in Air</i> | | | | | | |
| ln of 1-NP in Air | 0.13 (0.21) | 0.519 | (-0.27, 0.54) | 142 | 0.66 (0.15) | 0.88 (0.06) |
| Intercept | -4.84 (0.82) | <0.001 | (-6.43, -3.25) | | | |
| <i>Model 2: Pre-shift and Post-shift Urine with Predicted 1-NP in Air</i> | | | | | | |
| ln of 1-NP in Air | 0.08 (0.07) | 0.254 | (-0.06, 0.22) | 158 | 0.60 (0.14) | 0.89 (0.05) |
| Intercept | -4.63 (0.29) | <0.001 | (-5.20, -4.05) | | | |
| <i>Model 3: Pre-Shift and Post-Shift Urine with Predicted 1-NP in Air Accumulated over 2 Days</i> | | | | | | |
| ln of 1-NP in Air | 0.10 (0.06) | 0.115 | (-0.02, 0.22) | 138 | 0.60 (0.15) | 0.91 (0.06) |
| Intercept | -4.69 (0.30) | <0.001 | (-5.28, -4.10) | | | |
| <i>Model 4: Pre-Shift and Post-Shift Urine with Predicted 1-NP in Air Accumulated over 3 Days</i> | | | | | | |
| ln of 1-NP in Air | 0.11 (0.06) | 0.068 | (-0.008, 0.22) | 113 | 0.74 (0.19) | 0.89 (0.07) |
| Intercept | -4.72 (0.32) | <0.001 | (-5.35, -4.09) | | | |
| <i>Model 5: Pre-Shift and Post-Shift Urine with Predicted 1-NP in Air Accumulated over 4 Days</i> | | | | | | |
| ln of 1-NP in Air | 0.09 (0.05) | 0.045 | (0.002, 0.18) | 129 | 0.73 (0.19) | 0.89 (0.06) |
| Intercept | -4.65 (0.27) | <0.001 | (-5.17, -4.11) | | | |

Table 14: Results for mixed effects models for association between log-transformed 8-OHNP in ln(pg/mg creatinine) and log-transformed 1-NP in air in ln(pg/m³)

| | β (SE) | p | 95% Conf. Interval | Number of Observations | Subject-Specific Random Effect Est.(SE) | Residual Random Effect Est (SE) |
|---|--------------|--------|-----------------------|---------------------------|---|--|
| <i>Model 1: Post-Shift Urine with Predicted 1-NP in Air</i> | | | | | | |
| ln of 1-NP in Air | 0.07 (0.21) | 0.725 | (-0.34, 0.49) | 142 | 0.75 (0.15) | 0.77 (0.05) |
| Intercept | -5.28 (0.84) | <0.001 | (-6.91, -3.64) | 142 | 0.75 (0.15) | 0.77 (0.05) |
| <i>Model 2: Pre-shift and Post-shift Urine with Predicted 1-NP in Air</i> | | | | | | |
| ln of 1-NP in Air | 0.07 (0.06) | 0.248 | (-0.05, 0.19) | 158 | 0.72 (0.14) | 0.77 (0.05) |
| Intercept | -5.27 (0.28) | <0.001 | (-5.82, -4.71) | | | |
| <i>Model 3: Pre-Shift and Post-Shift Urine with Predicted 1-NP in Air Accumulated over 2 Days</i> | | | | | | |
| ln of 1-NP in Air | 0.09 (0.05) | 0.101 | (-0.02, 0.20) | 138 | 0.69 (0.15) | 0.79 (0.05) |
| Intercept | -5.34 (0.29) | <0.001 | (-5.90, -4.77) | | | |
| <i>Model 4: Pre-Shift and Post-Shift Urine with Predicted 1-NP in Air Accumulated over 3 Days</i> | | | | | | |
| ln of 1-NP in Air | 0.10 (0.05) | 0.051 | (-0.0005, 0.19) | 113 | 0.82 (0.18) | 0.74 (0.05) |
| Intercept | -5.37 (0.30) | <0.001 | (-5.95, -4.78) | | | |
| <i>Model 5: Pre-Shift and Post-Shift Urine with Predicted 1-NP in Air Accumulated over 4 Days</i> | | | | | | |
| ln of 1-NP in Air | 0.08 (0.04) | 0.034 | (0.006, 0.16) | 129 | 0.80 (0.18) | 0.75 (0.05) |
| Intercept | -5.31 (0.26) | <0.001 | (-5.82, -4.80) | | | |

