Evaluation of 1-Nitropyrene as a Surrogate Measure for Diesel Exhaust:

Assessment of Personal Air Monitoring Data from an Underground Mine

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Abstract

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Objectives

We investigate the hypothesis that 1-Nitropyrene (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. 1-NP content in personal air samples was measured for a cohort of underground miners and compared with measures of Elemental Carbon (EC), Organic Carbon (OC), and Total Carbon (TC) in the same samples. Additionally, data from surveys was assessed as potential modifiers of these comparisons, and used to form a predictive model for 1-NP.

Methods

Personal exposure data were collected on a cohort of 20 employees at a large underground metal mine during 4 different sample campaigns. Full-shift personal sampling was conducted using an MSHA compliant SKC DPM impactor downstream of a GS-1 cyclone pre-filter. Each DPM filter element was analyzed for EC and OC using NIOSH method 5040. After EC and OC analysis each DPM filter was extracted with methylene chloride and analyzed for 1-NP using an LC/MS/MS method.

Results

Regression analysis of 1-NP vs. EC yields a 5.3% increase in geometric mean (GM) 1-NP for each 10% increase in GM EC ($p<0.001$). Associations between 1-NP and OC or TC were not found to be statistically significant. Survey data variables for location in the mine, fuel type, and cigarettes smoked were investigated for effect modification upon the 1-NP/EC association and none were found statistically significant. Out of ten survey variable combinations examined as predictive models for 1-NP, a model predicting 1-NP from primary tasks conducted alone was found to yield the best model performance (cross-validated $r^2=0.254$).

Conclusions

1-NP was found to be associated with EC in personal air samples. 1-NP was not found to be associated with OC or TC with statistical significance, 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. Information on primary job tasks can be used to predict personal air concentrations of 1-NP.

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Background

Diesel Exhaust as an Occupational Health Hazard

Health effects

Diesel exhaust (DE) is an exposure presenting a number of health risks. Acute exposure can result in irritation of the eye, nose and throat, and at higher concentrations can induce inflammation of the airways and lung. Neurophysiological symptoms, such as headache, lightheadedness, nausea, vomiting, and numbness or tingling of the extremities can also result from acute exposure [USEPA, 2002]. Chronic exposure can lead to impaired lung function, and DE is classified as a group 1 human carcinogen [IARC, 2012]. Additionally, diesel exhaust exhibits immunological toxicity to induce allergic responses or worsen existing respiratory allergies. [USEPA, 2002].

Definition of Diesel Exhaust and Description of Components

Diesel exhaust is a complex mixture, making health effect etiology uncertain. Particulate matter comprising DE (DPM) includes elemental carbon (EC), adsorbed organic compounds, and small amounts of sulfate, nitrate, metals, trace elements, water, and unidentifiable compounds [USEPA, 2002]. About 80-90% of DPM is less than 1 µm by mass, in the fine particle range. Particles in this range have large surface area per mass facilitating the adsorption of organic compounds [Health Effects Institute, 1995]. Organic compounds found in DE of health concern include aldehydes, benzene, 1,3-butadiene, PAHs, nitro-PAHS (all of which are potentially carcinogenic), as well as aldehydes, alkanes, alkenes, nitrogen oxides, and sulfur oxides (which can cause respiratory irritation) [USEPA, 2002].

The ideal combustion scheme for diesel burning consists of diesel fuel and oxygen being converted to carbon dioxide, water, and energy [Bugarski, 2012]. However, within a typical engine's combustion chamber, elemental carbon, partially burned fuel, PAHs and carbon monoxide are formed in the fuel-rich center of the burning injection jet. NOx formation occurs at the jet's outer edges due to excess air and higher temperatures [IARC 2013]. Additionally, hydrocarbons are formed due to crevice-trapped fuel and lubricant insufficiently mixed with air [USEPA 2001].

Most aerosols emitted by diesel engines are submicrometer (<1 µm) in size and an order of magnitude smaller than aerosols generated by mechanical processes. Operations such as drilling, blasting, grinding, crushing, and transportation create respirable dust-containing particles with aerodynamic diameters between about 1 and 10 µm [Bugarski, 2012].

Prevalence of Diesel Exhaust in Underground Mining

Use of Diesel Engines and Vehicles in the Overall Mining Industry

Diesel engines have been in use for mining since 1939, and use increased in the 1970s. In 1998 about 78% of underground metal/nonmetal mines in the US used diesel engines [Pronk, 2009]. They are preferred over gasoline engines because they are more fuel efficient, and diesel fuel is relatively inexpensive [HEI, 1995]. Diesel-powered vehicles include transportation of personnel, haulage trucks, load and dump vehicles, drills, graders, and utility trucks. Worker tasks near diesel exhaust sources include underground drilling and blasting mine face, loading and scooping ore and debris, hauling ore and debris to transportation equipment/conveyor belts, maintenance in underground repair shops, and infrastructure maintenance in haulage and travel ways [Pronk, 2009]. Compared to those in other workplaces, workers in coal mines or non-coal mines using diesel equipment experience the highest occupational exposure to diesel particulate matter [USEPA, 2002].

Use of Diesel Engines in the Metal/Nonmetal Mining Industry

The underground metal and nonmetal mining sector uses diesel engines extensively, and is expected to continue for some time. In 2001, 196 out of the 264 total US underground metal/nonmetal mines used diesel equipment with a total of 3,998 engines. Of these engines, approximately 1,800 are used for loading and hauling (in contrast to the use in coal mines primarily for support equipment). Metal/nonmetal mines use large diesel engines compared with coal mines. Mass of DPM emissions produced per hour is directly proportional to horsepower size of the engine, so M/NM mines have higher DPM emissions than coal mines [MSHA, 2001].

Monitoring of Diesel Exhaust Exposure Using Total Carbon and Elemental Carbon

Current MSHA Regulations and Definition of Elemental Carbon and Total Carbon

In January, 2001, the Mining Safety and Health Administration (MSHA) published final rules in the Federal Register regulating DPM in coal and metal/nonmetal (M/NM) mines. For M/NM mines the DPM permissible exposure limit (PEL) is 160 micrograms of total carbon (TC) per cubic meter of air. Compliance is determined by taking personal and area air samples and determining TC content. In order to account for non-diesel organic carbon (OC) interferences on personal samples, elemental carbon (EC) is also determined from the personal and area samples, since TC is defined as the sum of EC and OC [MSHA 2001]. This method is specific to M/NM mines; the regulation for coal mines is based on the testing of engine emissions as opposed to worker exposures.

EC in DPM is formed by the pyrolysis of fuel and lubricating oil in the engine's combustion chamber, and is emitted from the engine as solid particulate matter. OC is formed by incomplete oxidation of hydrocarbons in fuel and lubricating oil, and is emitted as volatile and semivolatile organic material often absorbed onto the surface of larger EC particles [Bugarski, 2012].

In underground metal mines, EC concentrations average about 75% of the TC concentrations, and TC concentrations make up an average of 72% of total DPM concentrations. The ratio of EC/TC varies by mine and by area within a given mine [Bugarski, 2012]. However, this ratio was determined using old technology diesel engines and standard diesel fuel, and would differ in the case of newer engine technology and biodiesel blends.

MSHA Method of Determining Exposure Limit Compliance Using TC and EC

As illustrated in Figure 1, below, a worker is considered overexposed to DE when a combination of TC and EC conditions is found to be present. If personal sample TC and EC exceed 160 µg/m3 (multiplied by an error factor, EF), the worker is considered overexposed. If personal sample EC is below 160 µg/m3 (xEF), the worker is considered overexposed if personal sample TC exceeds 160 µg/m3 (xEF) and EC on the personal sample times the ratio of TC to EC from an area sample exceeds 160 µg/m3 (xEF). The error factors account for variability in the sampling and analysis procedure for EC and OC determination; variability exists in the volume of air pumped through DPM collection filters, the deposit area of particles on the filter, and the laboratory analysis of EC and OC density within the deposit. The error factor was incorporated into the MSHA regulation to ensure a citation is only issued when a sample measurement demonstrates noncompliance with at least 95% confidence [MSHA 2001].

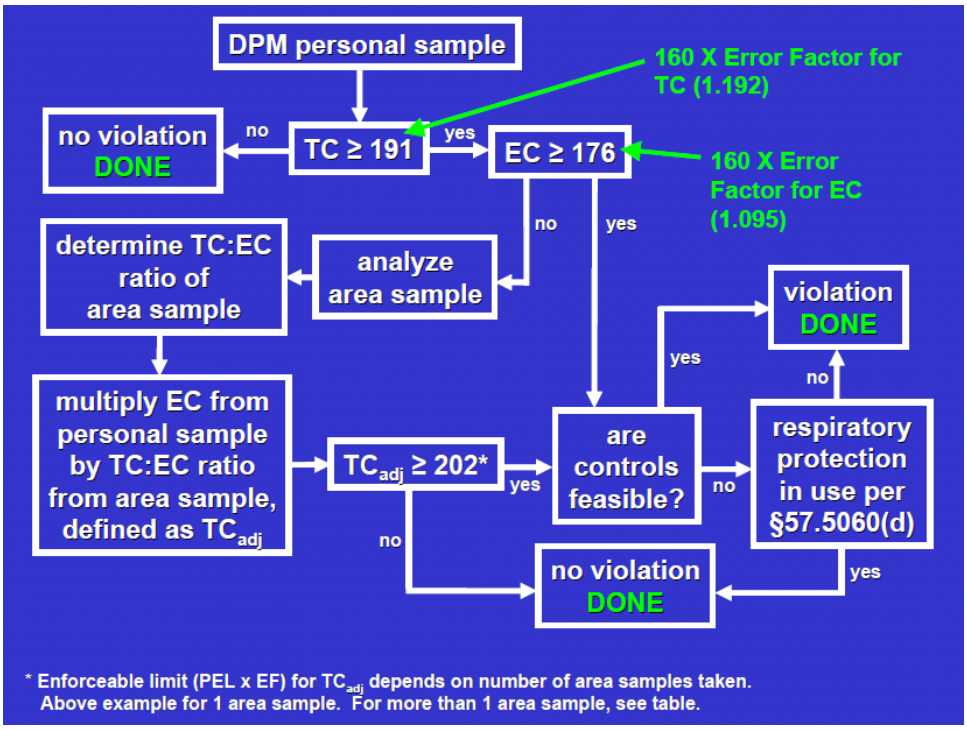

Figure 1: MSHA DPM Rule Compliance Schematic [MSHA, 2015]

Limitations of the Use of EC/TC to Determine DE Exposure

MSHA regulation requires EC and OC are measured by NIOSH method 5040 [MSHA 2001]. However, concerns have been raised as to the feasibility of EC monitoring for diesel exhaust quantification, including variations in analytical methods, lack of information on other EC sources, the ratio of EC to particulate mass for DE particulate matter, as well as the fundamental definition of EC itself [Schauer, 2003].

Elemental carbon is defined operationally, as the carbon components of diesel exhaust that are not carbonate carbon or organic carbon. EC itself is not specifically defined or identified, and its definition relies upon bulk analytical methods used to identify it by contrast with carbonate carbon and organic carbon. [Schauer, 2003]. Therefore, EC determination is susceptible to variability due to analytical variation.

The NIOSH EC determination method involves thermal-optical analysis and flame ionization detection. The method consists of a series of steps in which the sample is progressively heated and the carbon evolved at each step is detected. The carbon evolved in a helium atmosphere is operationally defined as OC, whereas the carbon subsequently evolved in an oxidizing atmosphere is defined as EC. Another commonly used method is the IMPROVE EC/OC method, or the total optical reflectance (TOR) method

[Schauer, 2003]. This method shares similarities with the NIOSH method, but differs in peak temperatures at a critical heating stage and hold times at various heating stages. These differences result in higher EC measurements by the IMPROVE method than by the NIOSH method for many samples. The variations between the two methods were found to exceed the precision of the EC measurements for a fixed ECOC analysis method [Schauer, 2003]. Not only does this raise concern about comparability of the methods, but it can lead to significant biases or errors if the two methods are integrated together. Additionally, EC determination techniques may or may not include correction for pyrolysis during the heating step, which is an adjustment to prevent particulate carbon being incorrectly reported as EC.

Additionally, EC as a surrogate for diesel exhaust may be confounded by alternate sources of EC. Atmospheric sources of EC, aside from diesel exhaust, include fireplace combustion, agricultural/brush burning, meat smoke, coal fly ash, fuel oil boiler emissions, jet engine emissions, church candles, cigarette smoke, and gas-powered engines [Schauer, 2003]. Additionally, in underground mines alternate sources of carbon include mineral dust, environmental tobacco smoke, and oil mist. Though EC and OC contamination from mineral dust is able to be removed using size-selective samplers, submicrometer aerosols cannot similarly be removed from tobacco smoke or oil mist [Noll, 2007]. If EC and TC are used alone to estimate DE particulate matter, contribution from multiple sources must be addressed proportionally. This presents an issue when additional sources are difficult to identify.

Due to the multiple feasibility issues of relying on EC as a marker of DE exposure, alternate, more reliable and specific markers are needed. Additionally, because EC is an established exposure metric, comparison with a new marker is warranted. For this reason, EC, OC, and TC will be compared with 1-NP to evaluate 1-NP as a potential surrogate measure for DPM.

Use of 1-Nitropyrene Air Concentration as a Surrogate Measure for DPM

Definition, Formation, and Sources of 1-Nitropyrene

1-Nitropyrene (1-NP) is one of a number of nitrated polycyclic aromatic hydrocarbons (nPAHs) formed in the combustion chamber of a diesel engine, due to high temperatures and excess air supply [IARC 2013]. 1-NP is formed when free pyrene radicals receive an addition of nitrogen oxide (NO) or nitrogen dioxide (NO_2) or by nitration of PAHs with HNO_3 in the exhaust manifold [Schuetzle 1986]. PAHs are typically present in both the gas and particulate phase in diesel exhaust and comprise a small percent of the soluble organic fraction of diesel particulate matter [Bugarski, 2012].

Other sources of 1-NP aside from diesel exhaust include cooking oil fumes, waste incineration or fly ash from industrial processes, propane burning fumes, kerosene heater emissions, and gas-powered vehicle exhaust fumes. 1-NP is unlikely to form in cigarette smoke because tobacco combustion is reductive and would not form cationic PAHs that could join with NOx species [IARC 2013].

Detection Methods for 1-NP Air Concentration

1-NP concentration in DE is able to be assessed by filter sampling. Because gas-phase 1-NP occurs at very low concentrations, 1-NP adsorbed onto particles is the primary target for sampling. A filter is used to collect particles from diluted exhaust fumes or ambient air, and pure Teflon or Teflon-coated filters have been shown to prevent conversion of trapped pyrene into 1-NP on the filter surface. There is limited influence from atmospheric conditions on the formation and stability of 1-NP adsorbed onto DE particles [IARC, 2013].

A number of analysis techniques have been used to detect 1-NP in filter samples, including HPLC with fluorescence detection, HPLC with chemiluminescence detection, and GC or liquid chromatography with various types of mass spectrometry [IARC, 2013]. A notable recent method for detection has been reported by Miller-Schulze and associates [Miller-Schulze, 2007] that utilizes ultrasonic extraction of DPM samples in organic solvent and two-dimensional HPLC analysis of the extract using MS/MS detection. The LOD obtained with this method represents about a 10-fold improvement over previous HPLC and GC-NICI-MS methods, and is suited for analysis of low-level 1-NP in environmental extracts [Miller-Schulze, 2007].

Previous Studies for 1-Nitropyrene Measurement

A literature review was conducted in order to gather a comprehensive list of previously measured 1-nitropyrene in air samples, the gathered information is provided in Table 1 below. Measurements date back to 1982. Studies have taken place all over the world, in primarily ambient settings, with emphasis on urban areas experiencing high levels of traffic exhaust pollution. Levels of 1-NP measured tend to be in the range of about 0-200 pg/m^3 in ambient settings, with a few exceptions in high-traffic settings. There are a few examples for which 1-NP was measured in workplaces where employees would operate diesel-fueled equipment indoors one of which took place at mine locations [Scheepers et al, 2003, and some studies reported in IARC, 2013]. Levels of 1-NP measured in these occupational settings are generally higher than in ambient studies; above 200 pg/m^3 in many cases, and in a few instances an order of magnitude higher. Overall, data is scarce on 1-NP measured in indoor occupational settings or mines in particular.

Table 1: Compilation of Past 1-NP Measurements

1-NP Concentration (pg/m^3)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
64000	-	-	-	1	GCMS (negative ion chemical ionization MS)	1986	Non-diesel source	-	IARC, 2013; table 1.4	Thrane & Stray 1986
147	-	-	-	1	HPLC (fluorescence detection)	1988	Non-diesel source	-	IARC, 2013; table 1.4	Kinouchi et al 1988
1620	-	-	-	1	HPLC (fluorescence detection)	1988	Non-diesel source	-	IARC, 2013; table 1.4	Kinouchi et al 1988
44	-	-	-	1	HPLC (fluorescence detection)	1988	Non-diesel source	-	IARC, 2013; table 1.4	Kinouchi et al 1988
20.8	-	-	-	1	-	1982	Ambient air, Industrial	-	IARC, 2013; table 1.6	Morita et al 1982
57	AM	-	-	5	-	1982	Ambient air, Industrial	River Rouge, MI, USA	IARC, 2013; table 1.6	Gibson 1986
29	AM	-	-	8	-	1980	Ambient air, Industrial	Dearborn, MI, USA	IARC, 2013; table 1.6	Gibson 1986
9	AM	-	-	3	-	1986	Ambient air, Industrial	Yuba City, USA	IARC, 2013; table 1.6	Atkinson et al 1988
29	AM	-	-	5	-	1986-87	Ambient air, Industrial	Concord, USA	IARC, 2013; table 1.6	Atkinson et al 1988
8	-	-	-	1	-	1987	Ambient air, Industrial	Mammoth Lakes, USA	IARC, 2013; table 1.6	Atkinson et al 1988
7	AM	-	-	3	-	1987	Ambient air, Industrial	Oildale, USA	IARC, 2013; table 1.6	Atkinson et al 1988

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
80	-	-	-	1	-	1999	Ambient air, Industrial	Oued Smar, Algeria	IARC, 2013; table 1.6	Yassaa et al 2001
-	-	-	-	1	-	1998	Ambient air, Industrial	Oued Smar, Algeria	IARC, 2013; table 1.6	Yassaa et al 2001
16	-	-	-	1	-	1981	Ambient air, Urban-suburban	Detroit, MI, USA	IARC, 2013; table 1.6	Gibson 1982
30	-	-	-	1	-	1981	Ambient air, Urban-suburban	Detroit, MI, USA	IARC, 2013; table 1.6	Gibson 1982
-	-	28-110	range	4	-	1981	Ambient air, Urban-suburban	Santiago, Chile	IARC, 2013; table 1.6	Tokiwa et al 1983
-	-	2.4-12	range	4	-	1985	Ambient air, Urban-suburban	Michigan, USA	IARC, 2013; table 1.6	Siak et al 1985
1500	-	-	-	1	-	1983	Ambient air, Urban-suburban	Bayreuth, Germany	IARC, 2013; table 1.6	Garner et al 1986
1700	-	-	-	1	-	1983	Ambient air, Urban-suburban	Bayreuth, Germany	IARC, 2013; table 1.6	Garner et al 1986
41	-	-	-	1	-	-	Ambient air, Urban-suburban	Tokyo, Japan	IARC, 2013; table 1.6	Tanabe et al 1986
130	-	-	-	1	-	-	Ambient air, Urban-suburban	Tokyo, Japan	IARC, 2013; table 1.6	Tanabe et al 1986
32	-	-	-	1	-	-	Ambient air, Urban-suburban	Tokyo, Japan	IARC, 2013; table 1.6	Tanabe et al 1986
62	-	-	-	1	-	-	Ambient air, Urban-suburban	Tokyo, Japan	IARC, 2013; table 1.6	Tanabe et al 1986
15	-	-	-	1	-	-	Ambient air, Urban-suburban	Tokyo, Japan	IARC, 2013; table 1.6	Tanabe et al 1986
38	-	-	-	1	-	-	Ambient air, Urban-suburban	Tokyo, Japan	IARC, 2013; table 1.6	Tanabe et al 1986
51	-	-	-	1	-	-	Ambient air, Urban-suburban	Tokyo, Japan	IARC, 2013; table 1.6	Tanabe et al 1986
80	-	-	-	1	-	-	Ambient air, Urban-suburban	Tokyo, Japan	IARC, 2013; table 1.6	Tanabe et al 1986
15	AM	-	-	7	-	1982	Ambient air, Urban-suburban	Warren, MI, USA	IARC, 2013; table 1.6	Gibson 1986
22	AM	-	-	5	-	1984	Ambient air, Urban-suburban	Warren, MI, USA	IARC, 2013; table 1.6	Gibson 1986
30	AM	-	-	15	-	1981	Ambient air, Urban-suburban	Detroit, MI, USA	IARC, 2013; table 1.6	Gibson 1986
40	-	-	-	1	-	-	Ambient air, Urban-suburban	Torrance, CA, USA	IARC, 2013; table 1.6	Arey et al 1987
30	-	-	-	1	-	-	Ambient air, Urban-suburban	Torrance, CA, USA	IARC, 2013; table 1.6	Arey et al 1987

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
20	AM	-	-	6	-	1986	Ambient air, Urban-suburban	Glendora, USA	IARC, 2013; table 1.6	Atkinson et al 1988
8	AM	-	-	2	-	1987	Ambient air, Urban-suburban	Reseda, USA	IARC, 2013; table 1.6	Atkinson et al 1988
16	-	-	-	1	-	-	Ambient air, Urban-suburban	Claremont, USA	IARC, 2013; table 1.6	Zielnska et al 1989
26	-	-	-	1	-	1989-90	Ambient air, Urban-suburban	Barcelona, Spain	IARC, 2013; table 1.6	Bayona et al 1994
36	-	-	-	1	-	1992	Ambient air, Urban-suburban	Nijmegen, Netherlands	IARC, 2013; table 1.6	Scheepers et al 1994a
12	-	-	-	1	-	1990-91	Ambient air, Urban-suburban	Hamilton, Canada	IARC, 2013; table 1.6	Legzdins et al 1995
10	-	-	-	1	-	1991-93	Ambient air, Urban-suburban	Madrid, Spain	IARC, 2013; table 1.6	Ciccioli et al 1995
12	-	-	-	1	-	1991-93	Ambient air, Urban-suburban	Montelibretti, Italy	IARC, 2013; table 1.6	Ciccioli et al 1995
220	-	-	-	1	-	1991-93	Ambient air, Urban-suburban	Milan, Italy	IARC, 2013; table 1.6	Ciccioli et al 1995
70	-	-	-	1	-	1991-93	Ambient air, Urban-suburban	Rome, Italy	IARC, 2013; table 1.6	Ciccioli et al 1995
16	-	-	-	1	-	1991-93	Ambient air, Urban-suburban	Sao Paulo, Brazil	IARC, 2013; table 1.6	Ciccioli et al 1995
120	-	-	-	1	-	1994	Ambient air, Urban-suburban	Damascus, Syrian Arab Republic	IARC, 2013; table 1.6	Dimashki et al 1996
140	-	-	-	1	-	1991	Ambient air, Urban-suburban	Milan, Italy	IARC, 2013; table 1.6	Cecinato et al 1998
590	-	-	-	1	-	1993	Ambient air, Urban-suburban	Rome, Italy	IARC, 2013; table 1.6	Cecinato et al 1998
80	-	-	-	1	-	1991-1993	Ambient air, Urban-suburban	Rome, Italy	IARC, 2013; table 1.6	Cecinato et al 1998
-	-	1.2-3.5	range	3	-	1998	Ambient air, Urban-suburban	Southampton, UK	IARC, 2013; table 1.6	Scheepers et al 1999
32	-	-	-	1	-	1994	Ambient air, Urban-suburban	Kananazawa, Japan	IARC, 2013; table 1.6	Scheepers et al 1999
40	-	-	-	1	-	1996	Ambient air, Urban-suburban	Athens, Greece	IARC, 2013; table 1.6	Marino et al 2000
140	-	-	-	1	-	1999	Ambient air, Urban-suburban	Algiers, Algeria	IARC, 2013; table 1.6	Yassaa et al 2001
5.9	median	-	-	7	-	2008	Ambient air, Urban-suburban	Payas, Turkey	IARC, 2013; table 1.6	Ari et al, 2010
2.4	median	-	-	7	-	2008	Ambient air, Urban-suburban	Payas, Turkey	IARC, 2013; table 1.6	Ari et al, 2010

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
4.1	median	-	-	8	-	2008	Ambient air, Urban-suburban	Iskenderun, Turkey	IARC, 2013; table 1.6	Ari et al, 2010
19.2	median	-	-	5	-	2008	Ambient air, Urban-suburban	Eskisehir, Turkey	IARC, 2013; table 1.6	Ari et al, 2010
9.6	-	-	-	1	-	1982	Ambient air, Rural	Bermuda	IARC, 2013; table 1.6	Gibson 1986
10.3	-	-	-	1	-	1983	Ambient air, Rural	Bermuda	IARC, 2013; table 1.6	Gibson 1986
0.5	AM	-	-	2	-	1987	Ambient air, Rural	Pt. Arguello, USA	IARC, 2013; table 1.6	Atkinson et al 1988
0.3	-	-	-	1	-	1987	Ambient air, Rural	San Nicolas Island, USA	IARC, 2013; table 1.6	Atkinson et al 1988
2	-	-	-	1	-	1992	Ambient air, Rural	Castelporziano, Italy	IARC, 2013; table 1.6	Ciccioli et al 1995
2	-	-	-	1	-	1993	Ambient air, Rural	Alta Floresta, Brazil	IARC, 2013; table 1.6	Ciccioli et al 1995
1.7	-	-	-	1	-	1992	Ambient air, Rural	Hoenderloo, Netherlands	IARC, 2013; table 1.6	Scheepers et al, 1994a
0.5	-	-	-	1	-	1998	Ambient air, Rural	Southampton, UK	IARC, 2013; table 1.6	Scheepers et al, 1999
0.6	-	-	-	1	-	1998	Ambient air, Rural	Southampton, UK	IARC, 2013; table 1.6	Scheepers et al, 1999
0.8	-	-	-	1	-	1998	Ambient air, Rural	Southampton, UK	IARC, 2013; table 1.6	Scheepers et al, 1999
16	-	-	-	1	-	1998-99	Ambient air, Rural	Svalbard Island, Norway	IARC, 2013; table 1.6	Cecinato et al 2000
10.6	AM	-	-	13	-	2002-2003	Ambient air, Rural	Sollieres, Maurienne Valley, France	IARC, 2013; table 1.6	Albinet et al, 2006
4.8	median	-	-	4	-	2008	Ambient air, Rural	Eskisehir, Turkey	IARC, 2013; table 1.6	Ari et al, 2010
2.9	median	-	-	4	-	2008	Ambient air, Rural	Eskisehir, Turkey	IARC, 2013; table 1.6	Ari et al, 2010
12	-	-	-	1	-	1992	Diesel engines at workplaces, outdoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
15	-	-	-	1	-	1992	Diesel engines at workplaces, outdoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
34	AM	1.9	-	3	-	1992	Diesel engines at workplaces, outdoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
790	-	-	-	1	-	1992	Diesel engines at workplaces, outdoor	Belgium	IARC, 2013; table 1.7	Scheepers et al 1994a