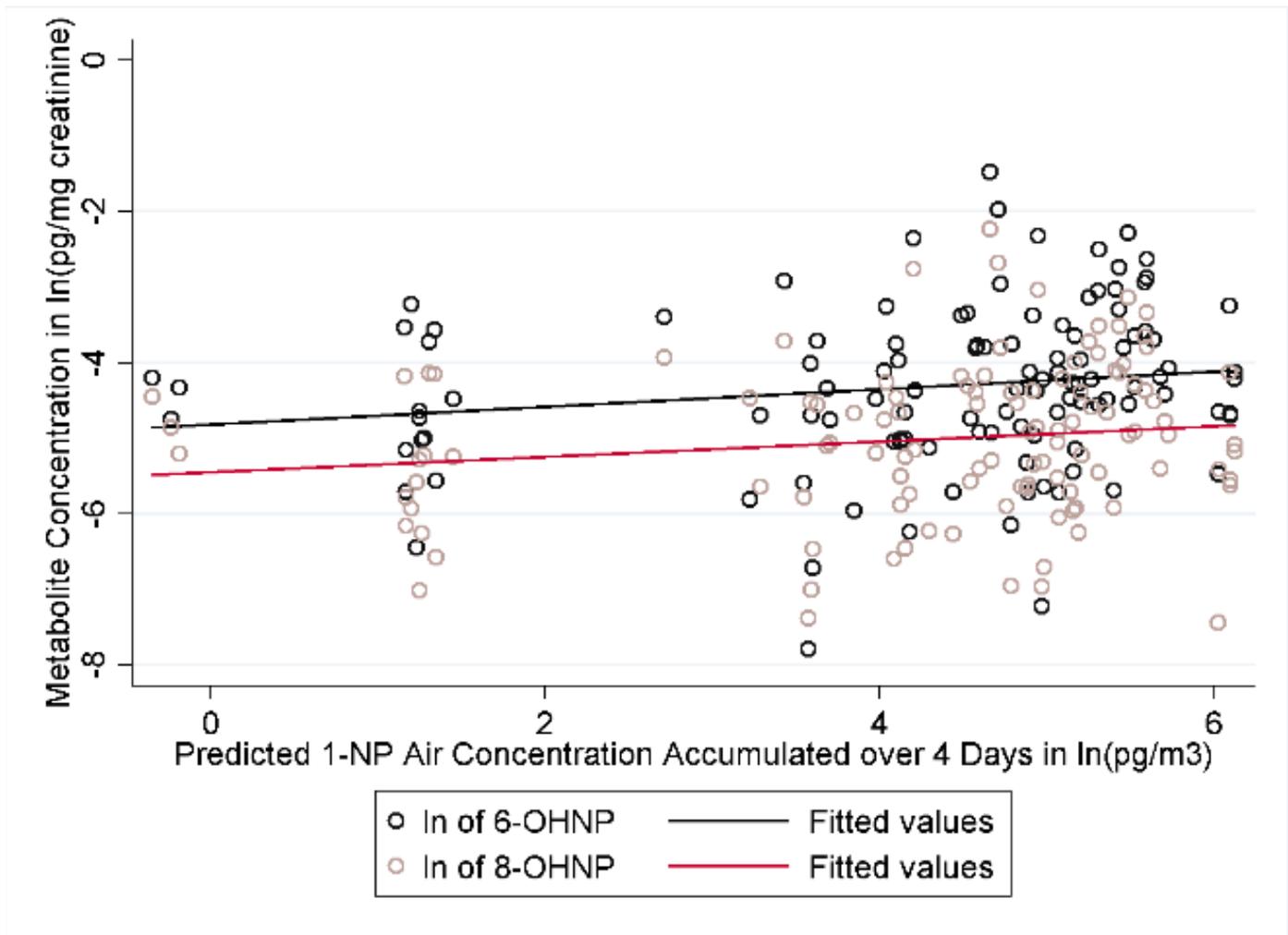


Figure 11: Estimated association between exposure to 1-NP in air and levels of 1-NP metabolites in urine (Model 5)

Predictive Metabolite Models

Two models were generated using exposure predictors from Job/Task Activity Surveys and measured 1-NP metabolite concentrations to predict post-shift levels of 6-OHNP and 8-OHNP. The predictors selected for inclusion were shift location (Face, Shop, or Surface), time spent working in areas with diesel exhaust (Time DE), time spent underground (Time UG), respirator use, day of work week, and time between urine voids. Model performance was once again evaluated using CV with random groups as described above and the out-of-sample model R^2 and RMSE were used to select a model. The model with the lowest error (RMSE) and highest R^2 was selected.