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
36	-	-	-	1	-	1992	Diesel engines at workplaces, outdoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
6.6	-	-	-	1	-	1992	Diesel engines at workplaces, outdoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
34	AM	1.4	ASD	3	-	1992	Diesel engines at workplaces, outdoor	Schiphol, Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
54	GM	2400	GSD	14	-	1998	Diesel engines at workplaces, indoor	UK	IARC, 2013; table 1.7	Scheepers et al 1999
53	GM	2000	GSD	13	-	1998	Diesel engines at workplaces, indoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1999
310	GM	1200	GSD	4	-	1992	Diesel engines at workplaces, indoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
1800	GM	2400	GSD	7	-	1994	Diesel engines at workplaces, indoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
80	-	-	-	1	-	1992	Diesel engines at workplaces, indoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
610	-	-	-	1	-	1992	Diesel engines at workplaces, indoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
710	-	-	-	1	-	1992	Diesel engines at workplaces, indoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
1100	GM	1100	GSD	4	-	1992	Diesel engines at workplaces, indoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
90	GM	1700	GSD	4	-	1992	Diesel engines at workplaces, indoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
220	GM	210	GSD	4	-	1992	Diesel engines at workplaces, indoor	Netherlands	IARC, 2013; table 1.7	Scheepers et al 1994a
700	-	-	-	1	-	2002	Diesel engines at workplaces, indoor	Germany	IARC, 2013; table 1.7	Seidel et al 2002
1300	-	-	-	1	-	2002	Diesel engines at workplaces, indoor	Germany	IARC, 2013; table 1.7	Seidel et al 2002
1000	-	-	-	1	-	2002	Diesel engines at workplaces, indoor	Germany	IARC, 2013; table 1.7	Seidel et al 2002
2.8	GM	3.7	GSD	8	-	1999	Diesel engines at workplaces, indoor	Czech Republic	IARC, 2013; table 1.7	Scheepers et al 2002, 2003
78	GM	3.3	GSD	8	-	1999	Diesel engines at workplaces, indoor	Czech Republic	IARC, 2013; table 1.7	Scheepers et al 2002, 2003
70	GM	2.1	GSD	6	-	1999	Diesel engines at workplaces, indoor	Estonia	IARC, 2013; table 1.7	Scheepers et al 2002, 2003
216	GM	2.0	GSD	5	-	1999	Diesel engines at workplaces, indoor	Estonia	IARC, 2013; table 1.7	Scheepers et al 2002, 2003

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
52	AM	21.0	ASD	4-5 workday samples	-	1998	Personal air sampling of diesel exhaust-exposed workers	UK	IARC, 2013; table 1.8	Scheepers et al 1999
19	AM	16.0	ASD	4-5 workday samples	-	1998	Personal air sampling of diesel exhaust-exposed workers	UK	IARC, 2013; table 1.8	Scheepers et al 1999
8	AM	9.2	ASD	4-5 workday samples	-	1998	Personal air sampling of diesel exhaust-exposed workers	UK	IARC, 2013; table 1.8	Scheepers et al 1999
36	AM	32.0	ASD	4-5 workday samples	-	1998	Personal air sampling of diesel exhaust-exposed workers	UK	IARC, 2013; table 1.8	Scheepers et al 1999
12.9	AM	6.9	ASD	4-5 workday samples	-	1998	Personal air sampling of diesel exhaust-exposed workers	UK	IARC, 2013; table 1.8	Scheepers et al 1999
1171	AM	867.0	ASD	4 workers sampled + stationary samples	-	2002	Personal air sampling of diesel exhaust-exposed workers	Germany	IARC, 2013; table 1.8	Seidel et al 2002
8	GM	2.3	GSD	9 workers, 1 shift	-	1999	Personal air sampling of diesel exhaust-exposed workers	Estonia	IARC, 2013; table 1.8	Scheepers et al 2002, 2003
45	GM	3.8	GSD	9 workers, 1 shift	-	1999	Personal air sampling of diesel exhaust-exposed workers	Estonia	IARC, 2013; table 1.8	Scheepers et al 2002, 2003
85	GM	2.7	GSD	42 workers, 3 shifts	-	2000	Personal air sampling of diesel exhaust-exposed workers	Estonia	IARC, 2013; table 1.8	Scheepers et al 2002, 2003
2483	GM	3.4	GSD	10 workers, 1 shift	-	1999	Personal air sampling of diesel exhaust-exposed workers	Estonia	IARC, 2013; table 1.8	Scheepers et al 2002, 2003
984	GM	2.7	GSD	10 workers, 1 shift	-	1999	Personal air sampling of diesel exhaust-exposed workers	Estonia	IARC, 2013; table 1.8	Scheepers et al 2002, 2003
637	GM	3.3	GSD	50 workers, 3 shifts	-	2000	Personal air sampling of diesel exhaust-exposed workers	Estonia	IARC, 2013; table 1.8	Scheepers et al 2002, 2003
110	GM	2.8	GSD	10 workers, 1 shift	-	1999	Personal air sampling of diesel exhaust-exposed workers	Czech Republic	IARC, 2013; table 1.8	Scheepers et al 2002, 2003

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
71	GM	7.6	GSD	9 workers, 1 shift	-	1999	Personal air sampling of diesel exhaust-exposed workers	Czech Republic	IARC, 2013; table 1.8	Scheepers et al 2002, 2003
197	GM	1.5	GSD	9 workers, 1 shift	-	1999	Personal air sampling of diesel exhaust-exposed workers	Czech Republic	IARC, 2013; table 1.8	Scheepers et al 2002, 2003
209	GM	3.6	GSD	9 workers, 1 shift	-	1999	Personal air sampling of diesel exhaust-exposed workers	Czech Republic	IARC, 2013; table 1.8	Scheepers et al 2002, 2003
186.9	AM	58.5	ASD	7	HPLC with fluorescence/UV detection	2002-2003	Ambient, alpine traffic	France (Chamonix valley)	Albinet et al, 2007b; Supplementary Table 1	-
53.7	AM	25.7	ASD	14	HPLC with fluorescence/UV detection	2002-2003	Ambient, alpine traffic	France (Chamonix valley)	Albinet et al, 2007b; Supplementary Table 1	-
2.4	AM	2.6	ASD	7	HPLC with fluorescence/UV detection	2002-2003	Ambient, alpine traffic	France (Chamonix valley)	Albinet et al, 2007b; Supplementary Table 1	-
9.9	AM	6.1	ASD	9	HPLC with fluorescence/UV detection	2002-2003	Ambient, alpine traffic	France (Chamonix valley)	Albinet et al, 2007b; Supplementary Table 1	-
2.8	AM	0.9	ASD	7	HPLC with fluorescence/UV detection	2003	Ambient, alpine traffic	France (Chamonix valley)	Albinet et al, 2007b; Supplementary Table 1	-
7.7	AM	4.8	ASD	14	HPLC with fluorescence/UV detection	2003	Ambient, alpine traffic	France (Chamonix valley)	Albinet et al, 2007b; Supplementary Table 1	-
0.6	AM	0.5	ASD	7	HPLC with fluorescence/UV detection	2003	Ambient, alpine traffic	France (Chamonix valley)	Albinet et al, 2007b; Supplementary Table 1	-
0.7	AM	0.4	ASD	14	HPLC with fluorescence/UV detection	2003	Ambient, alpine traffic	France (Chamonix valley)	Albinet et al, 2007b; Supplementary Table 1	-

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
24.8	AM	21.9	ASD	14	HPLC with fluorescence/UV detection	2002-2003	Ambient, alpine traffic	France (Maurienne valley)	Albinet et al, 2007b; Supplementary Table 1	-
70	AM	53.6	ASD	7	HPLC with fluorescence/UV detection	2002-2003	Ambient, alpine traffic	France (Maurienne valley)	Albinet et al, 2007b; Supplementary Table 1	-
10.8	AM	7.6	ASD	13	HPLC with fluorescence/UV detection	2002-2003	Ambient, alpine traffic	France (Maurienne valley)	Albinet et al, 2007b; Supplementary Table 1	-
4.7	AM	5.1	ASD	14	HPLC with fluorescence/UV detection	2003	Ambient, alpine traffic	France (Maurienne valley)	Albinet et al, 2007b; Supplementary Table 1	-
6.5	AM	7.2	ASD	11	HPLC with fluorescence/UV detection	2003	Ambient, alpine traffic	France (Maurienne valley)	Albinet et al, 2007b; Supplementary Table 1	-
6.5	AM	5.8	ASD	7	HPLC with fluorescence/UV detection	2003	Ambient, alpine traffic	France (Maurienne valley)	Albinet et al, 2007b; Supplementary Table 1	-
0.8	AM	0.7	ASD	14	HPLC with fluorescence/UV detection	2003	Ambient, alpine traffic	France (Maurienne valley)	Albinet et al, 2007b; Supplementary Table 1	-
60.7	AM	14.9-222.1	range	12	HPLC with fluorescence/UV detection	2004	Ambient, urban	France (Marseilles)	Albinet et al, 2007a	-
7.9	AM	1.1-16.4	range	14	HPLC with fluorescence/UV detection	2004	Ambient, suburban	France (Marseilles)	Albinet et al, 2007a	-
0.6	AM	0.0-3.3	range	14	HPLC with fluorescence/UV detection	2004	Ambient, rural	France (Marseilles)	Albinet et al, 2007a	-
27	AM	14-40	range	4	GCMS (negative ion chemical ionization MS)	2001	Ambient, urban	Baltimore, Maryland, USA	Bamford et al, 2003	-

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
8.1	AM	3.0-16	range	5	GCMS (negative ion chemical ionization MS)	2001	Ambient, urban	Baltimore, Maryland, USA	Bamford et al, 2003	-
20	AM	7.5-38	range	4	GCMS (negative ion chemical ionization MS)	2001	Ambient, suburban	Fort Meade, Maryland, USA	Bamford et al, 2003	-
1.4	AM	0.5-2.2	range	4	GCMS (negative ion chemical ionization MS)	2001	Ambient, suburban	Fort Meade, Maryland, USA	Bamford et al, 2003	-
67	AM	9.0	ASD	3	GCMS	2001	Ambient, urban	Barcelona, Spain	Castells et al, 2003	-
81	AM	7.0	ASD	3	GCMS	2001	Ambient, urban	Barcelona, Spain	Castells et al, 2003	-
33000	-	1000.0	-	?	GCMS (negative ion chemical ionization MS)	2003-2004	Industrial Workplace	France	Chaspoul et al, 2005	-
5000	-	1000.0	-	?	GCMS (negative ion chemical ionization MS)	2003-2004	Industrial Workplace	France	Chaspoul et al, 2005	-
16000	-	1000.0	-	?	GCMS (negative ion chemical ionization MS)	2003-2004	Industrial Workplace	France	Chaspoul et al, 2005	-
23000	-	1000.0	-	?	GCMS (negative ion chemical ionization MS)	2003-2004	Industrial Workplace	France	Chaspoul et al, 2005	-
74	AM	78.0	ASD	average 24-hr levels	GCMS	2000	Ambient, urban	Santiago de Chile City	del Rosario Sienra et al, 2006	-
600	AM	1200.0	ASD	average 24-hr levels	GCMS	2000	Ambient, urban	Santiago de Chile City	del Rosario Sienra et al, 2006	-
22	AM	13.0	ASD	average 24-hr levels	GCMS	2000	Ambient, urban	Santiago de Chile City	del Rosario Sienra et al, 2006	-
70	AM	120.0	ASD	average 24-hr levels	GCMS	2000	Ambient, urban	Santiago de Chile City	del Rosario Sienra et al, 2006	-
282	AM	145-492	range	10	HPLC with fluorescence detection	2005	Ambient	Strasbourg, France	Delhomme et al, 2007	-

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
54	AM	22-97	range	10	HPLC with fluorescence detection	2006	Ambient	Strasbourg, France	Delhomme et al, 2007	-
90	AM	-		25	GCMS (negative ion chemical ionization MS)	1995-1996	Ambient, Urban	Birmingham, UK	Dimashki et al, 2000	-
120	AM	60-180	range	6	HPLC with fluorescence detection	1994	Ambient, Urban	Damascus, Syria	Dimashki et al, 2000	-
193	AM	35-387	range	13	GCMS (negative ion chemical ionization MS)	1995	Ambient, Urban	Damascus, Syria	Dimashki et al, 2000	-
560	AM	440-690	range	8	GCMS (negative ion chemical ionization MS)	1995-1996	Ambient, Urban	Birmingham, UK	Dimashki et al, 2000	-
1900	AM	1800	ASD	4	GCMS (negative ion chemical ionization MS)	2010	Indoors, Residential	Zhuanghu, Hebei, China	Ding et al, 2012	-
900	AM	740	ASD	4	GCMS (negative ion chemical ionization MS)	2010	Ambient, rural	Zhuanghu, Hebei, China	Ding et al, 2012	-
770	AM	670	ASD	4	GCMS (negative ion chemical ionization MS)	2010	Indoors, Residential	Zhuanghu, Hebei, China	Ding et al, 2012	-
40	AM	8.0	ASD	3	GCMS (negative ion chemical ionization MS)	2010	Indoors, Residential	Zhuanghu, Hebei, China	Ding et al, 2012	-
0	AM	0	ASD	3	GCMS (negative ion chemical ionization MS)	2010	Ambient, rural	Zhuanghu, Hebei, China	Ding et al, 2012	-
0	AM	0	ASD	3	GCMS (negative ion chemical ionization MS)	2010	Indoors, Residential	Zhuanghu, Hebei, China	Ding et al, 2012	-
280	AM	390	ASD	4	GCMS (negative ion chemical ionization MS)	2010	Indoors, Residential	Zhuanghu, Hebei, China	Ding et al, 2012	-
1100	AM	1500	ASD	4	GCMS (negative ion chemical ionization MS)	2010	Indoors, Residential	Zhuanghu, Hebei, China	Ding et al, 2012	-
34	AM	25.0	ASD	3	GCMS (negative ion chemical ionization MS)	2010	Indoors, Residential	Zhuanghu, Hebei, China	Ding et al, 2012	-

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
0	AM	0	ASD	3	GCMS (negative ion chemical ionization MS)	2010	Indoors, Residential	Zhuanghu, Hebei, China	Ding et al, 2012	-
30	AM	15	95% CI	20	GCMS	1996	-	Copenhagen, Denmark	Feilberg et al, 2001	-
127	AM	44	95% CI	14	GCMS	1998-1999	-	Copenhagen, Denmark	Feilberg et al, 2001	-
2	AM	2.0	ASD	67	-	2010	Ambient, roadway	San Ysidro, CA, USA	Galaviz et al, 2014	-
1.7	AM	2.3	ASD	56	-	2010	Ambient, roadway	San Ysidro, CA, USA	Galaviz et al, 2014	-
0.22	AM	0.2	ASD	15	-	2010	Ambient, roadway	San Ysidro, CA, USA	Galaviz et al, 2014	-
173.075	AM	69.2	ASD	12	HPLC	1989-1992	Ambient, urban	Kanazawa, Japan	Hayakawa, 1995	-
8.1	AM	3.9	ASD	60	HPLC	2005-2006	Ambient, Urban	Ho Chi Minh City, Vietnam	Hien et al, 2007	-
9.1	AM	3.9	ASD	46	HPLC	2005-2006	Ambient, Urban	Ho Chi Minh City, Vietnam	Hien et al, 2007	-
73	AM	40.0	ASD	48	HPLC	2005-2006	Ambient, Urban	Ho Chi Minh City, Vietnam	Hien et al, 2007	-
59.2	AM	8.8	ASD	3	HPLC	1995	Ambient, urban	Kanazawa, Japan	Kakimoto et al, 2000	-
38.3	AM	14.6	ASD	3	HPLC	1995	Ambient, urban	Kanazawa, Japan	Kakimoto et al, 2000	-
26.7	AM	13.0	ASD	3	HPLC	1995	Ambient, urban	Kanazawa, Japan	Kakimoto et al, 2000	-
11.3	AM	4.1	ASD	3	HPLC	1995	Ambient, urban	Kanazawa, Japan	Kakimoto et al, 2000	-
413	AM	230.0	ASD	3	HPLC	1995	Ambient, urban	Sapporo, Japan	Kakimoto et al, 2000	-
197	AM	151.0	ASD	3	HPLC	1995	Ambient, urban	Sapporo, Japan	Kakimoto et al, 2000	-
206	AM	76.5	ASD	3	HPLC	1995	Ambient, urban	Sapporo, Japan	Kakimoto et al, 2000	-
149	AM	64.8	ASD	3	HPLC	1995	Ambient, urban	Sapporo, Japan	Kakimoto et al, 2000	-
163	AM	60.4	ASD	3	HPLC	1995	Ambient, urban	Tokyo, Japan	Kakimoto et al, 2000	-
120	AM	32.0	ASD	3	HPLC	1995	Ambient, urban	Tokyo, Japan	Kakimoto et al, 2000	-

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
130	AM	25.8	ASD	3	HPLC	1995	Ambient, urban	Tokyo, Japan	Kakimoto et al, 2000	-
75.3	AM	21.6	ASD	3	HPLC	1995	Ambient, urban	Tokyo, Japan	Kakimoto et al, 2000	-
14	AM	7.9	ASD	5	HPLC	1997	Ambient, Urban	Kitakysushu, Japan	Kakimoto et al, 2002	-
5.7	AM	2.7	ASD	5	HPLC	1997	Ambient, Urban	Kitakysushu, Japan	Kakimoto et al, 2002	-
270	AM	110.0	ASD	5	HPLC	1997	Ambient, Urban	Sapporo, Japan	Kakimoto et al, 2002	-
130	AM	57.0	ASD	5	HPLC	1997	Ambient, Urban	Sapporo, Japan	Kakimoto et al, 2002	-
170	AM	84.0	ASD	5	HPLC	1997	Ambient, Urban	Tokyo, Japan	Kakimoto et al, 2002	-
45	AM	19.0	ASD	5	HPLC	1997	Ambient, Urban	Tokyo, Japan	Kakimoto et al, 2002	-
26.2	AM	10.6-48.7	range	9	HPLC	2003	Ambient, Urban	Osaka, Japan	Kameda et al, 2004	-
58.1	AM	12.4-89.8	range	10	HPLC	2002	Ambient, Urban	Osaka, Japan	Kameda et al, 2004	-
5.7	AM	1.5-9.9	range	10	HPLC	2003	Ambient, Urban	Osaka, Japan	Kameda et al, 2004	-
8.7	AM	5.2-20.8	range	10	HPLC	2002	Ambient, Urban	Osaka, Japan	Kameda et al, 2004	-
880	AM	50.0	ASD	-	HPLC	2010-2011	Ambient, suburban	Dongguan, China	Lan et al, 2014	-
11900	AM	890.0	ASD	-	HPLC	2010-2011	Ambient, Urban	Dongguan, China	Lan et al, 2014	-
5210	AM	340.0	ASD	-	HPLC	2010-2011	Ambient, Urban	Dongguan, China	Lan et al, 2014	-
650	AM	30.0	ASD	-	HPLC	2010-2011	Ambient, suburban	Dongguan, China	Lan et al, 2014	-
410	AM	50.0	ASD	-	HPLC	2010-2011	Ambient, Urban	Dongguan, China	Lan et al, 2014	-
770	AM	20.0	ASD	-	HPLC	2010-2011	Ambient, Urban	Dongguan, China	Lan et al, 2014	-
300	AM	70.0	ASD	-	HPLC	2010-2011	Ambient, suburban	Dongguan, China	Lan et al, 2014	-
1750	AM	90.0	ASD	-	HPLC	2010-2011	Ambient, Urban	Dongguan, China	Lan et al, 2014	-
1980	AM	150.0	ASD	-	HPLC	2010-2011	Ambient, Urban	Dongguan, China	Lan et al, 2014	-
80.8	AM	26.0	ASD	23	HPLC/MS/MS	2007	Inside vehicle	Shenyang, China	Miller-Schulze et al, 2010	-
97.1	AM	31.1	ASD	24	HPLC/MS/MS	2007	Outside vehicle	Shenyang, China	Miller-Schulze et al, 2010	-
22.4	AM	11.4	ASD	24	HPLC/MS/MS	2007	Indoors, Residential	Shenyang, China	Miller-Schulze et al, 2010	-

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
11.2	AM	6.0	ASD	8	HPLC/MS/MS	2007	Ambient, Urban	Shenyang, China	Miller-Schulze et al, 2010	-
227.5	AM	101.4	ASD	8	HPLC	1993-1994	Ambient, Urban	Kanazawa, Japan	Murahashi et al, 1995	-
32.1	AM	-	-	5	HPLC	1994	Ambient, Urban	Kanazawa, Japan	Murahashi et al, 1997	-
1.7	AM	-	-	5	HPLC	1994	Ambient, Urban	Kanazawa, Japan	Murahashi et al, 1997	-
32.1	AM	9.9	ASD	12	HPLC	1994-1995	Ambient, Urban	Kanazawa, Japan	Murahashi et al, 1999	-
3.7	AM	2.0	ASD	12	HPLC	1994-1995	Ambient, Urban	Kanazawa, Japan	Murahashi et al, 1999	-
6.9	AM	2.9	ASD	14	HPLC with fluorescence detection	2010	Ambient, Urban	Cairo, Egypt	Nassar et al, 2011	-
7.8	AM	3.2	ASD	14	HPLC with fluorescence detection	2010	Ambient, Urban	Cairo, Egypt	Nassar et al, 2011	-
7.8	AM	3.1	ASD	14	HPLC with fluorescence detection	2010	Ambient, Urban	Cairo, Egypt	Nassar et al, 2011	-
10.5	AM	6.2	ASD	14	HPLC with fluorescence detection	2010	Ambient, Urban	Cairo, Egypt	Nassar et al, 2011	-
80.3	AM	61.6	ASD	6	GCMS	2010	Ambient, Urban	Paris, France	Ringuet et al, 2012	-
66	AM	39.0	ASD	7	GCMS	2010	Ambient, Urban	Paris, France	Ringuet et al, 2012	-
2.9	AM	3.1	ASD	9	GCMS	2009	Ambient, Suburban	Paris, France	Ringuet et al, 2012	-
1.6	AM	1.2	ASD	7	GCMS	2009	Ambient, Suburban	Paris, France	Ringuet et al, 2012	-
28	GM	3.7	GSD	8	GCMS/MS	1999	Mine Surface	Czech Republic	Scheepers et al, 2003	-
78	GM	3.3	GSD	8	GCMS/MS	1999	Mine Underground	Czech Republic	Scheepers et al, 2003	-
70	GM	2.1	GSD	6	GCMS/MS	1999	Mine Surface	Estonia	Scheepers et al, 2003	-
216	GM	2.0	GSD	5	GCMS/MS	1999	Mine Underground	Estonia	Scheepers et al, 2003	-
3.7	GM	8.8	GSD	18	GCMS/MS	1999	Mine Surface	Estonia	Scheepers et al, 2003	-

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
1049	GM	1.7	GSD	20	GCMS/MS	1999	Mine Underground	Estonia	Scheepers et al, 2003	-
110	GM	2.8	GSD	10	GCMS/MS	1999	Worker Breathing Zone, Mine Surface	Czech Republic	Scheepers et al, 2003	-
197	GM	1.5	GSD	9	GCMS/MS	1999	Worker Breathing Zone, Mine Underground	Czech Republic	Scheepers et al, 2003	-
8	GM	2.3	GSD	9	GCMS/MS	1999	Worker Breathing Zone, Mine Surface	Estonia	Scheepers et al, 2003	-
2483	GM	3.4	GSD	10	GCMS/MS	1999	Worker Breathing Zone, Mine Underground	Estonia	Scheepers et al, 2003	-
85	GM	2.7	GSD	42	GCMS/MS	1999	Worker Breathing Zone, Mine Surface	Estonia	Scheepers et al, 2003	-
637	GM	3.3	GSD	50	GCMS/MS	1999	Worker Breathing Zone, Mine Underground	Estonia	Scheepers et al, 2003	-
71	GM	7.6	GSD	9	GCMS/MS	1999	Worker Breathing Zone, Mine Surface	Czech Republic	Scheepers et al, 2003	-
209	GM	3.6	GSD	9	GCMS/MS	1999	Worker Breathing Zone, Mine Underground	Czech Republic	Scheepers et al, 2003	-
45	GM	3.8	GSD	9	GCMS/MS	1999	Worker Breathing Zone, Mine Surface	Estonia	Scheepers et al, 2003	-
984	GM	2.7	GSD	10	GCMS/MS	1999	Worker Breathing Zone, Mine Underground	Estonia	Scheepers et al, 2003	-
28.9	AM	24.5	ASD	9	HPLC with fluorescence detection	2001	Ambient, Urban	Shenyang, China	Tang et al, 2005	-
16.6	AM	19.0	ASD	14	HPLC with fluorescence detection	1999	Ambient, Urban	Vladivostok, Russia	Tang et al, 2005	-
25.2	AM	27.7	ASD	14	HPLC with fluorescence detection	1999	Ambient, Urban	Kanazawa, Japan	Tang et al, 2005	-
126.1	AM	35.9	ASD	20	HPLC with fluorescence detection	1997	Ambient, Urban	Kitakyushu, Japan	Tang et al, 2005	-
44.5	AM	12.1	ASD	20	HPLC with fluorescence detection	1997	Ambient, Urban	Tokyo, Japan	Tang et al, 2005	-
5.7	AM	2.2	ASD	20	HPLC with fluorescence detection	1997	Ambient, Urban	Sapporo, Japan	Tang et al, 2005	-

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
178.8	AM	19.0	ASD	9	HPLC with fluorescence detection	2001	Ambient, Urban	Shenyang, China	Tang et al, 2005	-
95.2	AM	76.6	ASD	14	HPLC with fluorescence detection	1999	Ambient, Urban	Vladivostok, Russia	Tang et al, 2005	-
173.8	AM	38.6	ASD	4	HPLC with fluorescence detection	2002	Ambient, Urban	Seoul, South Korea	Tang et al, 2005	-
56.4	AM	56.1	ASD	14	HPLC with fluorescence detection	1999	Ambient, Urban	Kanazawa, Japan	Tang et al, 2005	-
272.0	AM	110.0	ASD	20	HPLC with fluorescence detection	1997	Ambient, Urban	Kitakyushu, Japan	Tang et al, 2005	-
168.1	AM	61.8	ASD	20	HPLC with fluorescence detection	1997	Ambient, Urban	Tokyo, Japan	Tang et al, 2005	-
13.6	AM	8.7	ASD	20	HPLC with fluorescence detection	1997	Ambient, Urban	Sapporo, Japan	Tang et al, 2005	-
10	AM	2.7	ASD	7	HPLC with chemiluminescence detection	2010	Ambient, Urban	Chiang Mai, Thailand	Thanyarat et al, 2014	-
4.2	AM	1.4	ASD	7	HPLC with chemiluminescence detection	2010	Ambient, Urban	Chiang Mai, Thailand	Chuesaard et al, 2014	-
3.3	AM	1.7	ASD	7	HPLC with chemiluminescence detection	2010	Ambient, Urban	Chiang Mai, Thailand	Chuesaard et al, 2014	-
2.6	AM	1.4	ASD	7	HPLC with chemiluminescence detection	2010	Ambient, Urban	Chiang Mai, Thailand	Chuesaard et al, 2014	-
2.4	AM	0.8	ASD	7	HPLC with chemiluminescence detection	2010	Ambient, Urban	Chiang Mai, Thailand	Chuesaard et al, 2014	-
4.7	AM	2.1	ASD	7	HPLC with chemiluminescence detection	2010	Ambient, Urban	Chiang Mai, Thailand	Chuesaard et al, 2014	-
7.2	AM	4.5	ASD	7	HPLC with fluorescence detection	2010	Ambient, Urban	Hanoi, Vietnam	Thuy et al, 2012	-

1-NP Concentration (pg/m³)										
Measure of Central Tendency		Measure of Variability		N	Analysis Method	Year	Setting	Location	Obtained From	Original Source
42.0	AM	16.0	ASD	7	HPLC with fluorescence detection	2011	Ambient, Urban	Hanoi, Vietnam	Thuy et al, 2012	-
120.7	AM	21.3	ASD	7	HPLC with fluorescence detection	2010	Ambient, Urban	Hanoi, Vietnam	Thuy et al, 2012	-
43.3	AM	29.9	ASD	7	HPLC with fluorescence detection	2011	Ambient, Urban	Hanoi, Vietnam	Thuy et al, 2012	-
0.69	AM	0.21-4.10	range	136	HPLC	1997-1998	Ambient, Urban	Nagasaki, Japan	Wada et al, 2001	-
45	AM	11.0-81	range	4	GCMS	2010	Ambient, Urban	Shanghai, China	Wang et al, 2014	-
12	AM	0.3-19	range	8	GCMS	2010	Ambient, Urban	Shanghai, China	Wang et al, 2014	-
5.3	AM	3.3-9.1	range	4	GCMS	2010	Ambient, Urban	Shanghai, China	Wang et al, 2014	-
6.4	AM	3.4-12	range	8	GCMS	2010	Ambient, Urban	Shanghai, China	Wang et al, 2014	-
50	AM	19-88	range	4	GCMS	2010	Ambient, Urban	Shanghai, China	Wang et al, 2014	-
15	AM	3.4-25	range	8	GCMS	2010	Ambient, Urban	Shanghai, China	Wang et al, 2014	-
4.6	AM	1.5-9.2	range	4	GCMS	2010	Ambient, Urban	Shanghai, China	Wang et al, 2014	-
15	AM	2.9-26	range	8	GCMS	2010	Ambient, Urban	Shanghai, China	Wang et al, 2014	-
8.3	AM	0.7	ASD	46	GCMS	2008	Ambient, Urban	Beijing, China	Wang et al, 2011 SI; Table SI.4	-
10.2	AM	1.5	ASD	17	GCMS	2008	Ambient, Urban	Beijing, China	Wang et al, 2011 SI; Table SI.4	-
7.7	AM	5.1-10.2	range	25	GCMS	2008	Ambient, Urban	Xiamen, China	Wu et al, 2012	-
8.8	AM	5.6-16.0	range	25	GCMS	2008	Ambient, Urban	Xiamen, China	Wu et al, 2012	-
15.7	AM	7.7-32.9	range	25	GCMS	2008	Ambient, Urban	Xiamen, China	Wu et al, 2012	-
41.6	AM	6.7-230	range	25	GCMS	2008	Ambient, Urban	Xiamen, China	Wu et al, 2012	-
985	AM	630-1312	range	6	GCMS	2008	Ambient, Urban	Xiamen, China	Wu et al, 2012	-

Defense of 1NP as a potentially specific, reliable marker to compare against TC/EC

Substantial evidence exists indicating limitations in the current method of exposure monitoring and compliance for the MSHA DPM rule that relies on measures of EC and TC. Additionally, a wealth of studies of 1-NP in air samples impacted by diesel exhaust and traffic emissions have provided well-developed methods to detect 1-NP in diesel emissions. 1-NP has been reported to lack significant interference from other common combustion sources, unlike EC and TC. Additionally, this study has determined that 1-NP can be feasibly extracted from MSHA-compliant SKC DPM impactor quartz filter elements, equipment already easily obtained and in use by mine personnel for compliance determination.