Table 15: Parameter estimates for predictive models for log-transformed post-shift 6-OHNP in ln(pg/mg creatinine) [reference location is face, n=141]

| | β (SE) | p | 95% Conf. Interval | In-sample R ² (RMSE) | Out-of-sample R ² (RMSE) |
|-------------------------|--------------|--------|--------------------|---------------------------------|-------------------------------------|
| <i>Model A</i> | | | | 0.36 (1.02) | 0.28 (1.05) |
| Shop | -0.15 (0.23) | 0.529 | (-0.61, 0.31) | | |
| Surface | -0.89 (0.30) | 0.004 | (-1.48, -0.29) | | |
| Time DE (hr) | 0.10 (0.03) | <0.001 | (0.05, 0.16) | | |
| Respirator Use (%) | 0.85 (0.34) | 0.013 | (0.18, 1.52) | | |
| Time between Voids (hr) | 0.20 (0.06) | 0.001 | (0.08, 0.32) | | |
| Day of Week | 0.30 (0.08) | <0.001 | (0.14, 0.46) | | |
| Intercept | -3.75 (0.39) | <0.001 | (-4.53, -2.97) | | |
| <i>Model B</i> | | | | 0.38 (1.01) | 0.32 (1.03) |
| Time UG (hr) | 0.12 (0.03) | 0.001 | (0.05, 0.18) | | |
| Time DE (hr) | 0.09 (0.03) | 0.002 | (0.03, 0.15) | | |
| Respirator Use (%) | 0.85 (0.33) | 0.011 | (0.20, 1.50) | | |
| Time between Voids (hr) | 0.23 (0.06) | <0.001 | (0.11, 0.34) | | |
| Day of Week | 0.34 (0.08) | <0.001 | (0.18, 0.49) | | |
| Intercept | -4.98 (0.40) | <0.001 | (-5.76, -4.20) | | |

Table 16: Parameter estimates for predictive models for log-transformed post-shift 8-OHNP in ln(pg/mg creatinine) [reference location is face, n=141]

| | β (SE) | p | 95% Conf. Interval | In-sample R ² (RMSE) | Out-of-sample R ² (RMSE) |
|-------------------------|--------------|--------|--------------------|---------------------------------|-------------------------------------|
| <i>Model A</i> | | | | 0.32 (0.95) | 0.24 (0.98) |
| Shop | -0.55 (0.22) | 0.012 | (-0.98, -0.12) | | |
| Surface | -0.68 (0.28) | 0.016 | (-1.24, -0.15) | | |
| Time DE (hr) | 0.10 (0.03) | <0.001 | (0.04, 0.15) | | |
| Respirator Use (%) | 0.76 (0.32) | 0.017 | (0.14, 1.39) | | |
| Time between Voids (hr) | 0.13 (0.06) | 0.024 | (0.02, 0.24) | | |
| Day of Week | 0.21 (0.07) | 0.005 | (0.06, 0.36) | | |
| Intercept | -3.86 (0.37) | <0.001 | (-4.58, -3.13) | | |
| <i>Model B</i> | | | | 0.33 (0.94) | 0.26 (0.96) |
| Time UG (hr) | 0.11 (0.03) | 0.001 | (0.05, 0.17) | | |
| Time DE (hr) | 0.07 (0.03) | 0.009 | (0.02, 0.13) | | |
| Respirator Use (%) | 0.82 (0.31) | 0.009 | (0.21, 1.43) | | |
| Time between Voids (hr) | 0.17 (0.05) | 0.002 | (0.06, 0.27) | | |
| Day of Week | 0.26 (0.07) | 0.001 | (0.11, 0.40) | | |
| Intercept | -5.08 (0.37) | <0.001 | (-5.81, -4.33) | | |