Factors that Affect Air Concentration of 1-NP and EC/TC in DE

DE Emissions Control Technology Overview

The composition of diesel exhaust varies depending on a number of factors including engine type, fuel type, and engine operating conditions, as well as modifying factors such as use of a diesel particulate filter (DPF) or a diesel oxidation catalyst (DOC). Variations in these conditions results in the production or emission of different quantities of EC, OC, and TC, as well as 1-NP, resulting in different ratios of EC to OC and of 1-NP to EC or TC [Bugarski, 2012].

Engine Types and Operating Conditions

In general, a lower oxygen content, or air-to-fuel ratio, increases PM formation but decreases NOx formation [EPA, 2001]. As stated previously, 1-NP is produced due to an excess air supply as well as higher burning temperatures [IARC, 2013]. Additionally, partitioning between particle- and gas-bound PAHs is a function of engine operating conditions [Bugarski, 2012]. Various engine types and operating conditions can affect the fuel-air ratio and temperature of combustion and/or exhaust that determine 1-NP/TC or 1-NP/EC ratios.

The fuel-air ratio and chemistry of the combustion process are affected by fuel-air mixing. Mixing is reduced under increased engine speed and load, resulting in a higher EC fraction and lower OC fraction in DPM. Mixing is increased by advanced injection timing technology of newer engine types, and the EC emissions are thus reduced. However, newer engines or four-stroke engines (as opposed to two-stroke engines) burn fuel more efficiently and use less oil so the OC fraction is lower [Bugarski, 2012]. Newer technology engines have also been reported to generally reduce nitro-PAH emissions [IARC, 2013].

Engine and exhaust temperatures also play a role: light-load conditions, and thus lower operating temperatures, result in higher quantities of unburned fuel and lubricating oil, and the OC fraction is higher [Bugarski, 2012]. 1-NP formation is suggested to be linked to nitrogen dioxide formation in the combustion chamber, which is abundantly formed at higher combustion temperatures. However, higher speeds have been linked with lower PAH formation, so 1-NP formation is difficult to predict [IARC, 2013].

Fuel types, Particularly Biodiesel

Fuel types also have an impact on the composition of DE. Fuels with higher cetane number and lower aromatic content generally reduce the OC content of exhaust, thus reducing the OC/EC ratio and the total TC compared to standard fuel. Due to the role of unburned fuel in PAH production, PAH content of the fuel type affects the PAH, and thus 1-NP, emissions. Fuels with lower PAH content produce lower particulate and semivolatile phase PAHs [Bugarski, 2012].

Biodiesel is an emerging fuel type that is prized for being renewable, nontoxic, and biodegradable, as well as less dependent on imports than petroleum diesel. Biodiesel is typically used in blends with petroleum diesel, anywhere from 5% to 100% biodiesel; though the higher blends are not feasible for use with currently-used equipment, and not recommended by the US Department of Energy. 5-20% biodiesel blends are typical, and can be used in modern equipment with minor modifications. [Bugarski, 2012].

Generally, biodiesel produces lower EC and higher OC emissions [Bugarski, 2012]. As the percent biodiesel increases, the composition of DE changes: NOx content gradually increases to up to 10% more than is produced by petroleum diesel, and particulate matter, CO, and hydrocarbons decrease rapidly to around 50% or less than is produced by petroleum diesel [EPA, 2002]. PAH and nitro-PAH content is generally lower in biodiesel exhaust than in petroleum diesel due to the lower PAH content of biodiesel [Bugarski, 2012].

Diesel Particulate Filters (DPFs) and Diesel Oxidation Catalysts (DOCs)

Exhaust after treatment technologies significantly influence the ratios of DE components. Diesel particulate filters (DPFs) are filter systems fitted to existing equipment designed to capture diesel particulate matter (DPM), and are an important and widely-used control strategy for adhering to US DPM emission standards. DPFs are typically composed of a porous microstructure filtration medium, and an additional structure for regenerating the filter by oxidizing the captured DPM (usually using heat). Engines are often also fitted with a diesel oxidation catalyst (DOC), which is designed to control

carbon monoxide (CO) and hydrocarbon (HC) emissions. DOCs consist of a substrate and a catalyst (usually formulated by noble metals like platinum or palladium) that converts CO and HC to water vapor and CO_2 [Bugarski, 2012].

DPFs by definition reduce the TC content of DE, and are shown to reduce the EC fraction of DE more efficiently than the OC fraction. DOCs, on the other hand, effectively reduce the OC fractions and HC content of DE. Catalyzed DPFs more efficiently remove the OC fraction than noncatalyzed DPFs [Bugarski, 2012]. It has been suggested that DPF systems increase emissions of NO_2 and nitro-PAHs. Though DOCs appear to reduce concentrations of PAHs associated with the soluble organic fraction and OC, some types of DOCs may increase secondary NO_2 emissions [Bugarski, 2012]. The addition of a DOC appeared to increase 1-NP content of DE in one study [Sharp et al, 2000], but in two other studies the use of a DOC or DPF+DOC system significantly reduced 1-NP content of DE [Liu 2008, 2010].

Use of Job Titles & Tasks Data to Predict Exposures in Underground Mining

The variety of job tasks and locations within a metal mine results in discernable differences in diesel exhaust exposure. A number of studies have quantified diesel exposure for various job titles such as underground production workers, underground maintenance workers, and unspecified mining [Pronk, 2009]. Additionally, the impact of location, either above ground or below ground, has been assessed and found to have an impact upon DE exposure [Silverman, 2012]. For the 1992 NCI- and NIOSH-initiated Diesel Exhaust in Miners Study (DEMS), surveys were conducted to assess past DE exposures for miners. Information was gathered about each subject's mining facility, department, job, and years of work. The exposure assessment used a hierarchical grouping strategy to assign exposure levels to underground (or aboveground) jobs, based on job titles, the amount of time spent in different underground areas and on other similar air exposures, such as CO [Stewart, 2010]. However, this study relied on historical job title, task, and location data and scattered data on air concentrations of DE and its components. An assessment of current air exposures compared with current job description survey data can better determine the relationship between survey field and air concentrations of DE components.

Description of Specific Study Site & Control Technologies Implemented

The setting of this study is a large underground metal mine employing about 1,300 workers. During the last decade this mine has developed control strategies for DPM emissions reduction in order to comply with the new MSHA rule for M/NM mines [MSHA, 2001b]. These control strategies include diesel particulate filters and the use of biodiesel. At the time of this study, all underground diesel-powered vehicles were running on 70% biodiesel blend.

Specific Aims

There are few 1-NP studies in the literature that take place in a well-confined industrial setting, where DE emissions are high and 1-NP, EC, and TC levels are high enough to be easily detectable. This study seeks to collect and analyze data from such a setting and provide evidence of an association between 1-NP, EC, and TC in diesel-powered vehicle emissions. Additionally, the overall study contains a component that examines 1-NP metabolites in urine as a potential biological marker of DE exposure. 1-NP air measurements are examined alongside mine, worker, and work task information obtained from surveys, and a predictive model for 1-NP air concentration is developed. Specific aims of this project are as follows:

I. Aim 1: Determine the Association Between 1-NP and EC/OC/TC
II. Aim 2: Use Survey Information to identify potential modifiers of the 1-NP-EC Association
III. Aim 3: Develop a 1-NP Predictive Model using Survey Information Predictors

These aims will help determine whether 1-NP can be considered a specific and reliable surrogate measure for DE exposure. Additionally, work task data may be found to provide useful insight into individual DE exposure, and can thus provide an additional way to approach individual exposure assessment and intervention.

Methods

Study Design

This is an observational study examining current occupational exposures to diesel exhaust by underground mine employees. This study also has elements of a longitudinal cohort study design: a cohort of twenty employees was followed for four work weeks during March, June, August and October in 2014. The primary factors evaluated are measured 1-NP, EC, OC, TC personal air concentrations, as well as Questionnaire and Survey data including information about job titles, tasks and perceived exposure to DE.

Study Setting & Subjects

Data was collected at a large underground metal mine employing about 1,300 workers. A group of twenty mine workers of various job titles and predicted exposure categories (high, medium, or low exposure) were recruited and followed. 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. Subject characteristics are displayed in Table 2 below.

Table 2: Subject Characteristics

Study ID	Age at Start of Study (yrs)	Job Title	Yrs at Study Mine	Yrs in Mining	Exposure Category		
					High	Medium	Low
01	48	Muck Hauler	2.20	2.20		X	
02	45	Sandplant Operator	3.33	13.00		X	
03	50	Operator	9.25	9.25	X		
04	28	Miner I	5.80	5.80	X		
05	34	Miner III	2.50	2.50			X
06	30	Miner I	6.00	6.00	X		
07	42	Geologist	8.70	8.70		X	
08	37	Miner I	9.20	9.20	X		
09	58	Mechanic	14.10	34.00			X
10	55	Diamond Driller	14.00	14.00			X
11	45	Electrician	0.75	0.75		X	
12	35	Miner I	9.60	9.60	X		
13	40	Miner I	20.75	20.75	X		
14	36	Stationary Mechanic	2.00	2.00			X
15	27	Operator	1.50	1.50		X	
16	57	Raise Bore I - Driller	25.90	25.90	X		
17	35	Geologist	9.60	9.60			X
18	29	Beat Mechanic	2.00	8.00	X		
19	53	Surface Mill Operator	13.80	33.00			X
20	42	Miner	11.00	11.00	X		

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 shifts of workers at the mine, and sample collection only took place when B crew were on the day shift. To accommodate the 1-NP urinary metabolite analysis portion of the overall study, the initial recruitment excluded potential subjects who have physician-diagnosed kidney, liver, or bladder disorders, factors that would interfere with the metabolism and excretion of 1-NP and its metabolites. Volunteers were asked about tobacco use, and current and past smoking and tobacco-chewing status were recorded for each. During the study two subjects dropped out after the first week of monitoring because the moved to one of the other work

crews. On several other occasions individual study participants were not available for sampling, for example due to vacation of family/medical leave. A schedule of subject participation and attendance is provided in Table 3 below.

Data Collection

Data collection for this study took place in March, June. August and October of 2014. Data collection consisted of full shift personal sampling for four days per monitoring period. Urine samples (pre and post-shift) were collected from each participant on each day. Due to equipment limitations, personal air samples were collected on the first ten subjects (typically subject id numbers one through ten) on days one and three, and the other ten subjects (typically subject id numbers eleven through twenty) were monitored on days two and four. As shown in Table 2, both sets of ten subjects were approximately balanced with respect to *a priori* exposure category. Thus, 1-NP, EC, OC, TC, and respirable PM mass air exposure data was not collected for each day for which 1-NP metabolites urine data was collected. All subjects also completed a daily work-activity questionnaire at the end of their workshift each day. Personal monitoring took place during each subject's full shift, which typically exceeds 8 hours. Air concentration values for 1-NP, EC, OC, TC, and respirable PM mass represent values integrated over a full day's shift.

Table 3: Air Sampling & Subject Attendance

Air & Survey Monitoring Summary by Subject																	
Subject ID	Days Monitored, 2014																Total # Shifts Personal Air Monitored
	Campaign 1				Campaign 2				Campaign 3				Campaign 4				
	7-Mar	8-Mar	9-Mar	10-Mar	11-Jun	12-Jun	13-Jun	14-Jun	14-Aug	15-Aug	16-Aug	17-Aug	1-Oct	2-Oct	3-Oct	4-Oct	
1	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	8
2	A+S	S	A+S	S	A+S		A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	8
3	A+S	S	A+S	S	A+S	S	A+S	S			A+S	S	A+S	S	A+S	S	7
4	A+S	S	A+S	S	A+S	S	A+S		A+S	S	A+S	S	A+S	S	A+S	S	8
5	A+S	S	A+S	S													2
6	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	8
7	A+S	A+S*	S	S	A+S	S	A+S	S					A+S	S	A+S	S	5
8	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	8
9	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	8
10					A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	6
11		A+S	S	A+S	S	A+S		A+S	S	A+S		A+S	S	A+S	S	A+S	8
12	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	8
13		A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	8
14	S	A+S	S	A+S	S	A+S	S			A+S	S	A+S	S	A+S			6
15	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	8
16	S	A+S	S	A+S													2
17		S	A+S*	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	7
18	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	8
19	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S	S	A+S					6
20	S	A+S	S	A+S	S	A+S			S	A+S	S	A+S	A+S*	A+S			6
A= air data collected, S=survey collected; Air Data was scheduled to be collected on days 1&3 for subjects 1-10, days 2&4 for subjects 11-20. Grey boxes indicate absence due to cohort dropouts, sick days, FMLA, etc.													Average # Shifts Personal Air Monitored				6.75
													SD				1.9
**Air monitoring for some subjects was switched to non-scheduled days during certain campaigns (see pump calibration and gravimetric analysis field logs for details)*																	

Personal Air Sampling Devices

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. The workers also wore a second sampling train consisting of a 37mm PFTE filter housed within a polystyrene cassette downstream of a GS-3 cyclone.

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. 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. A second back-up filter can be used as a dynamic blank for correction of absorbed organic carbon [SKC 2014a]. About 80-90% of DPM is less than 1µm by mass, so these samplers meet NIOSH Method 5040 specifications for DPM collection and analysis through quantification of EC, OC, and TC [HEI, 1995; Noll, 2005]. Additionally, a supplementary analysis conducted as part of the present study examined the size distribution of 1-NP in area samples collected in the mine using a Sioutas cascade impactor. This study determined that overall greater than 90% of the 1-NP is found primarily in particles <0.5 µm. 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 incorporated). At the end of each subject's workshift flow through the sampling train was re-rested using the drycal calibrator 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. If pre and post flows differed by greater than 10%, a QC flag was applied to the resulting air concentrations.

Air was drawn through the GS3 cyclone sampling trains using SKC AirChek and PXCR personal sampling pumps at a nominal flow rate of 2.75 liters/minute to provide a nominal respirable cutpoint (4 µm) [SKC 2014b]. These samplers are also expected to capture the majority of 1-NP-containing particles present in the mine. Flow through the sampling trains was pre and post calibrated as described above.

It was found upon inspection of GS-3 Teflon filter samples in the lab that for many samples (about 50%), cassette leakage had occurred. In the cassettes, airflow had diverted around the filter and deposited particles onto the filter backing. Thus, for these filters a full shift sample of particulate and adsorbed 1-NP could not be relied upon. It was determined that for the remainder of this project, all samples from Teflon (GS-3) filter samples would be disqualified, and only 1-NP measurements obtained from quartz

(GS-1) filter samples would be considered and analyzed. Table 4-Table 6 below contain information for missing or disqualified quartz (GS-1) filter samples from 1-NP analysis.

Table 4: Missing Filters

Missing Quartz Filter 1-NP or EC Data from Field (no filter)			
Reason for missing data	**Count missing**	**Count total planned**	**% missing of total planned**
Cohort Dropout	22	160	14%
Equipment Error	2	160	1%
All	24	160	15%

Table 5: Not-Analyzed Filters

Missing Quartz Filter 1-NP Data from Lab (filters not analyzed)			
Reason for missing data	**Count excluded from analysis**	**Count total available for analysis**	**% excluded of total available**
Excluded due to broken-open cassettes	6	136	4%
Excluded due to pump flow rate post-pre calibration difference >10%	10	136	7%
Excluded due to note from pump calibration field log	11	136	8%
All	27	136	20%

Table 6: Filters Excluded After Analysis

1-NP Data Excluded from Statistical Analysis			
Reason for Exclusion	**Count Excluded**	**Count Total Available**	**% Excluded of total available**
Interfering peaks on MS chromatogram	1	109	0.92%
% Difference between pump flow rates recorded pre- and post-sampling >95th percentile of all flow rate % differences (p95=26%)	1	109	0.92%
Pump operation time <10th percentile of all pump operation times (p10=359 min)	2	109	1.83%
Subjects #5 and #16 dropped out after Campaign #1 and only have 1 valid air sample each; non-representative	2	109	1.83%
All	6	109	5.50%

Intake Questionnaire & Surveys

At the commencement of the project an intake questionnaire was administered 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. Mine management supplemented the demographic information with job titles for each worker, and assigned each worker a priori to one of three exposure groups. Exposure group is a designation of low, medium, or high DE exposure per worker as predicted by mine health and safety staff, based on their knowledge of working conditions associated with each job title.

Study subjects were asked to fill out work-activity surveys at the conclusion of each work shift. The survey consists of three sections that were completed daily, and a fourth section that was completed only on the first day of the work week. An example of a completed survey can be found in Appendix I; a description of the template is as follows: The first section consists of a chart with specific DE-related activities as rows and hours of the workday as columns; time spent doing activities or in areas associated with DE emissions is indicated by filling in the corresponding boxes. Part two of the survey consists of a list of job activities with check boxes and indications of percent time spent on each activity. The activities are as follows: Ore channeling, Jack leg drill operation, Load-Haul-Dump operation, Cage Tending, Diesel Engine Repair, Above-ground office work, and Other. An additional question in this section asks about the percentage of time spent wearing a respirator when exposed to DE emissions. Part three of the survey consists of three yes or no questions asking about cigarette smoking, use of chewing tobacco, or any off-the-job activities involving DE emissions conducted during the previous 24 hour period. Part four of the survey (to be filled out on the first day of the work week) is a repeat of part three, but refers to the period 24-48 hours prior to time of completing the questionnaire.

Data Analysis

Filter extractions & LCMS/MS procedure

EC, OC, and TC sample concentrations were determined from the DPM quartz filters by ALS Environmental using the NIOSH 5040 method. EC and OC concentrations were reported in units of µg/sample and added together to equal TC. Averaged field blank OC mass (17.8 µg/sample) was subtracted from each reported sample OC mass, and for QC purposes the LOD for OC was set as three times the average corrected blank SD (20.2 µg/sample). The outside lab provided a reporting limit of 1.7 µg/sample for EC, and censored all EC values less than 1.7 µg/sample. Treatment of reporting limits and LODs is further discussed in the Statistical Analysis section below. EC, OC and TC values were converted

to air concentrations (μg/m^3) 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 determined as described by Miller-Schulze [Miller-Schulze, 2007]. 1-NP determination including filter extraction and LC/MS/MS analysis occurred in five analytical batches. Details concerning the filters selected for analysis, extraction order, and QC filter inclusions for the final two batches can be found in Appendix II. Each analytical batch included two or more of each of the following quality control filters: laboratory blanks, filters spiked with a deuterated internal standard 1-dNP ("deuterated" filters), and filters spiked with 1-dNP and 1-NP ("fortified" filters). The last two batches included both quartz DPM filter elements and PTFE (Teflon) filters, so QC samples were prepared using both filter types. Additionally, control samples were included that contained 1-dNP and 1-NP but no filter element, and field blanks were included when available. Extracts from all batches were quantified using two-dimensional high performance liquid chromatography tandem mass spectrometry (2D-HPLC-MS/MS). The full SOP for this procedure can be found in Appendix III. The instrument LOD for 1-NP extract concentration was calculated by taking the average 1-NP extract concentration from deuterated and field blank filters plus three times the SD for the 1-NP extract concentrations from deuterated and field blank filters. Instrumental LODs (Units of fg/sample) varied by sample batch, and were converted to concentration-equivalent LODs (units of fg/m^3) by dividing by the sampled air volume for each sample. QC summary tables for all five batches can be found in Appendix IV, and treatment of values below the LOD are discussed in the Statistical Analysis section below.

Air data used in this study consists of a variety of forms, all from different sources, laboratories, and batches. The procedure used to compile data from numerous sources is described in Appendix V.

Statistical Analysis

Statistical analysis was conducted using Stata Version 13 [StataCorp, 2013]. All compiled air, survey, and intake questionnaire data was imported directly into Stata. Whereas some variables were preserved in their original entered state (such as t.DE, which was treated as a continuous variable), variables for subject job title, job tasks and task durations, number of cigarettes smoked per shift, and study session were condensed into pre-determined groupings as described below.

Job Location Groupings:

Information about subject-assigned job titles was collected from intake questionnaires; a summary can be found in Table 2 above. This information informs the association between 1NP and EC/OC/TC

personal air concentrations, and the prediction of 1NP personal air concentrations in the absence of direct sampling. However, the intake questionnaires contain a total of 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 the shift. Therefore, job title alone is not an ideal parameter to assess. In a previous mine study, Coble and associates assigned DE exposure groups based on location in the mine [Coble et al, 2010]. This approach has been modified, and a grouping variable based on shift-specific job locations was generated using information available in this dataset: job title and subject-reported time underground.

Job titles were initially sorted into one of three mine location groups. “Face” represents work at the ends of mine tunnels such as drilling and haulage operations, and is expected to involve high levels of DE due to poor ventilation at the end of mine tunnels and the operation of diesel-powered equipment nearby. This group includes the following job titles: Diamond Driller, Miner, Miner I, Muck Hauler, and Operator. “Shop” represents work in well-ventilated underground shops, primarily involving equipment repair; job titles include Beat Mechanic, Electrician, Mechanic, and Stationary Mechanic. “Surface” represents work done outside the mine or near enough to the surface to experience fresh-air ventilation; this group is expected to have the lowest DE exposures. This group includes the job titles Sandplant Operator and Surface Mill Operator. Geologist is one job title that does not obviously align with a job location group because some geology shifts take place underground and some aboveground. In addition, the distribution of subject-reported time underground per shift indicates that some subjects with jobs falling into “face” or “shop” groups spent entire shifts entirely aboveground (see Figure 2 below; each dot represents a single shift).

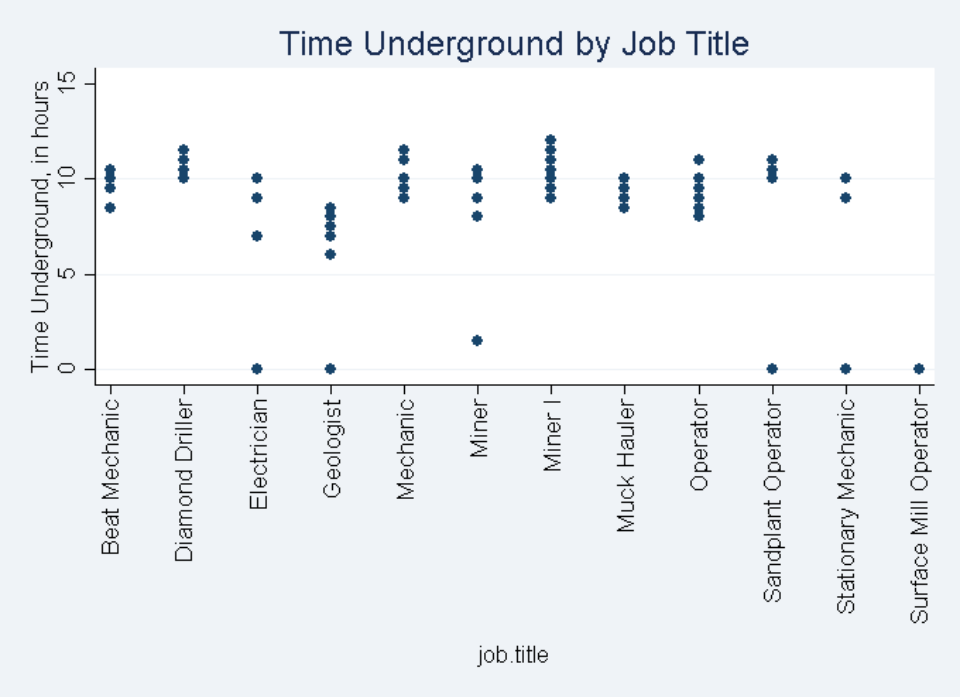

Figure 2: Subject-Reported Time Spent Underground, by Job Title

Thus, the formation of the location grouping variable was modified to include shift-specific information about time spent underground. If time spent underground was reported as 1.5 hours or less (the 10th percentile of the time underground variable values) for any given shift, the value of the location group variable was assigned as "surface" for that shift, regardless of the job title.

Job Task Groupings

Specific tasks reported by subjects in daily surveys similarly informs the 1NP-EC/OC/TC associations and 1NP predictions. Each day of sampling, subjects would fill out a survey and list between one and five job tasks, and the percent of that day's shift spent on each task (an example survey can be found in Appendix I). Each task could potentially be placed in one of the three location groups defined above, but the specificity of the eighteen total tasks reported can refine the DE estimates of the broad location groups, provided enough 1NP measurements are available to provide a sufficient sample size for analysis of individual tasks. Upon tabulation of 1NP measurements per reported job task, it was deemed 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. Geology was given its own group "face geology" for certain shifts if time underground was greater than 1.5 hours. Other tasks generally located at any of the three location groups were given designations of "other face task", "other shop task", or "other surface task" and thus grouped together

(geology was placed in the "other surface task" group if time underground was less than 1.5 hours). However, these tabulations utilized reporting information for any of the five possible tasks listed per shift, and these tasks were often reported together (see Appendix VI for a task reporting matrix). Information on each task's reported duration per shift was used to isolate a single primary task that was conducted for a majority of the shift. Thus, each shift was assigned a single unique value of the primary task grouping variable (out of eight total options). A summary is provided in Table 7 below.

Table 7: Tabulation of Primary Task & Task Group Per Shift

Task	Task Group	N (1NP Measurements)
Diesel Engine Repair	Diesel Engine Repair	12
Geology (face)	face geology	10
Jack leg drill operator	Jack leg drill operator	10
Load-Haul-Dump operation	Load-Haul-Dump operation	23
Ore channeling	Ore channeling	12
Utilities	other face	5
Bolting, loading, & drilling	other face	4
Sand filling	other face	1
Mucking	other face	2
total other face		12
Electrician	other shop	4
Maintenance & Repair	other shop	3
Mechanic	other shop	4
total other shop		11
Above ground office work	other surface	1
Sandplant	other surface	4
Pumphouse/Mill(Surface)	other surface	3
Pasteplant (Surface)	other surface	1
Out of Mine	other surface	1
Geology (surface)	other surface	3
total other surface		13
Total		103

Cigarettes Smoked per Shift Groupings:

Subjects reported the total number of cigarettes smoked during the previous shift on each survey. However, the same subject would often report the same number every day, so this data is not strictly continuous. A binary form of the variable, with values representing "no cigarettes" or "cigarettes" was

also available. These two variables were combined, and the "cigarettes" category was split into "low" and "high" categories based on numbers of observations at each level. These tabulations can be seen in Table 8 below.

Table 8: Cigarette Variables Tabulations

cig24no (continuous)	N (1-NP measurements)
0	77
1	1
2	1
5	4
6	1
7	1
10	7
11	1
15	4
20	6
Total	103

cig24group (grouped)	N (1-NP measurements)
high, >14	10
low, 1-14	16
none	77
Total	103

cig24 (binary)	N (1-NP measurements)
0	77
1-20	26
Total	103

Study Session & Fuel Type Groupings:

The mine study site had one major operational difference between study sessions (March, June, August, and October) that may cause differences in vehicle emissions of 1NP. In the summer months, the mine uses a different fuel additive than in the winter months for diesel-powered vehicles; this has to do with the mine's usage of biodiesel fuel and the seasonal temperatures affecting fuel application. Thus, a new variable "fuel type" was generated that combines session 1 and 4 (March and October), and 2 and 3 (June and August).

Aim 1: Association of 1-NP with EC and TC

The first aim of this project examines the association between 1-NP and EC, OC, and TC, in turn, when no modifying factors (covariates) are considered. These continuous variables were each assessed for lognormality using histograms and Shapiro-Wilks W statistical tests. 1-NP, EC, OC, and TC air concentration data were determined to be lognormally distributed; Histograms and statistical test results can be found in Appendix VII. Summary statistics were calculated for these variables, including arithmetic and geometric means and standard deviations, percent of values below the LOD, sample sizes, and minimum and maximum values. Additionally, 1-NP and EC

concentrations per subject were plotted on a box-plot, and intra- and inter-subject variability were observed. Thus, subject ID was included in the Aim 1 analysis as a random effect. The primary outcome of this aim is a set of measures of association, namely, ratios of geometric mean personal air concentration of 1-NP per unit change in geometric mean personal air concentration of EC, OC, and TC (in turn). A set of linear regressions on log-transformed 1-NP and EC, OC, and TC data was conducted to yield a set of ratios of geometric means. A mixed random effects model was used to account for correlation of the data, with the variable "subjectid" included as a random effect due to inter- and intra-subject variability. The restricted maximum likelihood (reml) option was used due to the small sample size (n=104 for matched 1NP-EC/OC/TC measurements) and the Wald statistic computed 95% CIs. A p-value was computed to test the null hypothesis that there is no association between 1-NP and EC/OC/TC (ratio of geometric means=1) to the 0.05 alpha level.

Four different data treatments and model formulations were used to account for values of 1-NP, EC, OC, and TC below the limit of detection. For Model 1, data below the LOD were replaced with the LOD divided by the square root of two (Hornung and Reed, 1990). For Model 2, data below LOD were omitted. For Model 3, data below the LOD was included in original form, and an interaction term was added to the model, a binary variable indicating whether a value was above or below LOD. For Model 4, data below the LOD was included in original form, and no substitutions, replacements, or interaction terms were incorporated.

The interpretation of the association between 1-NP and EC, OC, or TC for models 1, 2, and 4 was based on the model output coefficient for the primary predictor variable (lnEC, lnOC, or lnTC), called ß1 in the model equation: $\ln(1NP) = ß0 + ß1*\ln(EC)$. The association between 1-NP and EC/OC/TC was calculated by raising 1.1 to the ß1 power; the resulting value subtracted from 1 represents the percent change in 1-NP expected for a 10% change in EC/OC/TC.

For Model 3, the model equation takes the form: $\ln(1NP) = ß0 + ß1*\ln(EC) + ß2*flod + ß3*flod*\ln(EC)$, where *flod* is the interaction term binary variable indicating whether values are above or below the LOD. The interpretation of the association between 1-NP and EC, OC, or TC for this model is based on the ß1 coefficient as with the other models, though $1.1^{ß1}$ represents the relationship only when all values are above the LOD. The effect of the LOD indicator variable *flod* upon the ß1 coefficient also informs the association between 1-NP and EC/OC/TC in this

model, and was interpreted by assessing statistical significance of the ß3 coefficient to the 0.05 alpha level.

Aim 2: Effect of Survey and Intake Questionnaire Variables on Association of 1-NP with EC

The second aim of this project assesses the possible effect of modifying factors upon the association between 1-NP and EC. Originally, OC and TC were to be investigated as part of this aim, but the Aim 1 results from descriptive statistics and regressions indicate high proportion of OC and TC values below LOD and a resulting lack of significance for the 1-NP/OC and 1-NP/TC regressions; this suggests further analysis of these parameters would be uninformative. Thus, only EC will be considered as part of this aim.

Another observation from the Aim 1 results was the uninformative nature of a model for values below the LOD. For use in Aim 2, the dataset was trimmed to exclude all values for which EC or 1NP were below LOD (16 of the 103 values used in Aim 1 were excluded for Aim 2).

A subset of the variables obtained from the Intake Questionnaire and Survey data, were examined for their potential modifying effects upon the 1-NP-EC associations when plausible hypotheses for effect modification could be formulated, as summarized in Table 9 below.

Table 9: Covariate Options for Aim 2 (Association)

Intake Questionnaire & Survey Variables		
Variable name	**Description**	**Hypothesized effect upon 1-NP-EC/OC/TC associations**
Location.group	Approximate location worked in the mine, as estimated by job title and time spent underground	Different working locations may be associated with working conditions that have certain typical ratios of PAH's to EC/OC/TC in DE
Primary task group	Task conducted for the majority of the shift monitored, as reported by subject	Different job tasks may be associated with working conditions that have certain typical ratios of PAH's to EC/OC/TC in DE, such as different vehicles used, engine operating conditions, etc.
cig.24groups	Number of cigarettes smoked by subject during the shift; none/low/high	Cigarette smoke may interfere with detected EC/OC/TC concentrations, but not with 1-NP concentrations.
Fuel.type	Summer fuel blend vs. Winter fuel blend used by mine; Summer fuel used during June & August campaigns, Winter fuel used during March & October campaigns	Different fuel additives could result in different exhaust composition, and a different ratio of PAH's to EC/OC/TC in DE.
DOW	Day of work week during which sampling took place; 1-4	Rate of production in the mine may differ between the beginning and end of a subject's work week, which might affect engine operating conditions and PAH-EC/OC/TC ratios.

To address aim 2, log-transformed 1-NP data was compared with log-transformed EC data in a mixed model linear regression with subject ID as a random effect. Each of the variables listed above were assessed using summary statistics, and those chosen for inclusion were converted to dummy variables and added as covariates individually to different 1-NP/EC models. The effect of each covariate inclusion upon the regression coefficient was assessed by examining the output from each model. Additionally, a p-value was calculated to the 0.05 alpha level for each dummy variable coefficient to test a null hypothesis of no association between 1-NP and each covariate individually.

For these interaction term models, the model equations take forms similar to that of Model 3, in Aim 1 above: $\ln(1NP) = ß0 + ß1*\ln(EC) + ß2*covariate + ß3*covariate*\ln(EC)$, where *covariate* is the interaction term binary variable indicating whether an observation matches a certain criteria, such as “face” location group (yes/no) or “low” cigarettes group (yes/no). The effect of the covariate indicator variable upon the ß1 coefficient, and thus effect modification of the association between 1-NP and EC, was interpreted by assessing statistical significance of the ß3 coefficient to the 0.05 alpha level.

Aim 3: Predictive model for 1-NP using Survey and Intake Questionnaire Variables

Aim 3 consists of the development of a predictive model for personal air concentration of 1-NP using predictor variables from Intake Questionnaire and Survey data. The variables are listed along with their hypothesized effects upon the 1-NP personal air concentrations in Table 10 below:

Table 10: Covariate Options for Aim 3 (Prediction)

Intake Questionnaire & Survey Variables for 1-NP Prediction		
Variable name	**Description**	**Hypothesized effect upon 1-NP Air Concentrations**
Location Group	Approximate location worked in the mine, as estimated by job title and time spent underground	Different mine locations may be associated with working conditions that have higher levels of DE, such as different vehicles used, engine operating conditions, ventilation, etc.
Primary Task Group	Task conducted for the majority of the shift monitored, as reported by subject	Different job tasks may be associated with working conditions that have higher levels of DE, such as different vehicles used, engine operating conditions, etc.
exp.cat	DE exposure category as pre-determined by mine safety personnel, low-med-high	Certain job titles/locations used to determine exposure category may involve work with higher levels of DE
t.UG	time spent underground (hrs)	Higher amounts of time spent confined underground means higher levels of DE exposure
t.DE	subject-perceived time exposed to DE (hrs)	Subject-perceived higher amount of time spent exposed to DE may directly indicate higher exposure levels to DE
Fuel type	Summer fuel blend vs. Winter fuel blend used by mine; Summer fuel used during June & August campaigns, Winter fuel used during March & October campaigns	Different fuel additives could result in different exhaust composition, potentially including higher or lower emissions of 1-NP.
DOW	day of work week during which sampling took place; 1-4	Rate of production in the mine may differ between the beginning and end of a subject's work week, which would affect engine operating conditions and PAH-EC/OC/TC ratios.

Each variable listed in Table 10, above, was assessed for possible inclusion in the prediction model development using summary statistics. A series of multivariate linear regressions were then conducted, in which the chosen categorical dummy variables and continuous variables were added in a variety of combinations to the predictive models. The model formulations included subject ID as a random effect to account for correlation in the data, but the predictions and cross-validations themselves did not account for Subject ID, in order to produce generalizable predictions and assessments of covariate predictors.

The developed predictive models were each cross-validated using 10 randomly-chosen cross-validation groups from the study dataset. Observations from the same subject were evenly distributed across cross-validation groups, as shown in Table 11 below:

Table 11: Cross-Tabulation Groups by Subject

Tabulation of Cross-Validation Groups by Subject ID											
	CV Group:										
	1	2	3	4	5	6	7	8	9	10	Total
Subject ID:											
1	0	1	0	2	1	2	1	1	0	0	8
2	0	1	1	0	1	0	1	1	1	0	6
3	0	0	0	0	1	0	0	1	2	2	6
4	0	1	0	0	1	0	0	1	0	0	3
6	2	1	0	0	1	0	0	0	1	1	6
7	0	0	1	0	0	0	0	2	0	3	6
8	1	2	0	1	0	0	0	1	1	0	6
9	0	1	2	0	0	2	0	1	0	1	7
10	0	1	0	1	0	1	0	0	0	1	4
11	1	1	0	1	0	0	0	0	1	1	5
12	1	1	0	0	0	1	0	0	1	0	4
13	2	0	0	2	0	0	1	1	0	1	7
14	1	0	0	2	0	1	0	0	1	0	5
15	1	0	1	1	1	2	0	1	1	0	8
17	0	0	1	0	3	1	2	0	0	0	7
18	0	0	2	0	1	0	3	0	0	0	6
19	1	0	0	0	0	0	1	0	0	1	3
20	0	0	2	0	0	0	1	0	1	2	6
Total	10	10	10	10	10	10	10	10	10	13	103

Results

Aim 1: Association of 1-NP with EC and TC

Descriptive Statistics

The continuous variables assessed in Aim 1 are 1-nitropyrene (1NP), elemental carbon (EC), organic carbon (OC), and total carbon (TC). A Lognormality assessment for each can be found in Appendix VII, and summary statistics can be found in Table 12 below. Although EC typically comprises a majority of the mass of TC and OC contributes a small portion, the mean concentration of OC (19.0 μg/m^3) in this dataset is higher than that of EC (12.4 μg/m^3). This is likely due to the blank correction for the OC values and the resulting high LOD. Data points used for summary statistics were replaced with LOD/√2 if lower than the LOD, 79 of the total 103 samples had OC values less than the LOD (76.7%). The field blank filters used for the OC blank correction had a high average OC content (17.8 μg/sample) and standard deviation (6.7 μg/sample). 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. The effect of the LOD replacements in 76.7% of the OC and TC data can be seen in the regression output later in this section.

Table 12: Summary Statistics for 1-NP, EC, OC, and TC

Analyte	n	mean	SD	GM	GSD	% <LOD	min	max
1-NP (pg/m^3)	103	78.0	88.0	47.0	2.86	15.5	2.89	497
EC (µg/m^3)	103	12.4	12.5	8.46	2.51	6.80	1.04	101
OC (µg/m^3)	103	19.0	12.3	17.1	1.49	76.7	12.1	95.0
TC (µg/m^3)	103	35.4	24.5	29.6	1.77	76.7	13.4	135

1-NP and EC personal air concentration distributions are plotted in Figure 3 and Figure 4 below; this illustrates within- and between-subject variability in exposures to DE components. Sample sizes ranged from 3 to 8 usable air measurements per subject. Subjects 7 and 17 had high variability in both 1NP and EC because they both have the job title "geologist", which involves a variety of work locations including surface office work and underground work at the face. Subject 19 is a "sandplant operator", and spent every shift in a sandplant facility near the surface of the mine that had ventilation directly with the outdoors; thus this subject's 1NP and EC exposures were uniformly low. Other trends in 1NP and EC related to job title, location, tasks, and time underground are explained later in this section as part of Aim 2.

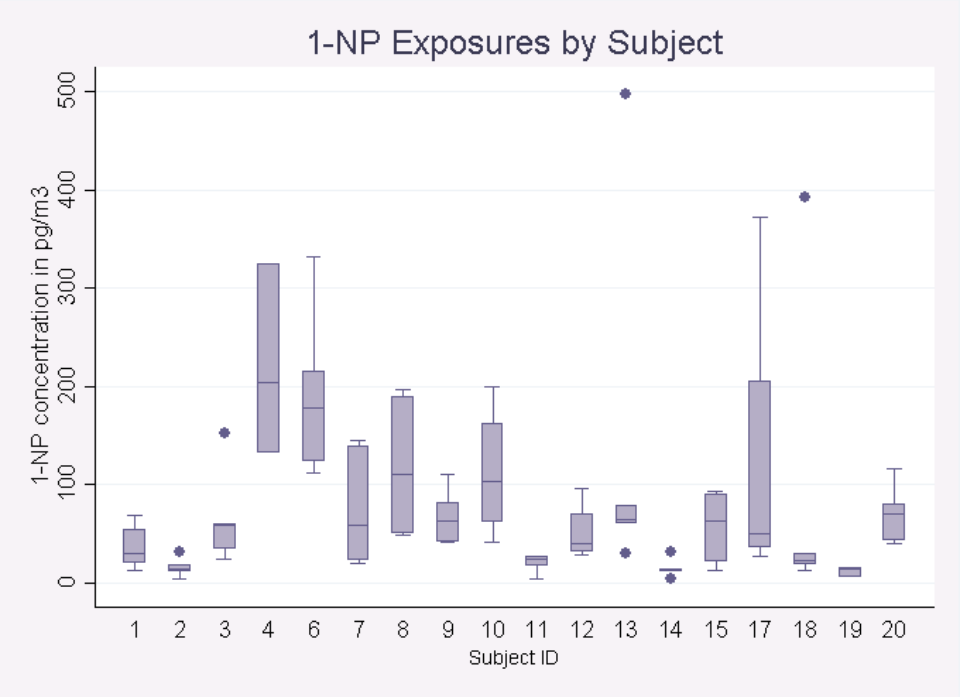

Figure 3: Boxplot of 1-NP Personal Air Concentrations by Subject

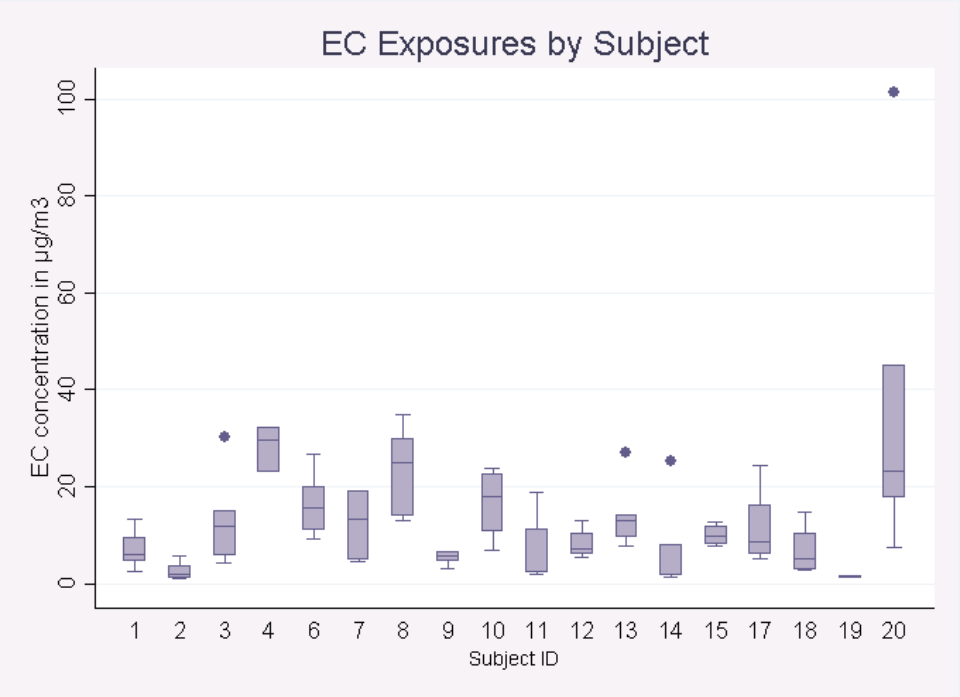

Figure 4: Boxplot of EC Personal Air Concentration by Subject

Regression Output

Mixed-model linear regressions on log-transformed EC, OC, and TC as predictors of 1-NP were conducted with four different model formulations differing in the treatment of values below the LOD. The results are presented in Tables 13-15 below.

Table 13: Aim 1 Regression Output for EC

EC Models												
Model Version:	1: Values below LOD replaced with LOD/√2 (n=103)			2: Values below LOD omitted from model (n=87)			3: Values below LOD kept "as is", interaction term for <LOD in model (n=103)			4: Values below LOD kept "as is", no adjustments/omissions (n=103)		
Model Equation:	ln(1NP) = ß0 + ß1*ln(EC)			ln(1NP) = ß0 + ß1*ln(EC)			ln(1NP) = ß0 + ß1*ln(EC) + ß2*flod + ß3*flod*ln(EC)			ln(1NP) = ß0 + ß1*ln(EC)		
Model Coefficients:	Value	SE	p-value	Value	SE	p-value	Value	SE	p-value	Value	SE	p-value
ß0 (intercept)	-11.126	0.250	<0.001	-10.742	0.281	<0.001	-10.779	0.295	<0.001	-11.854	0.313	<0.001
ß1 (lnEC)	0.542	0.103	<0.001	0.464	0.112	<0.001	0.482	0.119	<0.001	0.809	0.129	<0.001
ß2 (flod)	-	-	-	-	-	-	-1.981	0.405	<0.001	-	-	-
ß3 (flod*lnEC)	-	-	-	-	-	-	-0.348	0.229	0.129	-	-	-
% Variance explained by:												
Between-subject differences:	34%			27%			25%			32%		
Within-subject differences:	66%			73%			75%			68%		

Table 14: Aim 1 Regression Output for OC

OC Models												
Model Version:	1: Values below LOD replaced with LOD/√2 (n=103)			2: Values below LOD omitted from model (n=23)			3: Values below LOD kept "as is", interaction term for <LOD in model (n=103)			4: Values below LOD kept "as is", no adjustments/omissions (n=103)		
Model Equation:	ln(1NP) = ß0 + ß1*ln(OC)			ln(1NP) = ß0 + ß1*ln(OC)			ln(1NP) = ß0 + ß1*ln(OC) + ß2*flod + ß3*flod*ln(OC)			ln(1NP) = ß0 + ß1*ln(EC)		
Model Coefficients:	Value	SE	p-value	Value	SE	p-value	Value	SE	p-value	Value	SE	p-value
ß0 (intercept)	-11.002	0.680	<0.001	-8.787	1.102	<0.001	-8.388	2.152	<0.001	-11.478	0.445	<0.001
ß1 (lnOC)	0.359	0.228	0.115	-0.173	0.307	0.572	-0.323	0.607	0.595	0.501	0.143	<0.001
ß2 (flod)	-	-	-	-	-	-	-3.061	2.181	0.161	-	-	-
ß3 (flod*lnOC)	-	-	-	-	-	-	0.792	0.631	0.209	-	-	-
% Variance explained by:												
Between-subject differences:	51%			63%			48%			48%		
Within-subject differences:	49%			37%			52%			52%		

Table 15: Aim 1 Regression Output for TC

TC Models												
Model Version:	1: Values below LOD replaced with LOD/√2 (n=103)			2: Values below LOD omitted from model (n=23)			3: Values below LOD kept "as is", interaction term for <LOD in model (n=103)			4: Values below LOD kept "as is", no adjustments/omissions (n=103)		
Model Equation:	ln(1NP) = ß0 + ß1*ln(TC)			ln(1NP) = ß0 + ß1*ln(TC)			ln(1NP) = ß0 + ß1*ln(TC) + ß2*flod + ß3*flod*ln(TC)			ln(1NP) = ß0 + ß1*ln(EC)		
Model Coefficients:	Value	SE	p-value	Value	SE	p-value	Value	SE	p-value	Value	SE	p-value
ß0 (intercept)	-11.748	0.613	<0.001	-10.402	2.152	<0.001	-8.425	4.176	0.044	-14.702	1.148	<0.001
ß1 (lnTC)	0.521	0.173	0.003	0.238	0.504	0.637	-0.241	0.983	0.806	1.212	0.300	<0.001
ß2 (flod)	-	-	-	-	-	-	-6.391	4.307	0.138	-	-	-
ß3 (flod*lnTC)	-	-	-	-	-	-	1.478	1.028	0.15	-	-	-
% Variance explained by:												
Between-subject differences:	47%			63%			46%			45%		
Within-subject differences:	53%			37%			54%			55%		

For model 1, the coefficients associated with the lnEC and lnTC predictor variables for ln1NP were found to be statistically significant with p-values below 0.05. These results indicate that airborne 1-NP concentration is associated with EC and TC concentration, when all values below the LOD are replaced with LOD/√2. For a 10% increase in EC, or TC concentration, 1-NP is expected to increase by 5.3% and 5.1%, respectively.

For model 2, the coefficient associated with the lnEC predictor variable for ln1NP was found to be statistically significant with a p-value below 0.05. Those of lnOC or lnTC were not found to be statistically significant. These results indicate that airborne 1-NP concentration is associated with EC concentration, when all values below the LOD are omitted. For a 10% increase in EC concentration, 1-NP is expected to increase by 4.5%. However, 1-NP has not been shown to be associated with OC or TC concentration for the restricted dataset of only 23 observations above LOD.

For model 3, only the coefficient associated with the lnEC predictor variable for ln1np was found to be statistically significant with the p-value below 0.05. This suggests for a 10% increase in EC concentration, 1-NP is expected to increase by 4.7% when all values are above the LOD (flod=0). The coefficient associated with the flod*lnEC interaction term was not found to be statistically significant (p=0.129), indicating that there is no significant difference in the lnEC coefficient between values above and below the LOD.

For model 4, the association between 1-NP and EC, OC, and TC were all found to be statistically significant. By ignoring the scientific infeasibility of utilizing below-LOD values for association assessment, it appears that for every 10% increase of EC, OC, or TC, 1-NP is expected to increase by 8.0%, 4.9%, and 12.2%,

respectively. These associations cannot be trusted, however, they merely illustrate the severe association-obscuring effect of the necessary treatments of values below the LOD used for models 1, 2, and 3.

The EC models have a higher percent of variance explained by within-subject differences than by between-subject differences, and for the OC and TC models the variance is explained approximately equally by both components.

The results of all four models indicate that 1-NP is significantly associated with EC regardless of the treatment of values below LOD. In model 3, when the effect of values being below LOD is assessed by the model construction, no significant effect upon the 1-NP/EC association is found.

The association between 1-NP and OC was not found to be significant in models 1, 2, or 3 (those that involve reasonable accommodation for below-LOD values). The association between 1-NP and TC was found to be significant in only model 1 and 4 ($p=0.003$, and $p<0.001$, respectively). For Models 2 and 3, the primary indicator of association of either OC or TC with 1-NP (the coefficient associated with lnOC or lnTC) was calculated using a set of data with only 23 observations (ignoring the remainder below the LOD). This small sample size limits the power of these models.

Model assessment for association is provided in Figures 5-10 below.