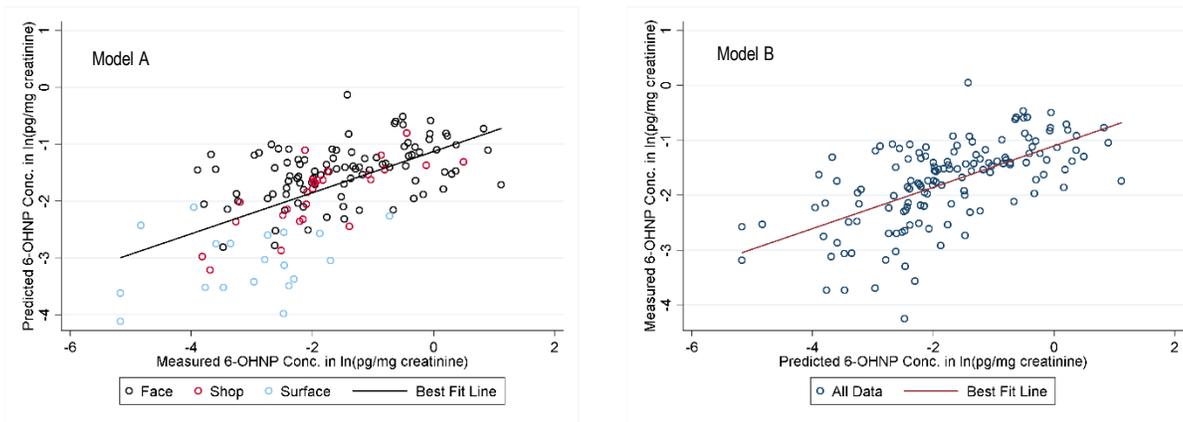


Figure 12: Comparison of predicted and measured log-transformed 6-OHNP concentrations for Models A and B

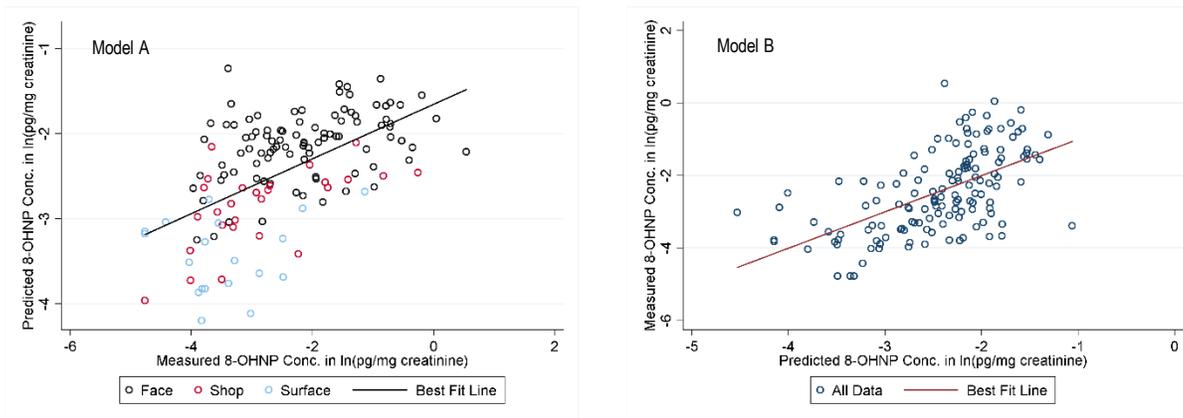


Figure 13: Comparison of predicted and measured log-transformed 8-OHNP concentrations for Models A and B

Subject responses to Job Task/Activity questionnaires appear to provide a limited ability to predict urinary metabolite levels in this study; however the models all had relatively poor predictive ability ($r^2 < 0.4$ in all cases). However, these models were capable of predicting differences in exposures between shift locations. Time spent underground was associated with higher metabolite levels as were underground face jobs. Biomarker levels predicted for shop jobs were lower than face jobs and higher than metabolite concentrations for surface jobs, as would be expected based on the measured differences in exposure (Table 15, Table 16, Figure 12, Figure 13).

An important limitation of the current study is that only 20 subjects were studied, with a relatively small number of independent observations. A larger study may allow for further refinement of predictions with specific task as well as location information that could not be reliably estimated with these data. Further refinement could also help identify if some covariates, such as respirator use, are reflecting mine-specific exposure patterns rather than generalizable trends. Contrary to what was expected, respirator use was associated with an increase in metabolite levels. However, in this mine subjects were required to wear respirators only for specific tasks that were associated with high DE exposures. Therefore, the respirator

use covariate could be acting as a surrogate for highly exposed jobs in this model rather than representing use of a control device to reduce exposures.