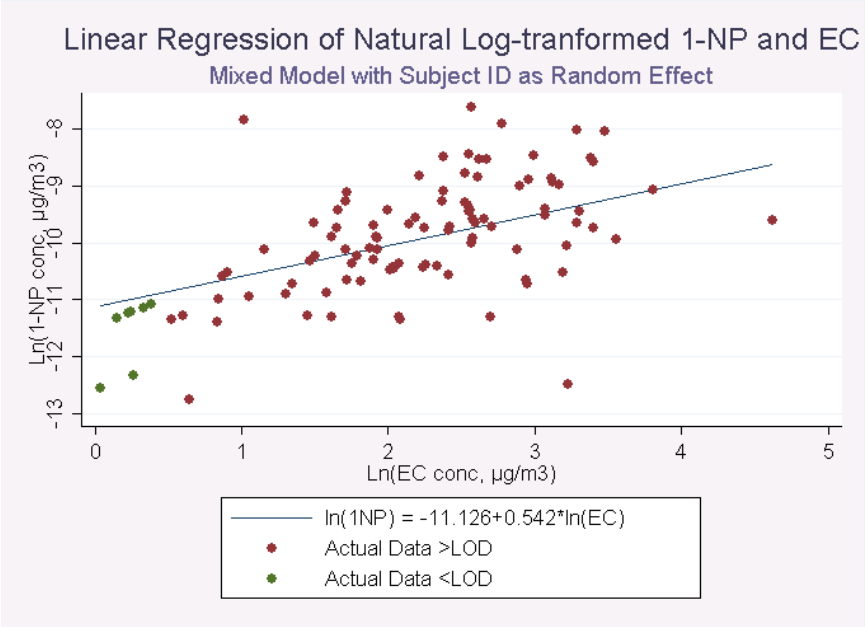

Figure 5: 1-NP/EC Association Model 1, with <LOD values replaced with LOD/√2

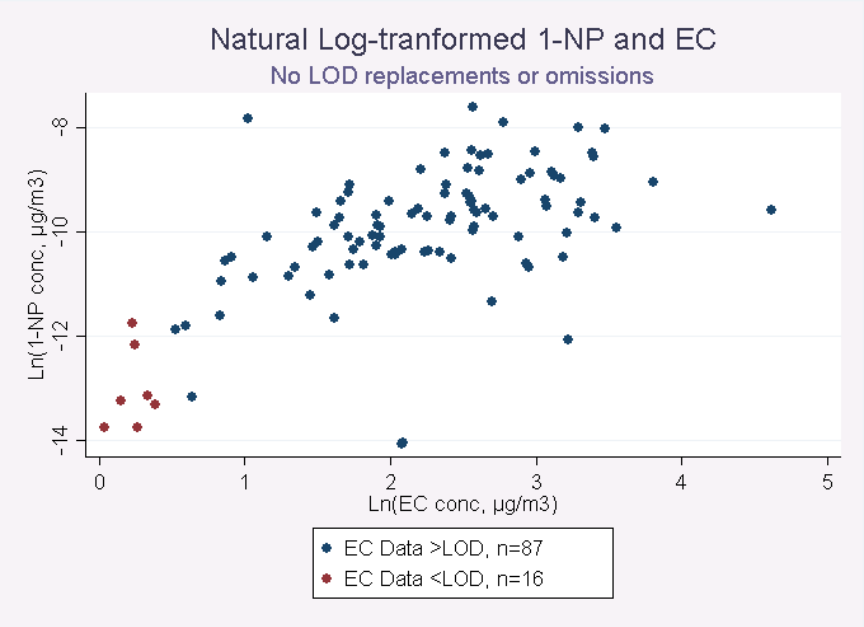

Figure 6: 1-NP & EC data used for Association models 2-4, <LOD values treated "As-Is"

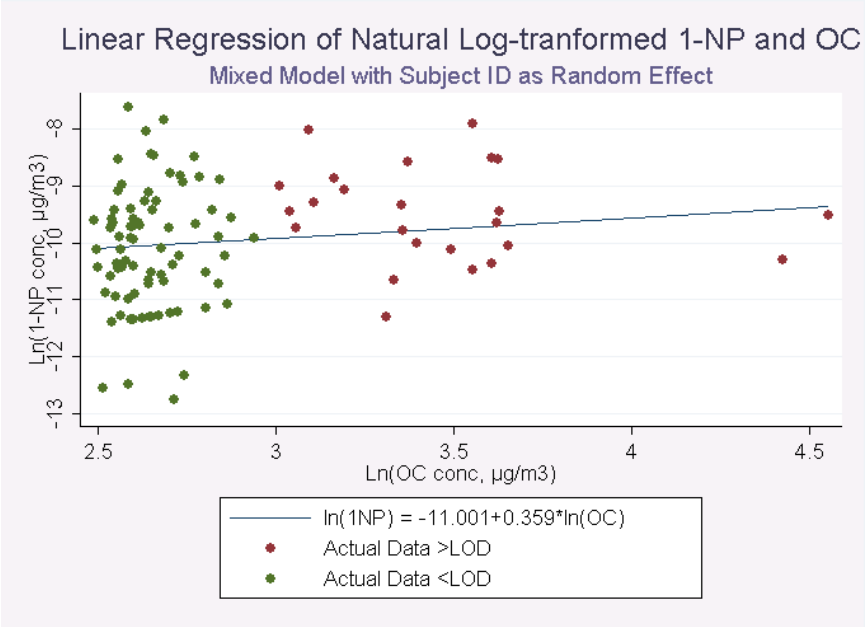

Figure 7: 1-NP/OC Association Model 1, with <LOD values replaced with LOD/v2

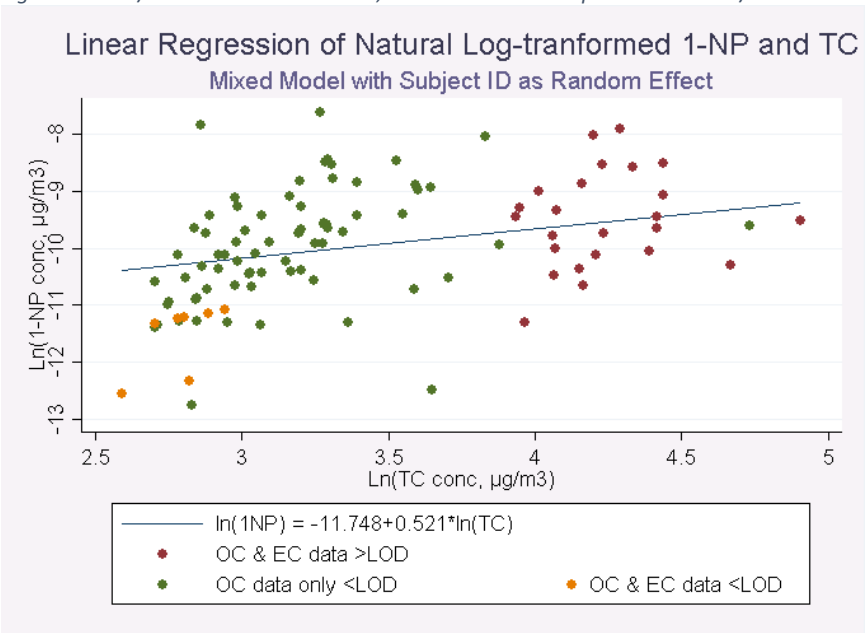

Figure 8: -NP/TC Association Model 1, with <LOD values replaced with LOD/v2

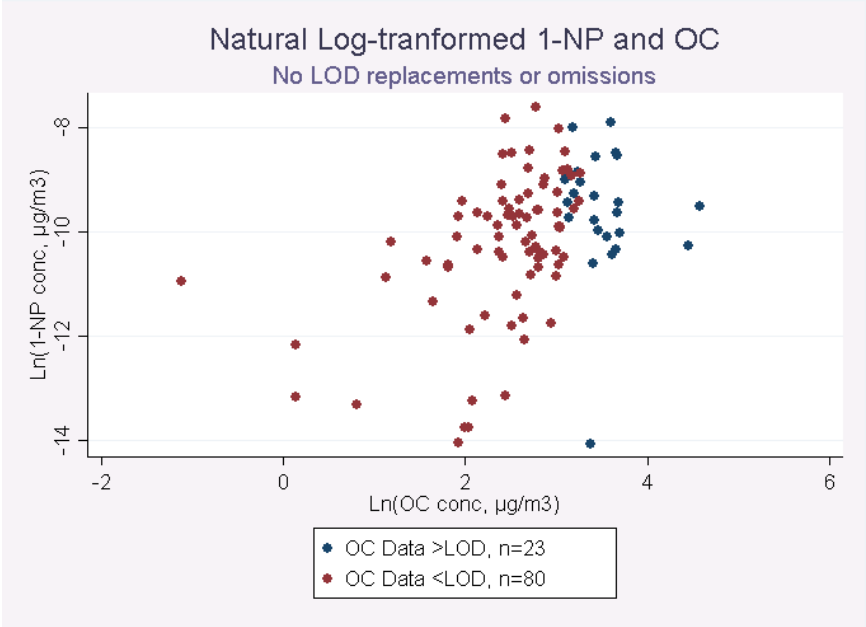

Figure 9: 1-NP & OC data used for Association models 2-4, <LOD values treated "As-Is"

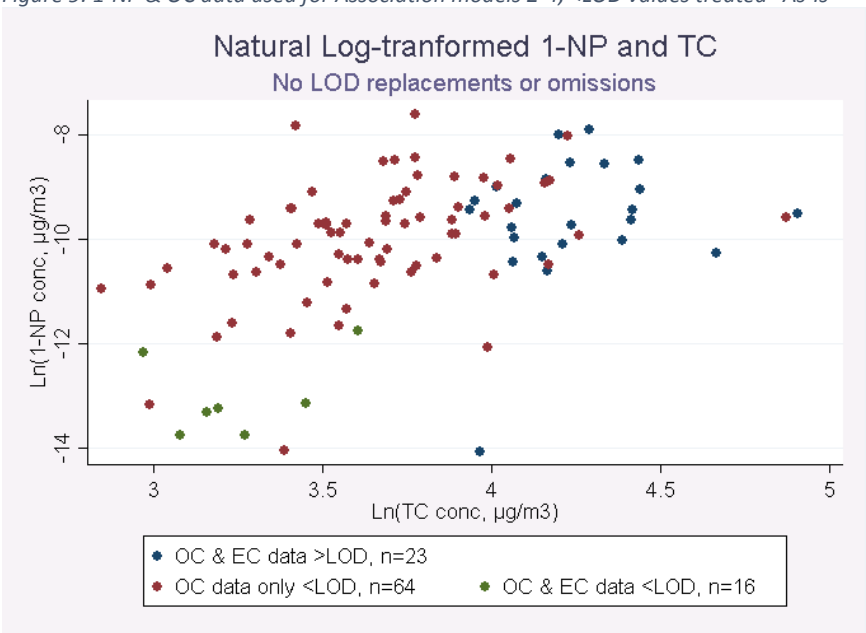

Figure 10: 1-NP & TC data used for Association models 2-4, <LOD values treated "As-I

Aim 2: Effect of Survey and Intake Questionnaire Variables on Association of 1-NP with EC

Descriptive Statistics

The primary variables of interest, 1-NP, EC, OC, and TC, have been described previously. The following variables were assessed for possible effect modification or confounding of the 1-NP-EC association: location group, cig.24.groups, fuel.type, exp.cat, and DOW. Prior to inclusion in an association for regression with 1-NP and EC, an exploratory analysis was conducted on each.

Location Group

1-NP and EC personal air concentrations were stratified by Location Groups and described in Figure 11, Figure 12, and Table 16 below.

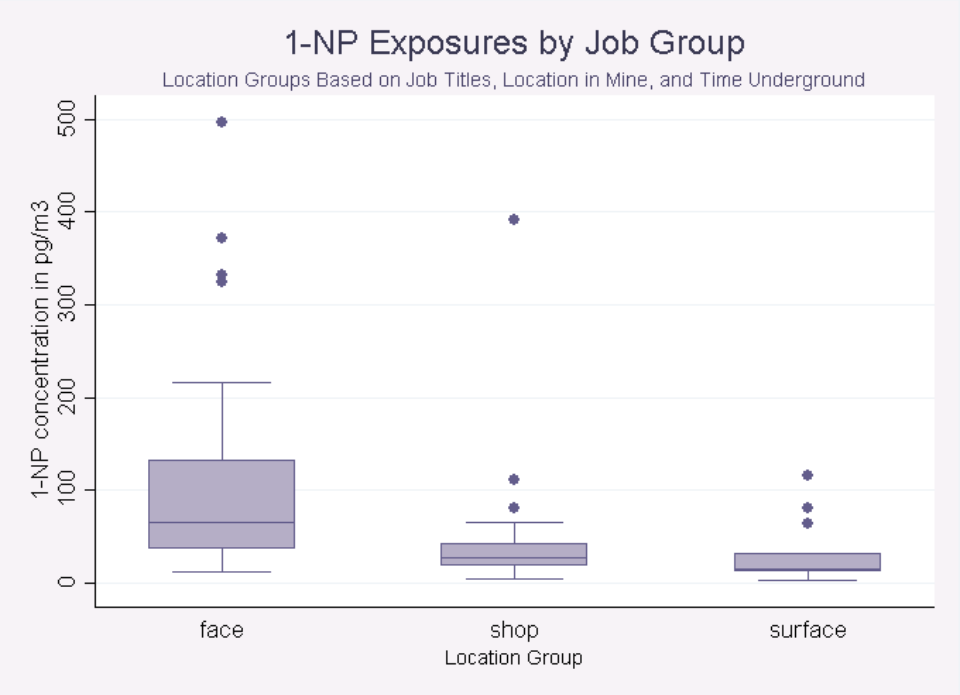

Figure 11: 1-NP Exposures by Location (job) Group

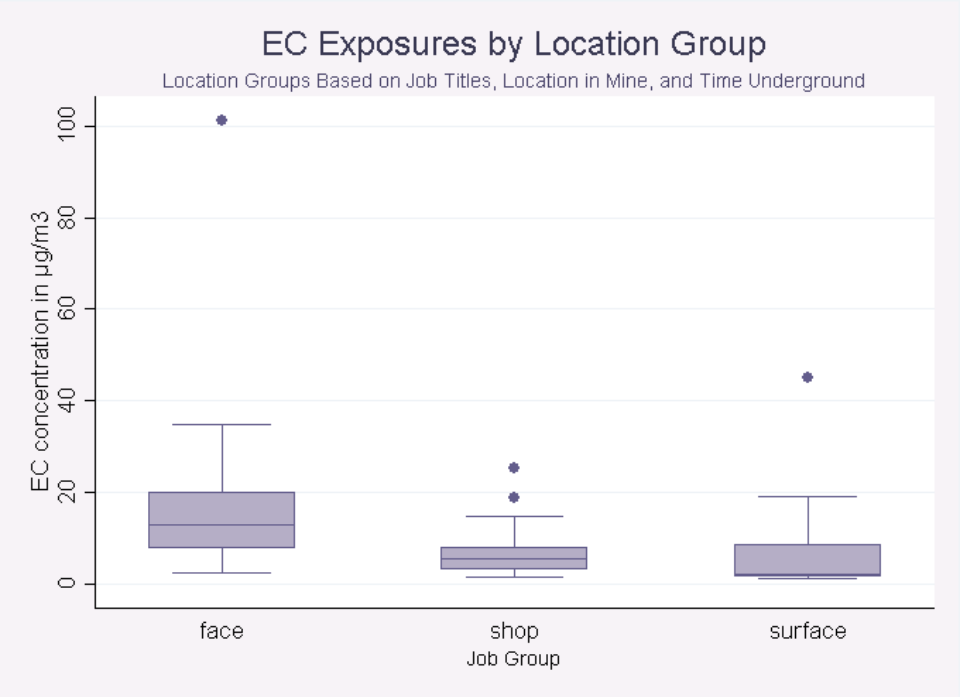

Figure 12: EC Exposures by Location (Job) Group

Table 16: Location Group Summary Statistics

1-NP and EC Personal Air Concentrations by Location Group

Job Group	n	Median 1-NP (95% CI), pg/m^3	Median EC (95% CI), µg/m^3
face	70	66.2 (51.8-81.4)	12.6 (9.89-13.5)
shop	23	25.9 (17.0-40.6)	5.26 (2.80-6.65)
surface	12	14.9 (11.2-30.6)	1.89 (1.29-8.24)

There appears to be a clear stratification of 1-NP and EC concentration medians and ranges between different location groups. Additionally, the range for the "face" location group is wider for 1-NP than for EC, and the range for the "surface" location group is wider for EC than for 1-NP. This suggests possible differences between 1-NP and EC presence in diesel exhaust in these locations, and warrants an investigation of possible effect modification conducted via inclusion in the 1-NP vs. EC regression model.

Cig.24 Group

1-NP and EC personal air concentrations were stratified by cigarette groups, as shown in Figure 13, Figure 14, and

Table 17 below.

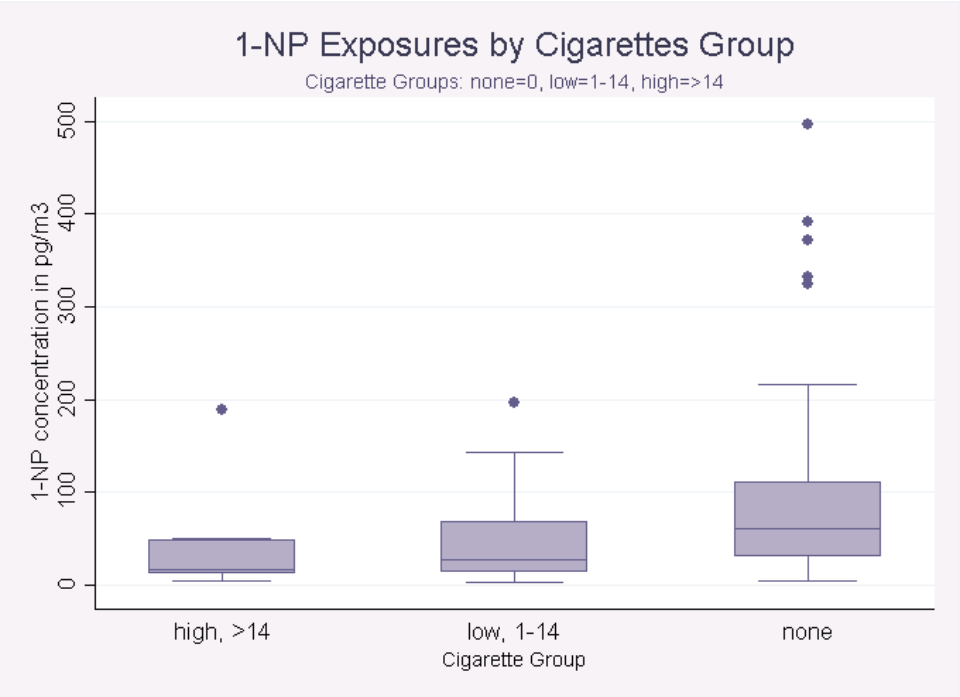

Figure 13: 1-NP Exposures by Cigarettes Group

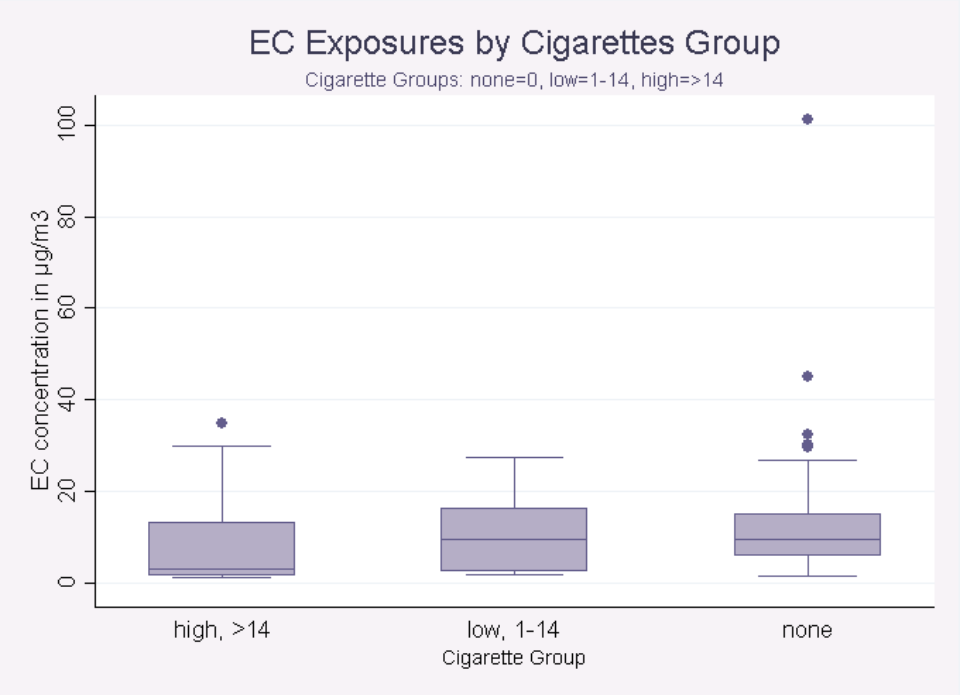

Figure 14: EC Exposures by Cigarettes Group

Table 17: 1NP and EC Summary Statistics by Cigarette Group

1NP					
Cigarette Group	N	mean	median	min	max
none	77	89.4	60	4.42	497
low, 1-14	16	47.4	26.4	2.89	197
high, >14	10	39.2	16.8	3.51	189
EC					
Cigarette Group	N	mean	median	min	max
none	77	13.1	9.58	1.28	101
low, 1-14	16	11.0	9.57	1.69	27.3
high, >14	10	9.45	2.99	1.04	34.8

Distributions of EC between different cigarette groups appear more uniform than the distributions of 1-NP between different cigarette groups. In particular, the distribution of EC concentrations in the "high" cigarette group appears much wider, with higher upper 95th percentile (shown on the boxplot) relative to the median than the 1-NP distribution in the "high" cigarette group. This suggests possible differences in 1-NP and EC content in personal air samples for different cigarette groups, and warrants an investigation into effect modification using the 1-NP vs. EC regression model.

Fuel Type

The sampling collection for this study occurred during 4 different campaigns in 2014, in August, and October. It was determined that the varying condition between these campaigns to affect the 1-NP/EC association is the different fuel additive used in March/October (winter) that used in June/August (summer). 1-NP and EC have been summarized by these two fuel types in Figure 15, Figure 16, and

Table 18 below.

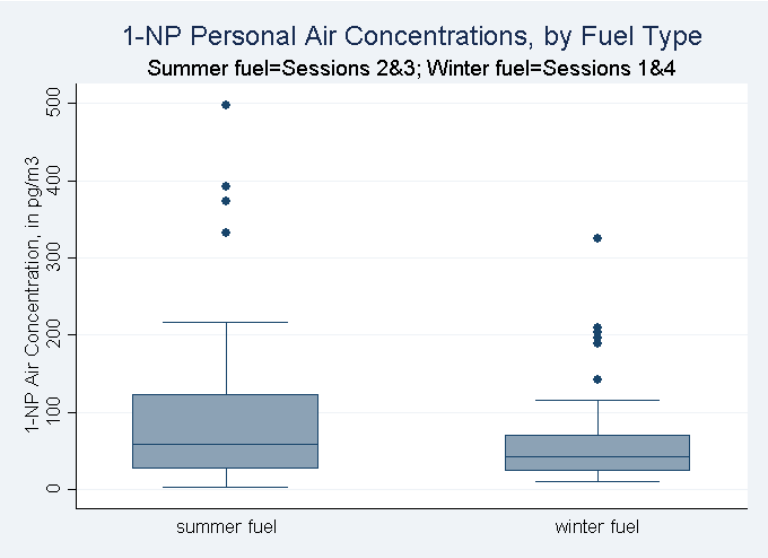

Figure 15: 1-NP Exposures by Fuel Type

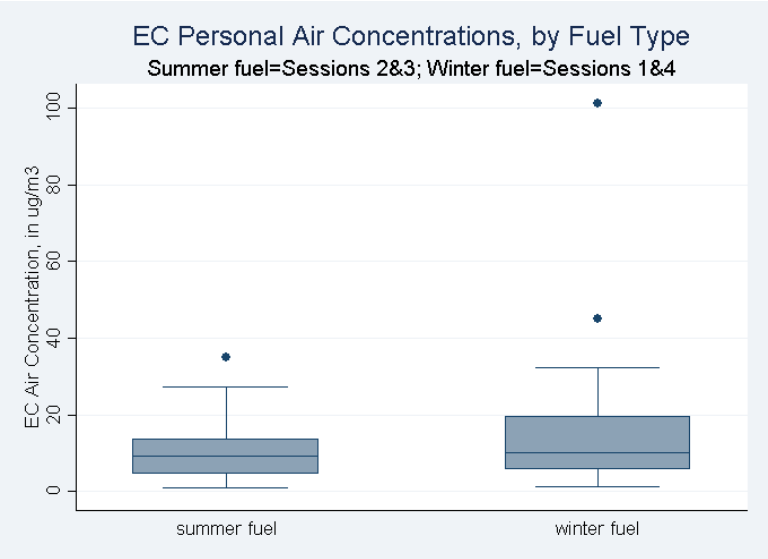

Figure 16: EC Exposures by Fuel Type

Table 18: 1NP and EC Summary Statistics Stratified by Session & Fuel Type

1-NP (pg/m^3)								
Session	n	mean	SD	GM	GSD	% <LOD	min	max
1	24	68.7	74.3	45.7	2.43	16.7	12.40	321
2	30	99.3	103	56.50	3.44	10.0	3.51	392
3	25	79.5	105	43.3	3.16	24.0	2.89	497
4	26	68.1	73.7	44.5	2.48	11.5	11.1	325
1+4 (winter fuel)	50	68.4	73.2	45.1	2.44	14.0	11.1	325
2+3 (summer fuel)	55	90.3	103.35	50.0	3.30	16.4	2.89	497
EC (µg/m^3)								
Session	n	mean	SD	GM	GSD	% <LOD	min	max
1	30	11.6	7.5	8.9	2.3	10.0	1.26	30
2	31	12.3	8	9.3	2.4	6.5	1.04	27
3	27	8.1	7	5.8	2.3	11.1	1.16	35
4	28	16.7	19.9	10.3	2.7	0.0	1.7	101
1+4 (winter fuel)	58	14.0	14.9	9.6	2.5	5.2	1.3	101
2+3 (summer fuel)	58	10.3	7.79	7.5	2.4	8.6	1.04	35

1-NP and EC do not appear to follow session-specific trends, as displayed in

Table 18 above. By fuel type, it appears that marginally higher 1-NP concentrations were detected for "summer" fuels, and higher EC concentrations for "winter" fuels. The scientific interest of investigating the effect of the fuel type upon DE composition drove the decision to investigate Fuel Type as an effect modifier with the 1-NP-EC regression.

Exposure Category

The preliminary exposure categories determined by mine staff members (according to job title) were investigated, as seen in Figure 17, Figure 18, and Table 19 below.

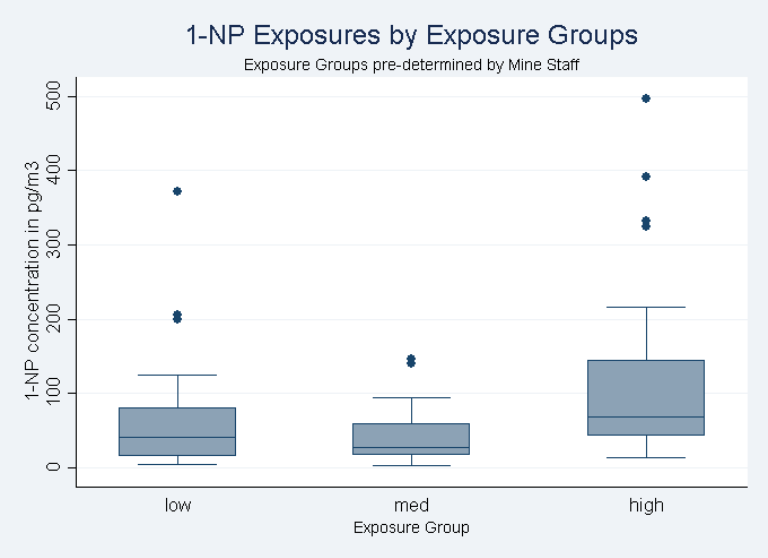

Figure 17: 1-NP Exposures by Exposure Group (exp.cat)

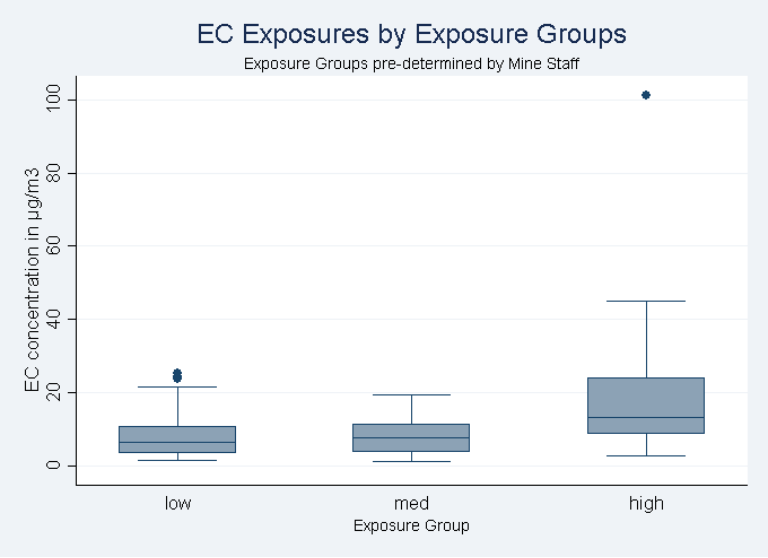

Figure 18: EC Exposures by Exposure Group (exp.cat)

Table 19: 1-NP and EC Summary Statistics by Exposure Category

Exposure Category	n	median 1-NP (95% CI), pg/m^3	median EC (95% CI), µg/m^3
low	27	41.6 (31.3-65.8)	6.51 (4.98-8.69)
medium	33	26.9 (18.4-45.5)	7.68 (4.62-10.3)
high	45	67.6 (53.0-105.5)	13.1 (11.0-18.0)

Exposure groups, as determined by mine staff, do not appear to accurately portray the 1-NP or EC distributions. The “low”, “med” and “high” DE exposure indications do not align with clearly distinguished 1-NP and EC concentration distributions. Little could be further learned by including this parameter in a 1-NP and EC regression model for association, so it was excluded from further analysis.

DOW

1-NP and EC concentrations were stratified by DOW, as seen in Figure 19, Figure 20, and Table 20 below.

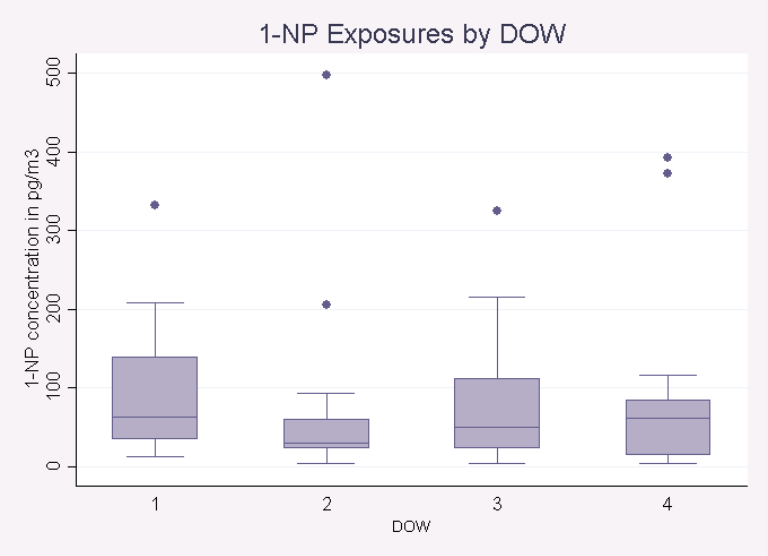

Figure 19: 1-NP Exposures by DOW

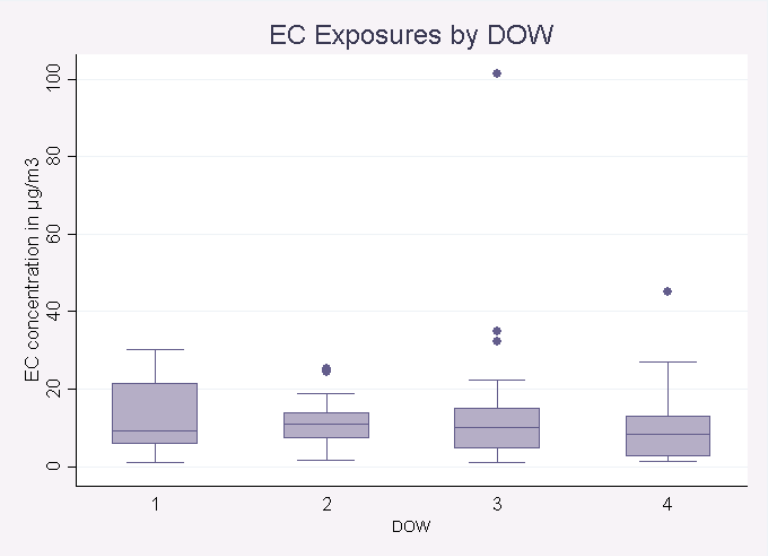

Figure 20: EC Exposures by DOW

Table 20: 1NP and EC Summary Statistics by DOW

1-NP (pg/m^3)					
DOW	N	mean	median	min	max
1	29	93.50573	62.06894	12.09731	331.4538
2	24	62.2266	29.69311	3.784057	497.0781
3	26	75.63743	49.54031	3.51296	324.6405
4	24	77.62971	61.20153	2.891108	392.2018
EC (µg/m^3)					
DOW	N	mean	median	min	max
1	29	13.12714	9.134334	1.158862	30.04943
2	24	11.43912	10.96178	1.691857	25.09154
3	26	14.45268	10.09117	1.037082	101.2594
4	24	10.20736	8.255338	1.256698	44.93994

Means, medians, and ranges for 1-NP and EC are generally uniform across different days of the week. It was determined that further investigation of this parameter for the 1-NP and EC association is not warranted.

Regression Output

Model 1: 1-NP vs. EC and Location Group

The first multivariate regression for the association between 1-NP and EC included Location Group as an interaction term; the model output can be seen in Table 21 below.

Table 21: 1-NP vs EC and Location Group Association Model

Regression Output: Natural Log-Transformed 1NP Concentration as a Function of Natural Log-Transformed EC with an Interaction Term for Location Group and Subject ID as a Random Effect					
Model Equation:	ln1NP = ß0 + ß1*lnEC + ß2*face + ß3*shop + ß4*face*lnEC + ß5*shop*lnEC + b(subj)				
Where "face" and "shop" represent dummy variable values of locationgroup; each 0 or 1. b(subjid) represents the random effect term.					
Model Coefficients:	Value	SE	p-value	95% CI	
ß0 (intercept)	-11.425	0.921	<0.001	-13.231	-9.620
ß1 (lnEC)	0.605	1.700	0.090	-0.093	1.303
ß2 (“face” location group)	0.584	0.983	0.553	-1.343	2.510
ß3 (“shop” location group)	1.602	1.061	0.131	-0.478	3.682
ß4 (face*lnEC)	-0.070	0.378	0.853	-0.812	0.672
ß5 (shop*lnEC)	-0.781	0.459	0.088	-1.680	0.118
% Variability explained by:					
Between subject differences:	26%				
Within subject differences:	74%				
number of observations: 87 (Excluded from original 103: 16 samples with 1NP and/or EC <LOD)					

The effect of the “face” or “shop” location group designations upon the relationship between EC and 1-NP are not statistically significant, as indicated by p-values for the ß4 and ß5 coefficients above 0.05 (p=0.85 & p=0.08, respectively).

Model 2: 1-NP vs EC & Fuel Type

The second multivariate regression for the association between 1-NP and EC included Fuel Type as an interaction term; the model output can be seen in Table 22 below.

Table 22: 1-NP vs. EC and Fuel Type Association Model

Regression Output: Natural Log-Transformed 1NP Concentration as a Function of Natural Log-Transformed EC with an Interaction Term for Fuel Type and Subject ID as a Random Effect					
Model Equation:	ln1NP = ß0 + ß1*lnEC + ß2*winter + ß3*winter*lnEC + b(subj)				
Where "winter"represents a dummy variable for winter fuel type (when the session=1 or 4); 0 or 1. b(subjid) represents the random effect term.					
Model Coefficients:	Value	SE	p-value	95% CI	
ß0 (intercept)	-10.609	0.373	<0.001	-11.340	-9.878
ß1 (lnEC)	0.485	0.159	0.002	0.174	0.796
ß2 (“winter” fuel group)	-0.511	0.493	0.300	-1.478	0.455
ß3 (winter*lnEC)	0.049	0.203	0.810	-0.349	0.446
% Variability explained by:					
Between subject differences:	27%				
Within subject differences:	73%				
number of observations: 87 (Excluded from original 103: 16 samples with 1NP and/or EC <LOD)					

The effect of the fuel type designations upon the relationship between EC and 1-NP is indicated by the statistical significance of the ß3 coefficient; it is not statistically significant to the 0.05 alpha level (p=0.810).

Model 3: 1-NP vs EC and Cigarettes Group

The third multivariate regression for the association between 1-NP and EC included Cigarette Group as an interaction term; the model output can be seen in Table 23 below.

Table 23: 1-NP vs. EC and Cigarettes Group Association Model

Regression Output: Natural Log-Transformed 1NP Concentration as a Function of Natural Log-Transformed EC with an Interaction Term for Cigarettes Group and Subject ID as a Random Effect					
Model Equation:	ln1NP = ß0 + ß1*lnEC + ß2*low + ß3*high + ß4*face*lnEC + ß5*shop*lnEC + b(subj)				
Where "low" and "high" represent dummy variable values of cig24group (low= 1-14 cigarettes per shift, high= >14 cigarettes per shift); each 0 or 1. b(subjid) represents the random effect term.					
Model Coefficients:	Value	SE	p-value	95% CI	
ß0 (intercept)	-11.479	1.024	<0.001	-13.487	-9.472
ß1 (lnEC)	0.572	0.386	0.138	-0.184	1.328
ß2 (“low” cigarettes group)	0.430	1.233	0.727	-1.986	2.846
ß3 (“high” cigarettes group)	0.885	1.074	0.410	-1.221	2.991
ß4 (low*lnEC)	-0.087	0.462	0.851	-0.992	0.818
ß5 (high*lnEC)	-0.135	0.407	0.739	-0.933	0.662
% Variability explained by:					
Between subject differences:	29%				
Within subject differences:	71%				
number of observations: 87 (Excluded from original 103: 16 samples with 1NP and/or EC <LOD)					

The effect of the “low” or “high” cigarette group designations upon the relationship between EC and 1-NP are not statistically significant, as indicated by p-values for the ß4 and ß5 coefficients above 0.05 (p=0.85 & p=0.73, respectively).

Aim 3: Predictive model for 1-NP using Survey and Intake Questionnaire Variables

Descriptive Statistics

The following variables were assessed for possible inclusion in a 1-NP predictive model: location group, primary task group, fuel.type, DOW, exp.cat, and time exposed to DE. Prior to inclusion in an association for regression with 1-NP and EC, an exploratory analysis was conducted on each.

Location Group

Descriptive statistics for 1-NP stratified by location group are provided above, in Figure 11 and Table 16. It is apparent that the distribution of 1-NP differs by location group, so this parameter was included in predictive model development.

Primary Task Group

The Primary Task Group was included as a parameter highly similar to the Location Group variable, but as a refinement of the "face" and "shop" Location Groups. Thus, it was not included in the same models as Location Group due to collinearity of tasks that were highly associated with certain locations. Summary statistics beyond task and task group frequency (as seen in Table 7 above) were not generated.

Fuel Type

Descriptive statistics for 1-NP stratified by Fuel Type are provided in Figure 13 and

Table 18 above. 1-NP distributions appear to differ (despite overlap of ranges) between fuel types, so this parameter was included in predictive model development.

Exposure Category

Descriptive statistics for 1-NP stratified by exposure category are provided above in Figure 15 and Table 19 above. As previously stated, 1-NP levels do not appear to be reflected by the exposure categories and there is no scientific basis for including them in a predictive model.

DOW

Descriptive statistics for 1-NP stratified by DOW are provided above in Figure 17 and Table 20. Despite the apparent uniformity of 1-NP distributions across days of the week, DOW was included in some prediction models for an exploration of possible model refinement.

Time exposed to DE

Subject-reported time exposed to diesel exhaust is the one continuous covariate considered for inclusion in the predictive model; summary statistics are provided in Table 24 below.

Table 24: Summary Statistics for Time Exposed to DE

	N	mean	SD	median	min	max
tDE, (hr)	103	6.43	3.33	8	0	10.5

Comparisons of tDE to 1-NP were not made prior to the regression for prediction; this parameter was automatically included in predictive model development for scientific reasons stated previously.

Regression Output

Regression model output for ten mixed models (with various combinations of covariate predictors), along with scatterplot displaying cross-validation predictions can be found in Appendix VIII; model assessment is summarized in Table 25 below. Models were numbered in the order they were conducted; they are ordered in the table in categories of similar covariate inclusions (Location group, Primary Task Group, and other) and in order of increasing complexity.

In addition to the ten mixed models, that all take the form *ln(1-NP) = ß0 + b(subj) + ß1*(covariate 1) + ß2*(covariate 2) + ßn*(covariate n)*, where *b(subj)* represents the subject ID random effect term, the first five models were also run and assessed excluding the subject ID random effect term. Model outputs and assessment for these additional analyses can be found in Appendix IX.

Table 25: Prediction Models Assessment

Model:	Covariates:	In-Sample Predictions:		Cross-Validated Predictions:	
		RMSE	MSE-based R^2	RMSE	MSE-based R^2
1	location group (1-3)	0.904	0.251	0.939	0.191
8	location group (1-3), time exp. to DE	0.901	0.256	0.941	0.188
7	location group (1-3), fuel type, time exp. to DE	0.901	0.255	0.945	0.180
3	location group (1-3), fuel type, DOW (1-4), time exp. to DE	0.882	0.287	0.959	0.156
2	primary task group (1-8)	0.826	0.374	0.902	0.254
10	primary task groups (1-8), time exp. to DE	0.826	0.375	0.904	0.251
9	primary task groups (1-8), fuel type, time exp. to DE	0.825	0.376	0.913	0.236
4	primary task groups (1-8), fuel type, DOW (1-4), time exp. to DE	0.814	0.393	0.936	0.197
5	location group (1-3) with nested primary task groups, fuel type, DOW (1-4), time exp. to DE	0.735	0.504	0.944	0.182
6	time-weighted task group (1-8), fuel type, DOW (1-4) , time exp. to DE	0.829	0.370	0.921	0.222

Out of all the cross-validated model r^2 values, the highest was produced by Model 2, which includes Primary Task Group as the only predictor. This model performs better than the equivalent alternative, Model 1 (with Location Group as the only predictor), with r^2 values of 0.254 and 0.191, respectively. The increasingly specified versions of Model 2 (Models 10, 9, and 4, in order of increasing number of covariates) all have higher r^2 values than any of the models using Location Group as opposed to Primary Task Group. As the models become more specified, the r^2 values decrease and RMSE values increase. A scatterplot of Model 2 cross-validated predictions is presented in Figure 21 below.

Also notable is the poorer prediction of Model 5 (r^2=0.182), with Primary Task Group refinements of the Location Group variable, than either of the Location Group or Primary Task Group models alone (Models 1, r2=0.191, and Model 2, r2=0.254, respectively). This is likely due to over-specification of the model. In addition, Time-Weighted Task Group, the more scientifically sophisticated variable than Primary Task Group, predicted 1-NP more poorly than the simpler variable (Model 6, r^2=0.222 vs Model 2, r^2=0.254). However, Model 6 performed better than any of the Location Group models.

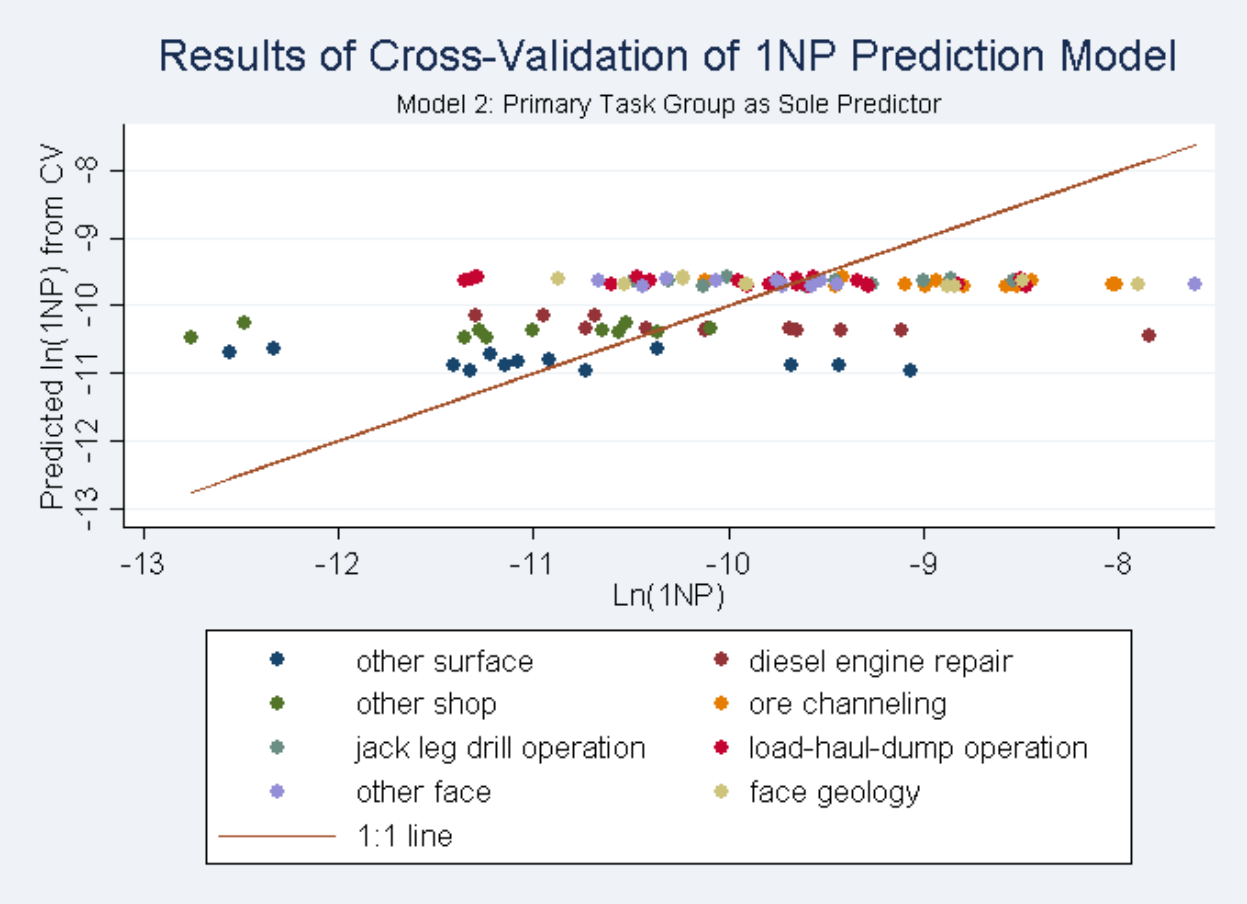

Figure 21: 1-NP Prediction Model Performance

The scatterplot visualization of Model 2 in Figure 21 above displays the overall poor predictive power of even the best-performing model; many predicted 1-NP values do not correspond well with the actual 1-NP values, as indicated by the disparity of points with the 1:1 line.

Additional Regression Output

As an additional analysis, the best-performing predictive model (Model 2, with Primary Task Group as the sole predictor) for 1-NP was also run using EC as the primary output variable instead of 1-NP. Model assessment is presented in Table 26 and Figure 22 below.

Table 26: EC Prediction Model Assessment

Model:	Covariates:	In-Sample Predictions:		Cross-Validated Predictions:	
		RMSE	MSE-based R^2	RMSE	MSE-based R^2
2 (EC)	Primary Task Group (1-8)	0.799	0.238	0.863	0.111

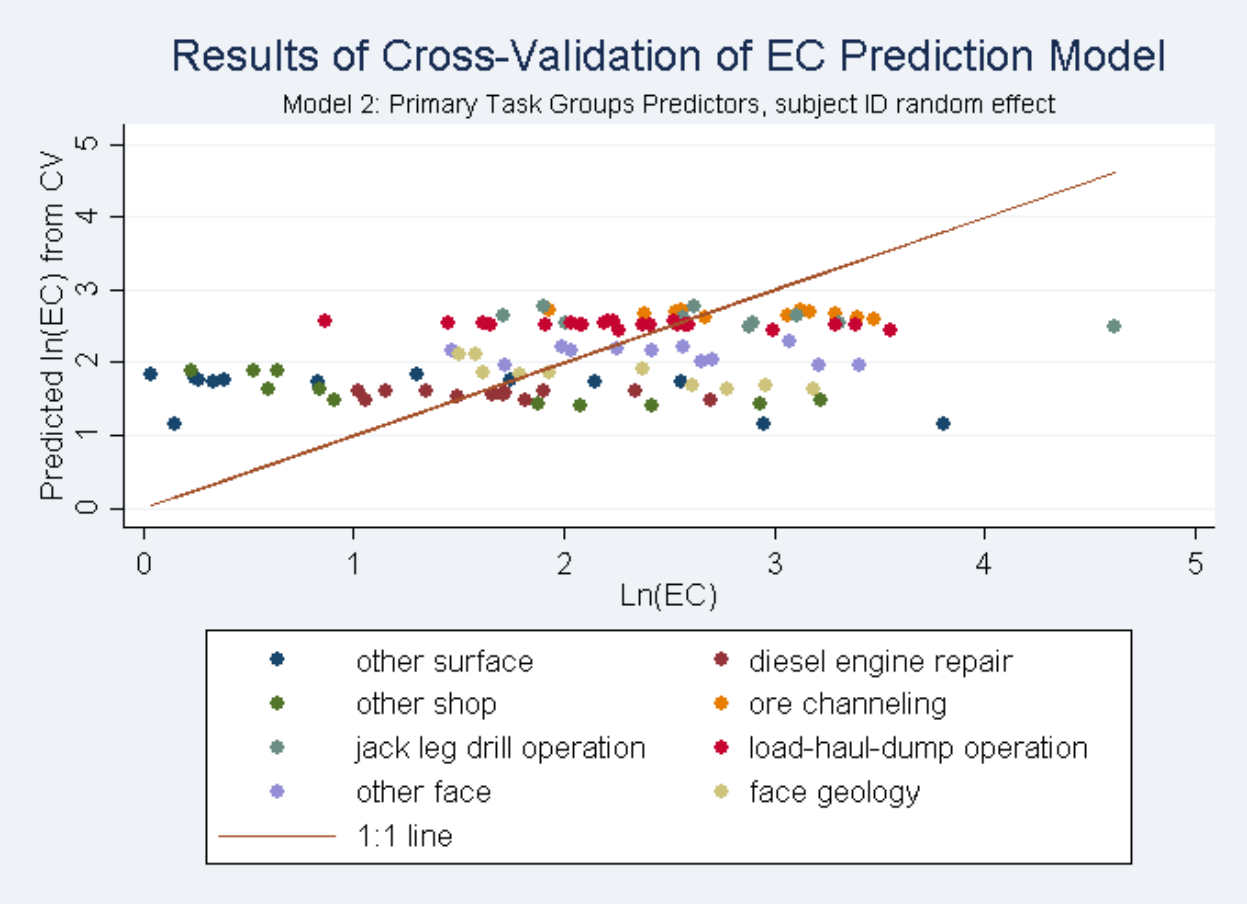

Figure 22: EC Prediction Model Performance

Overall, the EC prediction model with the same formulation as the 1-NP Model 2 did not perform as well as the 1-NP model (CV r^2=0.111 for EC, CV r^2=0.254 for 1-NP). This suggests that the task variables as obtained from this study survey may be better at predicting 1-NP than EC.

Discussion

Comparison of Data Collected to Regulation & Historical Studies

Of all valid samples examined in this study, no samples yielded a TC air concentration value over the MSHA DPM PEL of 160 µg/m^3 (TC maximum= 135 µg/m^3). This portrays the mine study site as an M/NM mine in compliance with MSHA standards, so ranges of the data collected for this study can be considered representative of similarly compliant underground M/NM mine settings.

1-Nitropyrene personal air measurements from this study have a mean of 78 pg/m^3 and a range of 2.89-497 pg/m^3 (GM 47 pg/m^3). Other indoor workplace DPM investigations of 1-NP found 1-NP values with

GMs ranging from 197-2483 pg/m^3 (personal samples from underground workers), and GMs from 2.8-1800 pg/m^3 (general indoor workplace samples) [Scheepers et al, 2003; IARC 2013]. The mean calculated from this study's data includes samples from aboveground workers, which pulls the mean and GM 1-NP values down lower than those found in the literature for underground settings, but the ranges approximately agree. Another observation to note is the recent implementations of DPM control measures by the study mine in recent years, which reduced their EC levels by about ten times. This could in part explain why 1-NP measurements made by this study are generally lower than those of other underground mine studies. Ambient air samples from the literature are notably lower and with few exceptions range tend to be about an order of magnitude lower than the reported maximum 1-NP concentration values measured in underground occupational settings.

Findings from 1-NP/EC/OC/TC Associations

The association between 1-NP and EC was found to be significant, with 1-NP expected to be 5.3% higher for every 10% increase in EC. The inclusion of an interaction term accounting for location in the mine, fuel type used, or number of cigarettes smoked did not produce statistically significant evidence of effect modification by any of the three factors. This indicates that 1-NP personal air concentration is positively associated with EC personal air concentration, regardless of the location worked,the fuel type used by the mine (either the summer fuel additive or the winter fuel additive), or the monitored subject's smoking status.

No associations between 1-NP and OC or 1-NP and TC were found to be statistically significant. These associations were heavily influenced by the large number of samples with OC concentrations below the limit of detection (76.7% below LOD for both OC and TC). This was a result of high OC content on field blank filters, which were averaged together from all campaigns to create a blank correction factor that was subtracted from all OC concentration values. Additionally the standard deviation of this field blank concentration was used to form the OC LOD; the SD for field blanks was high resulting in a high LOD. Overall, OC is expected to contribute very little to TC content in comparison with EC, so OC concentrations monitored were expected to be low. In regulation EC and TC are both trusted for monitoring DPM, but in this study the high LOD replacement for OC led to significantly biased TC values (as the sum of EC + OC) and the same proportion of unreliable TC values (76.7%). In future studies the collection of field blanks and analysis of OC filters could be more carefully investigated to identify and mitigate contributing factors to the high OC content of field blanks.

The original 1-NP/EC association model used 1-NP and EC data with values below the LOD replaced with LOD/√2; results are presented in Table 13. As a sensitivity analysis, a second 1-NP/EC association model was developed in which 1-NP and EC values below the LOD were left "as-is" (with all 1-NP values increased by 1 μg/m^3 prior to log-transformation); values below LOD were accounted for in the model by including an interaction term of a dummy variable indicating either the 1NP or EC value for a given sample is below the LOD; results are also presented in Table 13. The resulting expected change in 1-NP for a 10% change in EC is 5.3% and 4.7% for the LOD-replaced and LOD interaction term models, respectively. This represents a minor change in overall model output, and does not change the outcome. Both models appear equally robust, which is likely due to the small impact of the values below LOD, making up only 15.5% and 6.8% of all 1-NP and EC measurements, respectively.

Findings from 1-NP Prediction

Out of all the cross-validated model r^2 values, the highest was produced by Model 2, which includes Primary Task Group as the only predictor. This model performs better than the equivalent alternative, Model 1 (with Location Group as the only predictor), with r^2 values of 0.254 and 0.191, respectively. The increasingly specified versions of Model 2 (Models 10, 9, and 4, in order of increasing number of covariates) all have higher r^2 values than any of the models using Location Group as opposed to Primary Task Group. As the models become more specified, the r^2 values decrease and RMSE values increase.

Also notable is the poorer prediction of Model 5 (r^2=0.182), with Primary Task Group refinements of the Location Group variable, than either of the Location Group or Primary Task Group models alone (Models 1, r2=0.191, and Model 2, r2=0.254, respectively). This is likely due to over-specification of the model. In addition, Time-Weighted Task Group, the more scientifically sophisticated variable than Primary Task Group, predicted 1NP more poorly than the simpler variable (Model 6, r^2=0.222 vs Model 2, r^2=0.254). However, Model 6 performed better than any of the Location Group models.

The best-performing predictive model of the ten covariate combinations assessed was Model 2 (CV r^2=2.54), which included only one categorical variable predictor (split into 7 dummy variables in the model) representing the single task conducted during a given shift for a majority of the time. This variable is more precisely representative of a shift's exposure than the location group variable that simply lumps subjects into a "face", "shop" or "surface" group based on job title and time spent underground, and the model that includes the tasks group as opposed to the location group performs better (CV r^2=0.191 for the location group model). However, the tasks group splits the entire dataset up

into eight parts, some with as few as ten observations. Thus, the predictive model using the seven task group dummy variables is limited in its robustness.

The primary task group variable as the sole predictor led to better model performance than any models further specified by day of week, fuel type, or subject-reported time exposed to diesel exhaust. Adding these additional parameters most likely leads to over-specification of the model. Models were not investigated that contained only DOW, fuel type, and/or tDE, without either the location group or task group variables included, because location group or task group have the strongest scientific rationale for DE prediction by subject.

The task group variable was re-formatted to include time spent on each task, and a time-weighted task group variable was formed and run along with the DOW, fuel type, and tDE variables. This model did not perform markedly better than the models using the simpler (unweighted) primary task group variable (CV r^2=0.222).

Overall, the predictive model performance is poor (the highest CV r^2=0.254). The number of groups inherent to the best-performing model is too high for this study's small sample size and large amount of intercorrelated data due to repeat measurements on the same subjects. However, this model selection procedure does suggest that job tasks are useful predictors in 1-NP air concentration prediction.

This predictive model using the primary task group variable as the sole predictor was also used to predict EC personal air concentrations (as opposed to 1-NP). The model performance was poorer than that of 1-NP (CV r^2=0.111 for EC, CV r^2=0.254 for 1-NP). It would be expected that in a setting containing few (if any) interfering sources of EC or 1-NP, the tasks associated with different exposures to DE would predict EC and 1-NP personal air concentrations comparably well. In future, further investigation should be made into the ability of various survey data variables to predict 1-NP and EC in both an isolated setting (such as that of this study) and a setting with interference for EC or 1-NP. This could inform about the impact of interfering sources upon the ability to detect and predict either 1-NP or EC.