Conclusions

Conclusions Aim 1

Using the results of fixed location (area) monitoring and personal monitoring of a cohort of 30 underground miners, we evaluated DPM concentrations relative to the established MSHA exposure threshold and the association between the personal measures of EC and TC. We found DPM concentrations acquired from area and personal monitoring to be well below the MSHA PEL, and much improved over those observed during a similar study at this same mine in 2003. We also found that TC air concentrations were consistently and positively associated with EC personal air concentrations.

We also evaluated the association between NO_2 and PM_{10} , acquired in real-time, and TC. We found both measures to be positively correlated with TC, but this correlation was moderate at best and points to the need for more data to adequately evaluate the associations.

In summary, our findings demonstrate that control methods (i.e. ventilation, vehicle emission control devices, use of biofuels) aimed at reducing DPM contamination in the underground mining environment appear to have some utility. We have also demonstrated that EC may serve as a potential surrogate for TC, providing a measure of exposure that is free from environmental inferences and greatly simplifies the current MSHA protocol for DPM sampling. The MSHA sampling protocol requires acquisition of an additional area sample collected downstream of a personal sample to account for any OC interferences.

Conclusions Aim 2

Using portable wireless sensor devices, we performed environmental monitoring at four area mine locations. The results of this monitoring generally followed the anticipated concentration level designation previously assigned to each location. The acquisition of these results provides promise for the utility of this device. Concerning the personal wireless sensing device, while the sensors inside the unit appear to be operational, more work is needed on the housing hardware. Specifically, more work needs to be done to improve its robustness and enhance its ability to be worn by a worker performing a variety of movement-related tasks throughout the workday.

Conclusions Aim 3

We measured 1-NP, EC, OC and TC exposures in workers, and the associations between these parameters, in an underground metal mine. DPM exposures were well below the MSHA PEL, and we found 1-NP personal air concentrations were consistently and positively associated with EC personal air concentrations in a setting where few, if any, sources of interference with EC or 1-NP are present. This

association appeared unaffected by potential modifying covariates associated with specific job tasks or locations, or summer vs. winter fuel composition, however a larger study is needed to definitively characterize potential effect modification by these co-variates.

Metabolites of 1-NP were also measured in urine samples collected from the miners. Of the 1-NP metabolites detected, 6-OHNP and 8-OHNP were measured at the highest levels in urine samples which is consistent with prior studies. Metabolite levels showed a significant increase across the work week indicating that uptake, elimination, or both of 1-NP is slow relative the frequency and level of exposure for this cohort. A significant association between exposure to 1-NP in air and metabolite levels in urine was observed. However, due to the tendency for metabolites to accumulate the optimal exposure window appears to be longer than the shift preceding the spot urine sample. Subject responses to Job Task/Activity Surveys were predictive of urinary metabolite levels within this cohort, however these models had relatively poor out of sample predictive ability ($r^2 < 0.35$).

In summary, our findings demonstrate the potential utility of 1-NP and its metabolites as a surrogate measure of personal exposure to DPM. Measurements of 1-NP and its metabolites may be particularly valuable in setting such as coal mines or the ambient environment, where other potential surrogate measures of DPM exposure such as EC, TC and NO_x are confounded by non-diesel sources. Currently personal monitoring of exposure to DPM is not required in coal mines, largely due to the inability of current MSHA-approved methods to differentiate coal dust from DPM.

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<http://doi.org/10.1021/tx700015q>

Publications

Carpenter, E: [2015] Evaluation of 1-Nitropyrene as a Surrogate Measure for Diesel Exhaust: Assessment of Personal Air Monitoring Data from an Underground Mine. M.S. Thesis, University of Washington

Ramsey, J: [2015] Measurement of Urinary 1-Nitropyrene Metabolites as Biomarkers of Exposure to Diesel Exhaust in Underground Miners. M.S. Thesis, University of Washington

Bosch, R: [2015] Review of sampling and evaluation techniques for diesel exhaust particulate matter in the mining industry and the application of a vapor phase organic carbon correction factor. M.S. Thesis, Montana Tech.

Fortune, S: [2015] Comparison of integrated and direct reading sampling methods to measure biodiesel particulate matter in an underground metal mine. M.S. Thesis, Montana Tech.

Evans, D: [2015] An Evaluation of Carbon Concentrations Associated With Biodiesel Particulate Matter in an Underground Metal Mine. M.S. Thesis, Montana Tech.