Limitations

The most significant limitation of this study is the small sample size, and the high number of repeated measurements per subject and resulting correlated data. This resulted in a larger effect of noise in the data, obscuring associations between DE components. Additionally, it created problems with implementation of categorical survey data in the regressions, because with every division of the data the sample size diminished greatly, with some groups having as few as ten observations. With the high

amount of correlation in the data, such small sample sizes are problematic and led to poor predictive ability in the 1-NP prediction model.

Another significant limitation is the blank correction method used for the OC data, and the high LOD derived from the same procedure. A blank correction factor was calculated by taking the average of the OC concentration from two field blank filters from each campaign (eight total), and this average was subtracted from each sample OC concentration. The standard deviation of the field blank filters was multiplied by three to yield the LOD. The field blank OC concentrations and SDs were large in relation to the samples, so with the blank subtraction and the high LOD many samples had values below LOD. With the statistical analyses used, these low values added noise to the data and disrupted the associations.

Future Steps

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 detection of EC and 1-NP. In future, a larger study should be conducted, with cohort members chosen based on the job location and task groups identified by this study. This would provide enough data to better assess effect modification of various shift conditions upon the 1-NP/EC relationship, and upon the ability to predict 1-NP.

Another future study worth considering is one in a setting that has interfering sources of EC and/or 1-NP, such as a coal mine (with carbon-containing coal dust), or a roadway truck (or other vehicle) driver study (with road dust). This would allow for an investigation of the comparative specificity of 1-NP to diesel exhaust, as opposed to EC. In a mine study similar to the current study (but with interfering EC sources), 1-NP predictor data from similar worker surveys and location-based job groups could be collected, and the association between 1-NP and EC, as well as prediction of 1-NP and EC personal air concentrations, would be compared against those of the current study. It would be expected that with interfering sources of EC present, the 1-NP/EC association would be comparatively poor in a coal mine compared to a metal mine. This would indicate that 1-NP is a more specific surrogate measure for DE.

Based on the observations from this study, minor details of data collection in a future study design can be refined. In future, job location within the mine could be better tracked for individual air-monitored shifts. Additionally, specific equipment used and for what duration could be better tracked. The task questionnaire component need not be as complex and comprehensive in future, especially considering the small impact of time-weighting on the regression analysis. Seasonal and day of sampling did not appear to have an effect on the 1-NP/EC relationship or the 1-NP predictions, so future sampling is

unlikely to be restricted by scheduling. Additionally, in future, DPM impactors can potentially be used for both EC/OC/TC and 1-NP determination.

Overall, 1-NP personal air concentrations were found to be consistently and positively associated with EC personal air concentrations in an underground metal mine setting, in which few (if any) sources of interference with EC or 1-NP are present.

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Appendices

Appendix I

Example Completed Survey

Subject Id 10 Tech Initials MP/JR Date 8/14/14

DE Exposure – Subject Daily Activity Log

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

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

Notes:

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

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

1a. ☐ Ore channeling 95 % 1b. ☐ Jack leg drill operation 2 % 1c. ☐ Load-Haul-Dump operation _____%

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

1g. ☐ Other: _____ _____%

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

0 %

Miner Activity Log_rev020613

QC Date:_____
QC Initial:____

Technician: Questions related to biosampling

Part 3: Technicians: Ask these questions at the end of the day about the previous day's activities, using the phrase, "In the last 24 hours have you..."

1. Smoked cigarettes?

☐ Yes ☒ No ☐ Don't know

→ _____ (number of cigarettes smoked)

2. Used chewing tobacco?

☒ Yes ☐ No ☐ Don't know

→ ___/___ (number of cans of chewing tobacco used)

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

☐ Yes ☒ No ☐ Don't know

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

3b. What types of activities were you doing?

Activities: _____

_____

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

_____%

Subject Id 10 Tech Initials MP/JR Date 8/14/14

Technician: Only ask part 4 on the first day of the work week

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

1. Smoked cigarettes?

☒ Yes ☐ No ☐ Don't know

→ 5 (number of cigarettes smoked)

2. Used chewing tobacco?

☒ Yes ☐ No ☐ Don't know

→ 1 (number of cans of chewing tobacco used)

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

☐ Yes ☒ No ☐ Don't know

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

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

_____

3c. What percent of your time spent doing these activities did you use a respirator?
0 %

Notes:

QC Date:_____
QC Initial:____

Appendix II

Extraction Procedure for 1-NP Analysis Batches #4&5

Procedure for Quartz (& Teflon) Filter Analysis Choices, Batch 4:

Filters for this experiment consist of a total of 18 Teflon filters and 18 quartz filters containing 1-NP collected during 2014 Stillwater Mine campaigns #3 and #4 (August and October). 6 Teflon filters were chosen based on a visual observation of no sample leakage around the filter and onto the backing and matched with the 6 quartz filters collected from the same subjects or areas on the same days. 6 Teflon filters were chosen based on a visual observation of a moderate area of leakage (indicated by the code A1) and matched with the appropriate quartz filters, and 6 Teflon filters were chosen based on an observation of severe leakage area (coded A2 or A3) and matched with corresponding quartz filters. Within each leakage category, samples were randomized using the Microsoft Excel "RAND" function.

Procedure for Quartz Filter Analysis Choices, Batch 5:

1. Started with a list of all quartz filter IDs from original study documents
2. Eliminated all filter IDs for which quartz 1-NP air concentration data already exists (those already extracted/analyzed)
3. Eliminated all filter IDs for which no sample was collected (due to a cohort dropout, etc.)
4. Eliminated all filter IDs for those with cassettes that had broken open in transit or storage
5. Eliminated all filter IDs for those with a short pump operation time recorded in the pump calibration field logs: less ½ of a normal shift, or < ~300 minutes
6. Eliminated all filter IDs for those with a % error between pre- and post-shift pump flow rates greater than 10%, as recorded in the pump calibration field logs.
7. Eliminated all filter IDs for those with a % error between pre- and post-shift pump flow rates greater than 5%, as recorded in the pump calibration field logs.
8. Eliminated a few additional filter IDs that had notes from air pump calibration sheets indicating that the pump came back stopped.

Appendix III

1-NP Analysis SOP

STANDARD OPERATING PROCEDURE (SOP) FOR NPAH ANALYSIS IN PARTICULATE MATTER SAMPLES

Prepared By:	Justin P. Miller-Schulze Graduate Student	Date:	2/19/08
Revised By:	Mike Paulsen Vanessa E. Galaviz Mike Paulsen Mike Paulsen Emily Carpenter Emily Carpenter Mike Paulsen	Date:	8/18/09 6/17/11 7/19/11 1/2/13 3/25/14 1/15/15 2/12/15
Reviewed By:		Date:	
Approved By:		Date:	

Introduction

1. This document describes the standard operating procedure for the analysis of nitro polycyclic aromatic hydrocarbons (NPAHs) in particulate matter (PM) samples. The extraction method is an ultrasonic liquid extraction, and the analysis method is a two-dimensional high performance liquid chromatography with tandem mass spectrometry (2D-HPLC-MS/MS) method. Initially, this method was intended to quantify only 1-nitropyrene (1-NP), the most abundant NPAH in diesel exhaust particulate matter (DPM). However, this method has been expanded to include two additional NPAH species, 2-nitropyrene (2-NP) and 2-nitrofluoranthene (2-NFl). Both 2-NP and 2-NFl are formed primarily through atmospheric processes. The method has the potential to include additional NPAHs, most likely 4-nitropyrene (4-NP) and 3-nitrofluoranthene (3-NFl). The 2D-HPLC-MS/MS method employs a deuterated internal standard (d_9-1dNP) for quantification of all NPAHs at this time. **.**

2. Other documents to be consulted are:
   a. Miller-Schulze, J.P., et al. *J. Chromatogr. A.* **1167** (2007) 154-16.Forms to use with the assay:

3. Form: 'NPAH stock solns.xls'
   a. Location: simpsonlab on 'Storage-a\projects' (S:) \Lab\SOPs\NP SOP\ NPAH Stock Solns

4. Form: 'FilterBenchsheet_1NP_Template.xls'
   a. Location: simpsonlab on 'Storage-a\projects' (S:) \Lab\SOPs\NP SOP\ Template Bench Sheets folder

5. Known limitations and interferences:

a. The efficacy of the 1-dNP internal standard for each NPAH analyte is not uniform. For example, the 1-dNP was chosen as an internal standard for 1-NP, and does an adequate job of correcting for extraction and analysis losses for this analyte. However, the efficacy of the 1-dNP as an internal standard for 2-NP is noticeably less than for 1-NP, with 2-NFl somewhere in between 1-NP and 2-NP.

Sample Collection

1. For the most part, as of the writing of this SOP, this extraction and analysis method has been applied to PM samples that were either purchased from NIST (i.e., standard reference materials, SRMs), or collected by a 3rd party (NIEHS filter samples, KC metro samples, etc). As such, this SOP will not address procedures and/or issues associated with sample collection.

Materials

1. Chromasolve ethanol for HPLC (denatured) 95% + 5% IPA by volume, Sigma Aldrich cat# 270741-2L. Used for rinsing/cleaning glassware, making reconstitute extraction solution, making mobile phase for the HPLC-MS/MS, and NPAH stock solution used for calibrants and spiking solution
2. Acetic acid. Glacial, reagent ACS, spectrum cat# A1010. 500 ml. Density = 1.053 g/ml. Used for making buffer[1]
3. Sodium acetate anhydrous. Sigma. Cat: S8750-1KG. M = 82.03 g/mol. Used for making buffer
4. Methanol. Fisher Optima cat# A454-4. Used for rinsing/cleaning glassware, and making mobile phase for the HPLC-MS/MS
5. Formic acid. GRACS EM Science cat# FX0440-1. Used for making mobile phase for the HPLC-MS/MS
6. L-L-Ascorbic acid. 99+% Sigma Aldrich cat# 25564. 100g. M = 176.12 g/mol. Used for making mobile phase for the HPLC-MS/MS
7. Aminopyrene. Aldrich A77903-250MG. Used as a positive control for HPLC-MS/MS
8. Dichloromethane (Methylene Chloride). Baker analyzed A.C.S. Cat# 4L 9324-03. Used for extracting 1-NP from filters, and rinsing/cleaning conical-bottom and round-bottom screw top glass tubes and teflon-lined screw caps.
9. Five ml conical-bottom screw-top glass tube (Kimble Chase No.73785-5) and Teflon-lined screw cap (Kimble Chase No. 73802-13415, a less expensive alternative is Supelco 27141). (Note: Always use new glass tubes and caps for extraction to prevent contamination)
10. Amber autosampler vial with aluminum (PTFE-lined rubber septa) crimp cap (Autosampler vial does not need to be silanized prior to use because sample will be in a silanized insert.)
11. Silanized glass vial insert, with plastic "foot"
12. Glass 9" pasteur pipettes, silanized
13. Plastic 1 ml syringe (Fisher cat# 14-823-2E)
14. 13 mm Syringe filter with 0.2 μm PTFE membrane (Pall Life Sciences PN 4542)
15. Disposable flow control valve liner (Supelco cat 57059)

[1] Buffer is referring to 20 mM Sodium Acetate/Acetic Acid, pH 5.5 solution.

Equipment

1. One (1) ultrasonic water bath, with rack submerged in water
2. One (1) low-volume Turbovap evaporative concentrator and equipped with N_2 gas cylinder (N_2 tank lasts 2-6 weeks unused)
3. One (1) sample vortexer
4. Two (2) 25 µl positive displacement pipettors. For sensitive trace analysis, one pipettor should be segregated from general use so as to prevent high levels of NPAHs from being in contact with pipettor (particularly 1-NP and 1-AP) To be used ONLY for spiking 1-dNP on filters and calibrants
5. One (1) filter cutter for the separation of filters from PMP support rings
6. Diaper pads to cover lab bench to prevent contamination of samples, glassware, etc., by lab bench
7. One (1) Gilson pipettor (negative displacement type with plastic tips) for aqueous solvents, for dispensing volumes of 150 µl
8. Autosampler vial crimper and decrimper
9. Agilent 1100 HPLC
10. Agilent 6410 Triple Quad Mass Spectrometer
11. Two (2) external LC pumps
12. One (1) Helium gas cylinder with line split into 2, with inline filters attached, for sparging of reservoirs hooked up to external LC pumps
13. LC columns:
    a. AC-1: Waters Xterra RP18 2.1 x 150 mm, d_p=3.5 µm, with Waters guard column. P/N 186000410. Store in 80:20 MeOH:water
    b. AC-2: Agilent Zorbax SB-Phenyl, 2.1 x 150 mm, d_p=3.5 µm. Special order. Store in 100% ACN.
    c. TC: Waters Atlantis RP18 2.1 x 30 mm, d_p= 3 µm. 186001287. Store in 100% ACN.
    d. RC: Specially made reduction column, Pt/Rh, 4 x 10 mm. Store in 80:20 MeOH:water - DO NOT store in ACN.

Reagents

1. Extraction reagents
    a. 150 µl 75% ethanol/25% 20 mM sodium acetate/acetic acid, pH=5.5 sample matrix per sample extract. (Note: the sample matrix is different from the mobile phase, which is a mixture of 85:15 of the same solvents.) Solution is stable for 7 days. Store 4˚C. Total volume 100 ml.
        i. First make buffer. Total volume 0.5 L. Solution is stable for 7 days. Store 4˚C
            1. Base: 20 mM sodium acetate
                a. Using graduated cylinder measure 500 ml of DDI water and pour into a 500 ml glass bottle
                b. Weigh 0.82 grams of sodium acetate and add to DDI water. Shake until dissolved
            2. Acid: 20 mM acetic acid. (density = 1.05; MW = 60.05) Solution is stable for 7 days. Store 4°C

a. To a graduated cylinder add 0.57 ml of acetic acid then fill to 500 ml with DDI water then pour into 500 ml glass bottle. Swirl to mix
3. Calibrate pH meter using pH 4 and 7 buffers
4. Pour 300 ml sodium acetate solution into 1 L bottle
5. Pour 25 ml acetic acid solution
6. Using 9" glass pasteur pipette transfer needed solution until pH is 5.5

ii. Transfer 75 ml of ethanol into a 100 ml glass bottle
iii. Transfer 25 ml of buffer into 75 ml of ethanol and shake

2. LC/MS mobile phases
   a. The 2D-HPLC-MS/MS analysis method requires the following reagents/solvents (All glass bottles are reused and prior to each use should be rinsed with DDI water):
      i. One (1) liter methanol w/ 0.01% formic acid (Volume is dependent on length of sequence, which is dependent on number of samples). Stable for 7 days. Store at room temperature. Dispose in "bulk HPLC waste" jug.
         1. Measure 1 L of methanol with graduated cylinder and pour into 1 L glass bottle.
         2. Pipet 100 µl formic acid into 1 L methanol with positive displacement pipet.
         3. Shake
      ii. One (1) liter DDI H_2O w/ 0.01% formic acid (See above regarding volume). Stable for 7 days. Store RT. Dispose of down drain
         1. Measure 1 L of DDI water with graduated cylinder and pour into 1 L glass bottle
         2. Pipet 100 µl formic acid into 1 L DDI water with positive displacement pipet.
         3. Shake
      iii. ½ Liter (0.5 L) 85% ethanol/15% 0.020 M sodium acetate/acetic acid, pH=5.5. Stable for 7 days. Store 4°C. Dispose in "bulk HPLC waste" jug.
         1. Transfer 425 ml of ethanol into a 500 ml glass bottle
         2. Transfer 75 ml of buffer into 425 ml of ethanol and shake
      iv. One (1) liter 0.01 M L-Ascorbic Acid in H_2O. Reactive so make day of running HPLC-MS/MS. Dispose of down drain
         1. 0.01 moles/L = (176.12 g/ x mole) * 1 L → 1.76 g
         2. Using graduated cylinder measure 1 L of DDI water and pour into 1 L glass bottle
         3. Weigh out 1.76 grams of L-Ascorbic acid then add to 1 L glass bottle. Shake until dissolved.

Spike solutions and calibration standards

1. 1-dNP filter spiking solution. This solution should be prepared such that the concentration of 1-d-NP in the final extract (150 µl volume) is the same as the concentration in the calibrants (assuming 100% recovery from the spiked filter)
   a. Presently (3/25/14) we are spiking as follows:
      i. Calibrants with 15.6 µl of 10^{-7} M (1000 µl final volume)
      ii. Filters with 23.4 µl of 10^{-8} M 1-dNP (150 µl final extract volume)

Preparation of d-NP spiking solutions from 10^{-6} M stock

	Stock Conc (µg/µl)	Volume to add (µl)	Total Volume (µl)
Use for Calibrants	2.57E-06		

1dNP 10^{-7}M			
Stock 1dNP 10^{-6}M	0.000257	200	
100%EtOH		1800	2000
Use for Filter Spiking			
1dNP 10^{-8}M	2.57E-06		
Stock 1dNP 10^{-6}M	0.000257	100	
100%EtOH		9900	10000

2. A set of calibrants, with ascending levels of NPAH, i.e., from 0.5 fg/µl to 5000 fg/µl 1-NP, 2-NP, 2-NFl, etc., and constant level of 1-dNP internal standard, i.e., 400 fg/µl. Exact [1-dNP] will depend on anticipated NPAH levels in PM. Calibrants should be made day of or day before running the LCMS. Run 50 fg/ul calibrant and blank every 15 samples. Use silanized 4 ml and 2 ml glass amber vials for preparing calibrants. Rinse 4 ml vials with chromasolve ethanol before each re-use. Use new 2 ml silanized autosampler vials for each set of calibrants.

a. Stock solution is 6667 fg/µl each: 1-NP, 2-NP, 2-NFl in 100% chromasolve ethanol [Stored at -20°C in 40 ml amber vial.] Stock solution is used to make both calibrants and 1000 fg/uL spiking solution. Make enough stock and spiking solution to be used for all samples in a study.

	Stock Conc (µg/µl)	**Stock Conc (ng/µl)**	**Volume to add (µl)**	**Spike Solution Conc (fg/µl)**
1-NP	2.47E-04	0.247	270	6669
2-NFl	2.79E-03	2.794	23.9	6678
2-NP	3.46E-03	3.46	19.3	6678
100%EtOH			9687	

b. Reuse 4 ml vials for calibrants. Prior to each use empty leftover calibrants from low to high concentration into a clean beaker. Rinse vials with MeOH from squirt bottle, shake, pour into the beaker, and tap vial on Kimwipe to remove MeOH. Clean one vial at a time, working from low to high concentration taking extra precautions not to cross-contaminate vials. If in doubt, use new vials.

c. Prepare calibrants in 4 ml vials accord to the table below. Transfer 1 ml of each calibrant to an empty, silanized HPLC vial. For zero calibrant, transfer 1 ml of EtOH/buffer (75/25, pH=5.5) into silanized HPLC vial.

d. Remove 15.6 µl from each vial (DON'T USE SEGREGATED 25 µl POSITIVE DISPLACEMENT PIPETTOR). Work from low to high concentration using the same tip, then thoroughly clean the pipet.

e. Transfer 300 µl internal standard spike solution to a silanized autosampler vial. This will be used to spike the calibrants, working from low to high concentration, and then discarded. Use care to avoid contamination of the stock internal standard spike solution with calibration standards.

f. Add 15.6 µl internal standard solution (10^{-7} M 1-dNP) (USE SEGREGATED 25 µl POSITIVE DISPLACEMENT PIPETTOR)

Calibration Standards

	NPAH Cal	Stock Vol	Stock Conc	EtOH/buffer	Total Vol	Cal Conc
Cal #	Conc (fg/uL)	(uL)	(fg/uL)	Vol (mL)	(mL)	(fg/uL)
10	5000	1125	6667	0.375	1.5	5000
9	1000	225	6667	1.275	1.5	1000
8	500	112.5	6667	1.3875	1.5	500
7	100	60	6667	3.94	4	100
6	50	30	6667	3.97	4	50
5	10	80	500	3.92	4	10
4	5	40	500	3.96	4	5
3	2.5	100	50	1.9	2	2.5
2	1	80	50	3.92	4	1
1	0.5	40	50	3.96	4	0.5
0	0	0	0	4	4	0

Note: The 5000 fg/ul standard is only needed for very high concentration samples. The diesel miner filters from March 2014 had a few filters higher than the 1000 fg/ul standard, so future samples from this project should include all standards shown above.

3. Preparation of NPAH spike mix 1000 fg/ul from 6667 fg/ul stock. This is for spiking the "Fortified" and "CN" quality control samples.

NPAH 1000 Spike Mix

	Concentration (fg/µl)	Volume to add (ml)
Chromosolve EtOH	100%	8.5
NPAH mix	6667	1.50

4. Preparation of aminopyrene (AP) reduction standard. This standard is used to assess the efficiency of the online catalytic reduction column in the HPLC-MS system. Make fresh prior to each HPLC-MS/MS run.

AP Reduction Standard

Dilution	Solvent	Concentration
Stock	N/A	5000 ug/ml
Dilute 25ul to 25ml	Chromasolv EtOH	5 ng/ul
Dilute 25ul to 25ml	Chromasolv EtOH	5 pg/ul
Dilute 200ul to 20ml	75% ChromasolvEtOH: 25% buffer	50 fg/ul

Stored in headspace vials in white rack, top shelf of freezer.

5. Instrument blank consisting of EtOH (make 3 of them or more for longer sequences). No need for exact volume.

6. Prepare 3 needle wash vials filled with EtOH. Use autosampler vials with a screw cap and remove cap when starting the analysis.

Extraction Procedure (takes about 4 hrs)

1. Use benchsheet to record ID numbers (filter, QC, and standards) and notes relating to sample processing and dates prepared.
2. Extraction procedure should be done in a chemical fume hood.
3. When using a positive displacement pipettor, rinse tip with EtOH from squirt bottle. Put capillary tip on and clean pipette with EtOH, dispensing into waste beaker (DON'T touch waste beaker with tip). Do this a couple of times.
4. Before transferring filters into silanized glass vials rinse tweezers and scissors with methylene chloride from squirt bottle and wipe down with kimwipe to prevent touching filter with wet scissors and/or tweezers.
5. Quality Control (QC) samples are to be prepared with each set of filter samples. Specifically, these QC samples should include, in duplicate:
   a. 1-dNP Controls/Deuterated Filters. This QC sample is a blank filter spiked with the same 1-dNP spike volume and solution as the rest of the samples. The 1-dNP control is designed to give a measure of the recovery of the extraction procedure.
      Spike as follows:
      i. 23.4 µl of 10^{-8} M 1-dNP
   b. Positive Controls/Fortified Filters. This QC sample is a blank filter spiked with both the equivalent 1-dNP spike, as described for the 1-dNP Controls, as well as a moderately high concentration spike of the NPAH analytes being measured in the assay. The positive control is designed to give a measure of the recovery for each NPAH and possible interaction of the NPAH analytes with each other.
      Spike as follows::
      i. 23.4 µl of 10^{-8} M 1-dNP (150 µl final extract volume)
      ii. 25 ul of 1000 fg/ul NPAH (150 ul final extract volume)
   c. Filter Blanks. This QC sample is simply an unspiked filter that is put through the entire extraction procedure. The extract blank is designed to give a measure of the contamination of the filter, glassware and/or solvents used in the extraction procedure.
   d. Controls (CN): This QC sample consists of 1-dNP and NPAH analytes (as described for the positive controls/fortified filters) spiked into a silanized 5 ml conical-bottom screw-top glass tube. This QC sample is designed to give an idea of the recovery for just the 2D-LC-MS/MS analysis, i.e., excluding the extraction. **Prepare the CN samples at the time of evaporating the methylene chloride.**
      Spike as follows:
      i. 23.4 µl of 10^{-8} M 1-dNP (150 µl final extract volume)
      ii. 25 ul of 1000 fg/ul NPAH (150 µl final extract volume)
6. If filters have PMP support rings, cut filter out of ring using extraction solvent-rinsed (methylene chloride) filter cutter, and with solvent-rinsed forceps deposit detached filter into silanized, labeled, extraction-solvent rinsed 5 ml conical screw-top tube. (Note: PMP support rings may or may not need to be cut off depending on analytes to be measured and detection system to be used. PMP support ring interferes with PAH (by GC-MS) analysis. It does not

appear to interfere with the NPAH and the LG assays. (Personal communication with Chris Simpson)

7. Assign sample numbers to filters in randomized order for extraction and print labels for each step of analysis.
8. Spike filters with 23.4 µl of 1-dNP spiking solution (using the aforementioned calculated spike concentration and volume) using a segregated 25 µl positive displacement pipettor.
9. [continue formatting here]

i. As quality control filters, include:

1. 2 deuterated filters with 23.4 µl of 10^{-8} M 1-dNP
2. 2 fortified filters with 23.4 µl of 10^{-8} M 1-dNP and 25 ul of 1000 fg/µl NPAH spiking solution
3. 2 blank filters without any spikes (can be field or lab blanks)
10. Let spiked samples age for 30 minutes. (EtOH is evaporating and NPAHs are binding to filter and air particles on the filter.
11. Add 7 ml of CH_2Cl_2 extraction solvent to each sample tube with 10 ml graduated pipette or with a pipette dispenser. These 7 ml volumes need not be quantitative.
12. Sonicate all capped tubes for 60 minutes at full power. Use the lid on the sonicator to exclude light. Use care to avoid spilling samples and wipe tubes with Kimwipe after taken out of bath.
13. Remove filter from 5 ml tube using a 9" silanized glass Pasteur pipet. Use care to not break the pipet. If the pipet breaks, transfer the extract to a new tube.
14. Place tubes with extract into low volume Turbovap (equilibrated ~30 minutes prior such that water bath is at T=45ºC). Position tubes such that the nozzles extend slightly into the vials (the tube should be several mm off the bottom of the rack). Start with low nitrogen flow to avoid splattering samples. Increase flow gradually while observing liquid to confirm the sample is not splattering. Nozzles should be wiped with methanol or ethanol-soaked Kimwipe prior to use.
15. Evaporate CH_2Cl_2 extraction solvent just to dryness.

a. Include two control (CN) samples at this step.

16. If extraction procedure is to be suspended at this point, all tubes should be evacuated with N_2. If N_2 evacuated tubes have been stored in freezer overnight, allow them to temperature equilibrate for ~30 minutes.
17. econstitute dried extracts with 150 µl 75% EtOH/OAc sample matrix using 200 µl Gilson pipettor (negative displacement type with plastic tips). Pipettor tip need not be changed after dispensing a single aliquot unless tip touches glass during process.
18. Sonicate all extract tubes for 10 minutes at full power in ultrasonic bath.
19. Vortex all extract tubes in rack for 10 minutes at power level = 3 using sample vortexer.
20. Using sample matrix (75% EtOH:25% 20 mM pH 5.5 acetate buffer)-rinsed glass 9" or 5.5" pasteur pipette, transfer entire reconstituted extract volume from 5 ml tube into plastic 1 ml syringe equipped with 0.2 µm syringe filter. A fresh syringe and filter set should be used for each sample.
21. Depress plunger into syringe to expel reconstituted volume into silanized glass autosampler vial insert situated in "foot" inside labeled amber autosampler vial. Use care to avoid spilling sample. NEW INDIVIDUALS SHOULD PRACTICE THIS STEP BEFORE PROCESSING SAMPLES; IF ONE FINDS IT DIFFICULT, SPE EXTENTION TUBES CAN BE USED.

22. After expelling entire volume into vial insert, cap vial with aluminum crimp cap with PTFE-lined rubber septa.
23. Analyze extract or store in -20°C freezer (EtOH content high enough to not freeze sample.)