Presentations

UW/UBC/SFU Joint conference on environmental, occupational and public health, Semiahmoo, WA (January 2015). Evaluation of 1-Nitropyrene as a Surrogate Measure for Diesel Exhaust: Assessment of Personal Air Monitoring and Job Task Survey Data. E. Carpenter, M. Paulsen, E. Zamzow, L. Sheppard, N. Seixas, T. Spear, D. Stephenson and C.D. Simpson

American Industrial Hygiene Conference and Exposition. Salt Lake City, UT (June 2015)

Evaluation of 1-Nitropyrene as a Surrogate Measure for Diesel Exhaust: Assessment of Personal Air Monitoring Data from an Underground Mine E. Carpenter, S. Dhakal, C. Simpson, M. Paulsen, N. Seixas, L. Sheppard, D. Stephenson, T. Spear.

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Evaluation of Metabolites of 1-Nitropyrene in Urine as a Suitable Biomarker for Exposure to Diesel Exhaust J. Ramsay, C.D. Simpson, L. Sheppard, M. Paulsen, N. Seixas, D. Stephenson, T. Spear.

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Estimating Exposure to Diesel Particulate Matter in an Underground Metal Mine. D. Stephenson, E. Zamzow, T. Spear, C.D. Simpson

Northwest Occupational Health Conference Bremerton, WA (October 2015)

Estimating Exposure to Diesel Particulate Matter in an Underground Metal Mine. E. Zamzow, D. Stephenson, T. Spear, C.D. Simpson

International Society for Exposure Science Annual meeting. Henderson, NV (October 2015)

Evaluation of 1-Nitropyrene as a Marker of Exposure To Diesel Exhaust in an Underground Mine. C.D. Simpson, D. Stephenson, T. Spear, M. Paulsen, E. Zamzow, E. Carpenter, L. Sheppard, N. Seixas.

International Society for Exposure Science Annual meeting. Henderson, NV (October 2015)

Evaluation of the Use of Environmental Surrogates to Estimate Exposure to Diesel Particulate Matter (Total Carbon) in an Underground Metal Mine. D. Stephenson, C.D. Simpson, T. Spear, E. Zamzow, M. Paulsen, L. Sheppard

Inclusion of children

The proposed study concerns DE exposures among mine workers. Subjects will be recruited from the workforce at the Stillwater mine. Since children are not employed in underground mining operations in the Stillwater mine, children will not be eligible to participate in the current study.

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Diesel Exposure in Mines: Biomarkers in Urine and Realtime Air Monitoring
Total Enrollment: 20 **Protocol Number:** #193-MED13-005 (BSU)
Grant Number: R21OH010362

| PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race | | | | |
|--|---------|-------|------------------------------------|-------|
| Ethnic Category | Females | Males | Sex/Gender Unknown or Not Reported | Total |
| Hispanic or Latino | 0 | 0 | 0 | 0 ** |
| Not Hispanic or Latino | 2 | 18 | 0 | 20 |
| Unknown (individuals not reporting ethnicity) | 0 | 0 | 0 | 0 |
| Ethnic Category: Total of All Subjects* | 2 | 18 | 0 | 20 * |
| Racial Categories | | | | |
| American Indian/Alaska Native | 0 | 0 | 0 | 0 |
| Asian | 0 | 0 | 0 | 0 |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 | 0 |
| Black or African American | 0 | 0 | 0 | 0 |
| White | 2 | 18 | 0 | 20 |
| More Than One Race | 0 | 0 | 0 | 0 |
| Unknown or Not Reported | 0 | 0 | 0 | 0 |
| Racial Categories: Total of All Subjects* | 2 | 18 | 0 | 20 * |
| | | | | |
| PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative) | | | | |
| Racial Categories | Females | Males | Sex/Gender Unknown or Not Reported | Total |
| American Indian or Alaska Native | 0 | 0 | 0 | 0 |
| Asian | 0 | 0 | 0 | 0 |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 | 0 |
| Black or African American | 0 | 0 | 0 | 0 |
| White | 0 | 0 | 0 | 0 |
| More Than One Race | 0 | 0 | 0 | 0 |
| Unknown or Not Reported | 0 | 0 | 0 | 0 |
| Racial Categories: Total of Hispanics or Latinos** | 0 | 0 | 0 | 0 ** |

* These totals must agree.
 ** These totals must agree.