Instrumental Analysis

1. Analyze all extracts and QC samples using 2D-HPLC-MS/MS method with internal standard calibration. Run time per sample is 38 minutes
2. To prepare 2D-HPLC system, use the following procedure:
    a. Use the "degas" function of the ultrasonic bath to "degas" mobile phases associated with external HPLC pumps: 0.01 M L-Ascorbic Acid, and 85% EtOH/15% OAc. To do this:
        i. Insert mobile phases, two at a time, into ultrasonic bath, and verify that water level is such that at least ¼ of reservoir bottle is submerged.
        ii. Remove cap from reservoir and place lead donut over reservoir bottle.
        iii. Attach vacuum line with #4 black rubber stopper to reservoir bottle.
        iv. Turn on ultrasonic bath and start the "degas" function. Red indicator should then illuminate, showing a reversed "6".
        v. Turn vacuum on.
        vi. egassing should take about 9 minutes. R
    b. Once mobile phases have been degassed, all pumps should be purged with the appropriate solvent. The Agilent pump should be purged with MeOH w/0.01% FA (Channel B) and H_2O w/0.01% FA (Channel A). The Biorad external pump should be purged with the 85/15 EtOH/OAc mobile phase, and the Shimadzu external pump should be purged with the 0.01 M L-Ascorbic Acid mobile phase. The Agilent pump can be purged simply by opening the purge valve on the pump and the setting the flow rate to 5 ml/min and running at this flow rate for ~5 minutes. The external pumps have to have their PEEK tubing outlets run into a beaker and run at ~9 ml/min. for 3-5 minutes.
    c. To prep the reduction column (RC) for the analysis, it should be "back flushed" (have mobile phase run through the column in the reverse direction) with the 85/15 EtOH/OAc mobile phase for 10 minutes at 0.15 ml/min. This can be done with the autosampler, guard column, and AC-1 (all situated in the "forward" direction) upstream of the RC. Back flushing times much longer than 10 minutes should be avoided.
    d. After back flushing the reduction column and purging all pumps with the appropriate mobile phases, the system should be configured as shown in the attached figure 1.
3. Additional notes regarding HPLC operation
    a. Present method is 2010 Methods\NPAH_100812h.m
    b. Biorad pump → autosampler (port 1) → AC1
        i. Flow is 0.1 ml/min
        ii. This pump has pressure limits that have to be re-set each time the power is cycled. Set high limit to 325 and low limit to 7.
    c. Shimadzu pump (10 mM L-Ascorbic acid)
        i. Diluent
        ii. 0.5 ml/min

d. Pressure during trapping
  i. BioRad: 220-260 bar
  ii. Shimadzu: 120 bar
  iii. Agilent: 140-150

e. Pressure during non-trapping period
  i. BioRad: 110-130 bar
  ii. Shimadzu: 5 bar
  iii. Agilent: 190-200

f. Trapping window is 4-12 minutes (changed from 4.5-11.5 at the start of sequence MP041414 during troubleshooting low sensitivity)

g. Be careful with the backpressure needle valve because it is sensitive to small adjustments! [no adjustment has been needed since the method was established]

4. Instrument initial evaluation to assess LC/MS and the reduction efficiency
  a. AP (50 fg/µl X 50 µl injection = 2500 fg on column)
  b. Standard 50 (50 fg/µl X 50 µl injection = 2500 fg on column)
  c. Blank

	MW	STD Conc (fg/ul)	Inj. Vol. (ul)	Mass on column (fg)	fMoles on column	Relative Molar Conc.
1-NP	247	50	50	2500	10.12	100%
1-AP	217	50	50	2500	11.52	114%

Therefore, AP50 should provide a 14% higher response than NP50

5. Worklist order after evaluating system performance
  a. Calibration curve from low to high standard
  b. 2 blanks (first blank is likely to have detectable carryover from the high standard)
  c. 15 samples
  d. Check standard
  e. Blank
  f. Repeat 15 samples, check standard and blank until complete
  g. Include at least two instrument duplicates

6. Instrument shutdown
  a. Turn temperature off and remove columns from heater
  b. Turn flows down
    i. Agilent: decrease from 0.225 to 0.1
    ii. BioRad: decrease from 0.1 to 0.05
    iii. Shimadzu: decrease from 0.5 to 0.2
  c. Flow slowly until the system is cool.
  d. Stop flow
  e. Agilent A and B into 100% ACN purge.
  f. Purge Shimadzu and BioRad with 80:20 MeOH: H_2O.

g. Flush AC-2 and trap with ACN.
h. Flush AC-1 and Reduction column with 80:20 MeOH: H_2O.
i. Monitor pressure until stable plus 10 minutes.
j. Remove flush solvents from pumps.
k. Wrap inlet and sparge lines with clean aluminum foil for storage
l. Turn off He.
*Must not leave Shimadzu or BioRad helium sparge tubing in solvent without gas flow or solvent will be drawn into the line.

System Component	Upstream Connection	Downstream Connection
BioRad pump *Shimadzu pump ("pump a") starting 12/09/14	**85/15 EtOH/OAc**	**Agilent autosampler port 1**
Guard col/AC-1/RC	**Agilent autosampler port 2**	**Mixing T (side port)**
Shimadzu pump ("pump c")	**10 mM L-Ascorbic acid**	**Mixing T (side port)**
Switching valve port 1		**AC-2**
Switching valve port 2	**Agilent pump**	
Switching valve port 3	**Trapping column outlet**	
Switching valve port 4		**Waste**
Switching valve port 5	**Mixing T**	
Switching valve port 6	**Trapping column inlet**	
AC-2	**Switching valve port 1**	**Detector**
Agilent pump	**MeOH/water/ 0.01% formic acid**	**Switching valve port 2**

Backpressure Log

Pump	BioRad	BioRad	Shimadzu	Shimadzu	Agilent	Agilent
Switch Valve Position	Eluting: Column 1	Trapping: Column 2	Eluting	Trapping	Eluting: 0.2mL/min	Trapping
Pressure Date: 8/26/09	143-155	238-263	60	135	185	142
9/4/2009					182	140
New AC-1 9/17/09	148-166	195-230	60	130	182	142
2/4/2010	150	160 w/pump#2 0.3 ml/min & 260 w/pump#2 0.5 ml/min	0	90	117	90
5/13/11	124-142		185			
5/17/11	125-136	288-263	2	145	154-158	115-117
6/24/11	120-134	269-296	20	180	198	136

1/23/13 (17:28)	112-120		5		191	
1/23/13 (17:30)		226-248		125		138
1/23/13 (17:55)	111-123		5		187	
1/23/13 (18:09)		224-243		125		137
1/24/13 (11:06)	110-122		5		182	
1/24/13 (16:00)		227-251		135		136
1/24/13 (17:33)	111-123		10		185	
1/25/13 (11:15)	111-124		10-15		186	
C Pyke filter training set						
3/20/14 (17:39)	114-128	219-246	5	120	166	134
3/20/14 (19:40)	109-126		5		188	
3/21/14 (02:39)	116-129		5		197	
3/21/14 (02:48)	114-128		5		189	
3/21/14 (02:58)		219-245		120		
3/21/14 (03:05)	114-125		5		186	
3/21/14 (10:24)	114-127		5		194	
Diesel mine filters March 2014						
4/17/2014 (10:42)		228-255		120		146
4/17/2014 (10:44)	121-130		5			
4/17/2014 (10:45)	116-130					196
4/18/2014 (13:00)	111-119				188	
Problem with signal after run 71. Replaced guard column and holder. Restarted at run 81 with std 50 (13:48)						
4/18/2014 (16:02)	120-129		5		188	
4/18/2014 (23:11)	117-131		5			191
Diesel mine filters June 2014						
7/21/14 (14:45)	110-122	220-239	5	110	193	142
7/21/14 (15:55)	110-123		5		192	
7/21/14 (16:53)	116-125	227-253	5	120	197	147
7/21/14 (21:36)	117-126	216-240	5	110	193	141
7/21/14 (22:00)	116-125		5		196	
7/22/14 (06:26)	117-126	229-247	5	120	195	144
7/22/14 (07:36)	115-123		5		188	
7/22/14 (12:31)	119-127		5		200	
7/22/14 (13:34)	111-124		5		191	
7/22/14 (17:33)	115-124		5		196	
7/23/14 (05:41)	117-126	232-251	5	120	199	145
7/23/14 (12:02)	117-127		5		200	
7/23/14 (16:36)		223-250		120		143
7/23/14 (18:06)	112-125		5		195	
7/24/14 (05:25)	115-124	234-263	5	125	193	145

7/24/14 (11:40)		229-258		130		148
7/24/14 (14:53)	113-127		5		204	
7/24/14 (17:32)	113-126		5		195	
7/25/14 (05:30)	114-123		5		195	

Diesel Mine Fillters Leakage Check Aug-Oct 2014						
Replaced Biorad pump with a Shimadzu pump ("pump a") due to problems with backpressure falling to "zero" after each pump stroke. Suspect either a leak inside the pump or a problem with the pressure monitor. "New" Shimadzu pump is making a periodic (~1/min) loud click sound accompanied by a movement of the pump head.						
Pump	Shimadzu "pump a"	Shimadzu "pump a"	Shimadzu "pump c"	Shimadzu "pump c"	Agilent	Agilent
Switch Valve Position	Eluting: Column 1	Trapping: Column 2	Eluting	Trapping	Eluting: 0.2mL/min	Trapping
Pressure Date:	(kg/cm2)	(kg/cm2)	(kg/cm2)	(kg/cm2)	(bar)	(bar)
12/10/14 (11:22)	65-67	145-160	0-5	105-118	204	147
12/11/14 (8:47)	80		5		205	
Problem with high background starting at Line 25 (Run 25) Seto filter 6. ISTD area also decreased. System gradually recovering. ~20 minutes flush system after run 35. Agilent pump set to 100% B. Pump C set to 0.1 mL/min. Pump A set to 0.2 mL/min 85% EtOH. Trapping configuration sensitivity restored after flushing.						
12/11/14 (10:40)	75		5		199	
12/11/14 (12:09)	75-80	160	5	115	203	147
12/12/14 (10:46)	80	160	5	115	194	147
Flush agilent components w/100% MeOH. System is in eluting configuration, so trap also gets flushed w/MeOH. Between data files _74 and _75.						
12/12/14 (14:04)	75		5		202	
Flush agilent with 100% MeOH after running Seto-6 and 9 1:10 dilutions (after data file _80)						
12/12/14 (16:27)		155		115		147
12/12/14 (22:50)	80		5		202	
12/13/14 (11:03)	85		5		198	

Note: Shimadzu pressure depends partly on the positioning of the backpressure valve

NPAH LC/MS/MS Backpressure Log Form

Eluting or Trapping	Worklist Line	Date	Time of Day	Run Time	Agilent Pressure (bar)	Shimadzu A Pressure (kgf/cm2)	Shimadzu C Pressure (kgf/cm2)	Notes

Data Analysis

1. File/New Batch (choose file location and name)
2. File/Add Samples (select all samples for batch and edit sample type to sample/cal, etc and assign cal levels
3. Select the highest calibrant (highlight)

Stata Calibration Curve Regression

- Stata regression command (replace "onenp_ratio" and "onenp_conc" with the variable name for the area to internal standard ratio, and standard concentration respectively.

regress onenp_ratio onenp_conc [aweight=1/onenp_conc]

- Scatter plot with linear fit line, title, and axis labels. Replace "1-NP calibration curve" with appropriate title.

twoway (scatter onenp_ratio onenp_conc) (lfit onenp_ratio onenp_conc), ytitle(Response ratio) xtitle(Standard Conc (fg/ul)) title(1-NP calibration curve) legend(off)

Calculate concentrations in Excel: concentration = (ratio-_cons)/coef.

LCMS columns

Column	SN	Date In	Date Out
AC-1[a]	17432024123-02		
AC-2[b]	USBNH01017		
Trap[c]	146311711		
Reduction[d]	10053-11287		
Guard[e]	017-332-0371		

a. AC-1: Waters Xterra RP18 2.1 x 150 mm, d_p=3.5 μm, with Waters guard column. P/N 186000410. Store in 80:20 MeOH:water

b. AC-2: Agilent Zorbax SB-Phenyl, 2.1 x 150 mm, d_p=3.5 μm. Special order. Store in 100% ACN.

c. TC: Waters Atlantis RP18 2.1 x 30 mm, d_p= 3 μm. 186001287. Store in 100% ACN.

d. RC: Specially made reduction column, Pt/Rh, 4 x 10 mm. Store in 80:20 MeOH:water - DO NOT store in ACN.

e. Guard: Waters 186 000 634

Enter summary table with sample concentrations from historic studies

Limit of Detection calculation

To determine the LOD, the mean and standard deviation of the measured concentration in the deuterated standards were calculated. The LOD was defined as the Mean concentration of the deuterated samples plus 3 standard deviations. [Note: the LOQ we published for the Healthy Border study was blank + 2SD and concentrations below the LOQ were replaced with the LOQ]

LOD=Avg + 3 SD

LOD values

Date	Study	Analyte	Mean Blank (fg/ul)	SD	LOD fg/ul	LOD fg/m3
	Hlthy Bdr					
	Hlthy Bdr					
	Hlthy Bdr					
	Thailand	1-NP	0.737	0.536	2.344	
	Thailand	2-NP	0.798	0.185	1.352	
	Thailand	2-NFl	2.133	0.409	3.361	
Mar-14	DE Mine	1-NP	1.23	0.13	1.62	
Mar-14	DE Mine	2-NP	1.43	0.16	1.92	
Mar-14	DE Mine	2-NFl	0.74	0.18	1.27	
Jun-14	DE Mine	1-NP	1.31	0.13	1.69	
Jun-14	DE Mine	2-NP	1.38	0.05	1.51	
Jun-14	DE Mine	2-NFl	1.23	0.08	1.48	
Aug-14	DE Mine	1-NP				
Aug-14	DE Mine	2-NP				
Aug-14	DE Mine	2-NFl				
Oct-14	DE Mine	1-NP				
Oct-14	DE Mine	2-NP				
Oct-14	DE Mine	2-NFl				
	Seto China	1-NP				
	Seto China	2-NP				
	Seto China	2-NFl				

HPLC-MS/MS Parameters

Acquisition Method Info

Method Name	NPAH_100812h.m
Method Path	E:\MassHunter\methods\2010 Methods\NPAH_100812h.m
Method Description	

Device List

ALS
Quat
Pump
MS QQQ

QQQ Mass Spectrometer

Ion Source	ESI
Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump
Stop Time	1
Time Filter	On
Time Filter Width	0.07

Time Segments

Time Seg #	Time	Scan Type	Ion Mode	Polarity	Div Valve	Delta EMV	Store
1	0	MRM	ESI	Positive	To Waste	0	☐
2	20	MRM	ESI	Positive	To MS	1400	☑
3	34.4	MRM	ESI	Positive	To Waste	0	☐

Time Segment 1

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)
Compound1	☐	350	Unit	200	Unit	200	135	0

Source Parameters

Parameter	Value
Gas Temp (°C)	350
Gas Flow (l/min)	3
Nebulizer (psi)	50

Capillary (V) 2500

Time Segment 2

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)
d9-1-NP	☑	227.1	Wide	210.2	Wide	200	150	30
1-NP,2NP, 2NFL	☐	218.2	Wide	201.1	Wide	200	150	30

Source Parameters

Parameter	Value
Gas Temp (°C)	350
Gas Flow (l/min)	12
Nebulizer (psi)	50
Capillary (V)	3000

Time Segment 3

Scan Segments

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)
Compound1	☐	350	Unit	200	Unit	200	135	0

Source Parameters

Parameter	Value
Gas Temp (°C)	350
Gas Flow (l/min)	3
Nebulizer (psi)	50
Capillary (V)	2500

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	0	10000000

Instrument Curves

Actual

#N/A

Autosampler

Name	ALS	Model	G1313A
Ordinal #	1	Options	

Stop Time (min) As Pump Post Time (min) Off

Injection Type	Needle Wash	Injection Volume	50
Overlap Time	Overlap when flushed	Draw Position	3
Draw Speed	400	Eject Speed	400
Wash Vessel	91		
Ready Temp. Range		Temp.	

Contact 1	0
Contact 2	0
Contact 3	0
Contact 4	0

Quaternary Pump

Name	Quat Pump	Model	G1311A
Ordinal #	1	Options	

Stop Time (min)	34.5	Post Time (min)	Off

Flow (µl/min)	0.225	Pressure Min (bar)	0
Pressure Max (bar)	300	Max Flow Gradient (ml/min)	100

Solvent A	Water w/0.01% Formic	Solvent B	MeOH w/0.01% Formic
Solvent C		Solvent D	
Solvent Ratio A	55	Solvent Ratio B	45
Solvent Ratio C	0	Solvent Ratio D	0
Compress. A (*10-6/bar)	50	Stroke A	Auto
Primary Channel	0		

Contact 1	0
Contact 2	0
Contact 3	0
Contact 4	0

Pump Time Table

Time	Flow	Pressure	Solv Ratio B	Solv Ratio C	Solv Ratio D
0	0.225	300	45	0	0
11	No Change	No Change	45	0	0
31.5	No Change	No Change	55	0	0
31.6	No Change	No Change	60	0	0
32.5	No Change	No Change	60	0	0
33	No Change	No Change	45	0	0

Signals Selected

Description

Pressure

Flow

Solvent% A

Solvent% B

Appendix IV

Quality Control Summary Tables for All 5 1-NP Analysis Batches

QC Summaries for All 1NP Analysis Batches

1-NP, Teflon:

Batch 1: Spring 2014 analysis of Campaign 1 filters

Sample	Peak Area	Mean Conc. (fg/uL)	Std. Dev.	C.V.	Recovery	% of control
Blank	49	-	-	-	-	-
Deuterated	89	1.2	0.1	12%	-	-
Fortified	57272	147.6	4.1	3%	89%	102%
Control	58738	145.0	4.9	3%	87%	-

Batch 2: Summer 2014 analysis of Campaign 2 filters

Sample	Peak Area	Mean Conc. (fg/uL)	Std. Dev.	C.V.	Recovery	% of control
Blank	789			-	-	-
Deuterated	838	1.3	0.13	10%	-	
Fortified	150569	150	3.40	2%	90%	101%
Control	133427	149	8.40	6%	89%	-

Batch 3: Fall 2014 analysis of Campaign 2 filters

Batch 4: December 2014 analysis of Campaign 3&4 filters

Sample	Peak Area	Mean Conc. (fg/uL)	Std. Dev.	C.V.	Recovery	% of control
Field Blank (FB)	584.5	1.15	0.23	20%	-	-
Lab Blank (LB)	1108	1732	1244	72%	-	-
Deuterated (D)	289	0.25	0.38	153 %	-	-
Fortified (F)	83813	164	22	14%	98%	118%
Control (CN)	94925.14	138.3 9	4.66	3%	83%	-

1-NP, Quartz:

Batch 1: Spring 2014 analysis of Campaign 1 filters

Batch 2: Summer 2014 analysis of Campaign 2 filters

Batch 3: Fall 2014 analysis of Campaign 2 filters

Sample	Peak Area	Mean Conc. (fg/uL)	Std. Dev.	C.V.	Recovery	% of control
Blank	238			-	-	-
Deuterated	311	3.9	0.54	14%	-	
Fortified	13068	139	5.78	4%	83%	90%
Control	20332	154	16.21	11%	92%	-

Batch 4: December 2014 analysis of Campaign 3&4 filters

Sample	Peak Area	Mean Conc. (fg/uL)	Std. Dev.	C.V.	Recovery	% of control
Field Blank (FB)	964.5	1.93	1.71	88%	-	-
Lab Blank (LB)	1175	246	78	32%	-	-
Deuterated (D)	414	1	0.30	59%	-	-
Fortified (F)	85693	151	6	4%	90%	109%
Control (CN)	94925.14	138.3 9	4.66	3%	83%	-

Batch 5: February 2015 analysis of Campaigns 1-4 filters						
Sample	Peak Area	Mean Conc. (fg/uL)	Std. Dev.	C.V.	Recovery	% of control
Field Blank (FB)	738.5	2.22	2.08	94%	-	-
Lab Blank (LB)	890	-	158	-	-	-
Deuterated (D)	719.50	4.72	0.81	17%	-	-
Fortified (F)	22243	136	11.54	9%	81.4%	112%
Control (CN)	28165.77	121.59	9.53	8%	73.0%	-

Batch 5: February 2015 analysis of Campaigns 1-4 filters						
Sample	Peak Area	Mean Conc. (fg/uL)	Std. Dev.	C.V.	Recovery	% of control
Field Blank (FB)	1120.9	5.30	6.19	117%	-	-
Lab Blank (LB)	1273	-	613	-	-	-
Deuterated (D)	2.40	2.40	1.35	56%	-	-
Fortified (F)	29001	127	6.07	5%	76.4%	105%
Control (CN)	28165.77	121.59	9.53	8%	73.0%	-

LOD Calculations:

Batch 1: Spring 2014 analysis of Campaign 1 filters

Instrument LOD (deuterated)

Sample	Conc. Mean (fg/ul)	Std. Dev.	LOD (+3SD)	LOQ (+10SD)
1-NP	1.23	0.13	1.62	2.54
2-NP	1.43	0.16	1.92	3.07
2-NFl	0.74	0.18	1.27	2.51

Batch 2: Summer 2014 analysis of Campaign 2 filters

Instrument LOD (deuterated)

Sample	Conc. Mean (fg/ul)	Std. Dev.	LOD (+3SD)	LOQ (+10SD)
1-NP	1.31	0.13	1.69	2.59
2-NP	1.38	0.05	1.51	1.83
2-NFl	1.23	0.08	1.48	2.06

Batch 3: Fall 2014 analysis of Campaign 2 filters

Instrument LOD (deuterated)

Sample	Conc. Mean (fg/ul)	Std. Dev.	LOD (+3SD)	LOQ (+10SD)
1-NP	4.11	0.55	5.76	9.60
2-NP	1.03	0.47	2.45	5.76
2-NFl	2.78	0.41	4.02	6.90

Batch 4: December 2014 analysis of Campaign 3&4 filters

Instrument LOD (field blank)

Sample	Conc. Mean (fg/ul)	Std. Dev.	LOD (+3SD)	LOQ (+10SD)
1-NP	0.96	0.97	3.87	10.66
2-NP	0.85	0.49	2.34	5.79
2-NFl	2.02	1.36	6.09	15.57

Batch 5: February 2015 analysis of Campaigns 1-4 filters

Instrument LOD (deuterated)

Sample	Conc. Mean (fg/ul)	Std. Dev.	LOD (+3SD)	LOQ (+10SD)
1-NP	4.28	4.49	17.75	49.17
2-NP				
2-NFl				

Appendix V

Compilation Procedure for Air Data Files

Air data used in this study consists of a variety of forms, all from different sources, laboratories, and batches. 1-NP sample concentration data was obtained by a series of filter extraction and LCMS/MS analyses conducted and compiled by the Simpson laboratory in Seattle, WA, in a total of five batches conducted over a span of time from 2014-2015. The first batch consisted of Teflon filters collected during the March campaign (session 1). The second batch analyzed Teflon and Sioutas filters collected during the June campaign (session 2). The third batch analyzed Quartz filters (DPM filter elements) collected during the June campaign. The fourth batch analyzed a small selection of both Teflon and Quartz filters collected during the August (session 3) and October (session 4) campaigns. The fifth batch consisted of a large selection of Quartz filters collected during all 4 campaigns, and a small number of Teflon filters collected from the August and October campaigns, as well as some Sioutas filters collected during all 4 campaigns. 1-NP data from each batch was saved to a separate excel worksheet. EC, OC, and TC sample concentration data was obtained from an external laboratory in the form of 4 excel worksheet reports, one for each campaign. Sample air concentration data was obtained by importing pump calibration field log data into excel worksheets (one for each campaign), conducted by the lab of Dale Stephenson at Boise State University, Idaho.

These separate excel worksheets were checked for errors and then locked from editing and saved in a central location. Then all relevant data was imported into Stata software [StataCorp, 2013] and merged into a single dataset. Air volume data was combined with 1-NP, EC, OC, and TC sample concentration data to yield air concentration data of all analytes for each sample. This final compiled dataset was then exported to a single excel worksheet for use in statistical analyses.

Handwritten paper surveys from all campaigns were collected, scanned as PDFs, and stored by the Simpson laboratory. Data from survey PDFs was then manually entered into a single excel worksheet. Fields left blank by subjects were guessed by members of the Simpson laboratory based on extrapolation of each subject's previous and other field responses, and flagged. After data entry into the excel worksheet, this table was given a quality control check by a separate member of the Simpson laboratory by review of the table entries and PDF survey scans for 10% of all surveys entered. During the QC review, a few errors in entry were discovered, representing an error rate of 1.04% erroneous fields of all fields checked. This excel worksheet was then locked from editing and stored in a central location for statistical analysis.

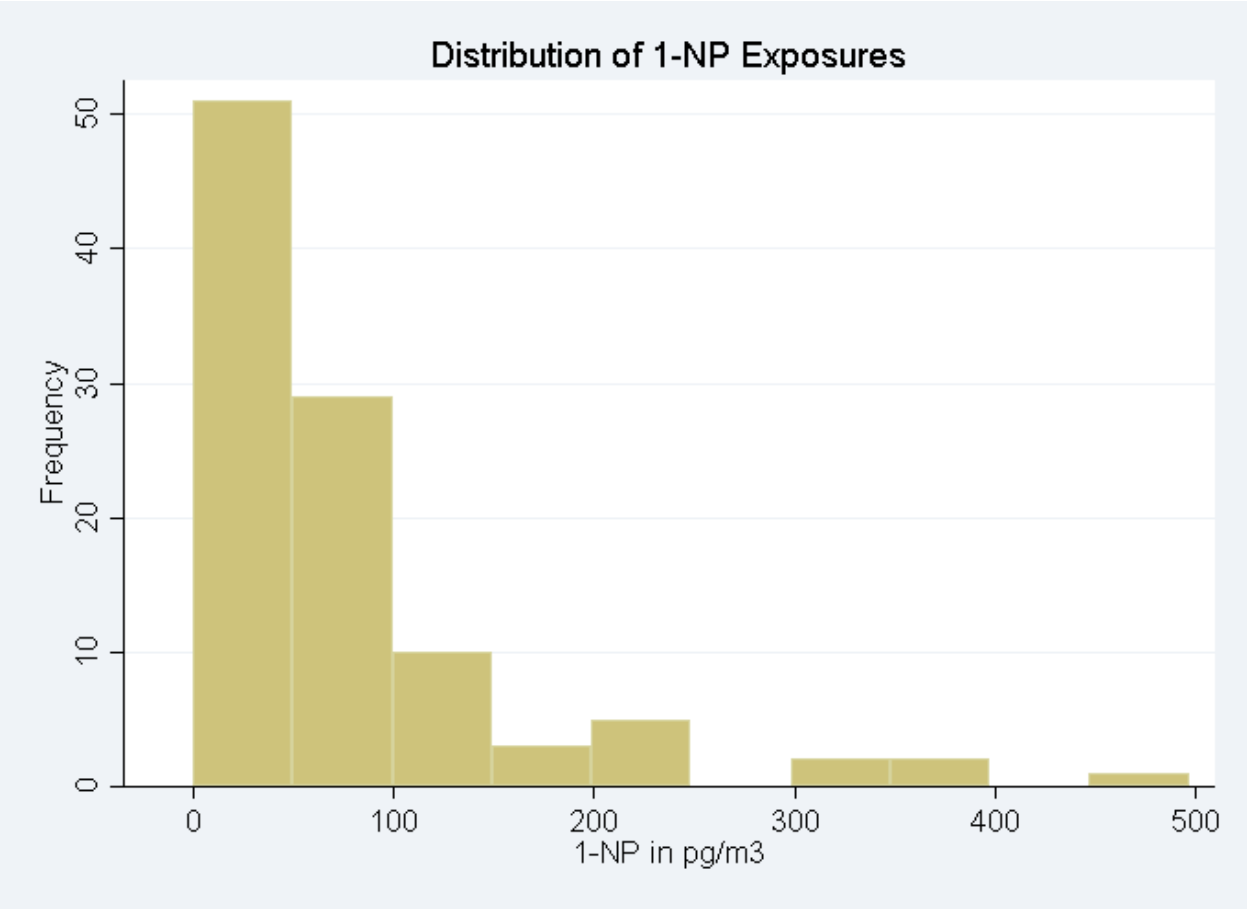
Appendix VI

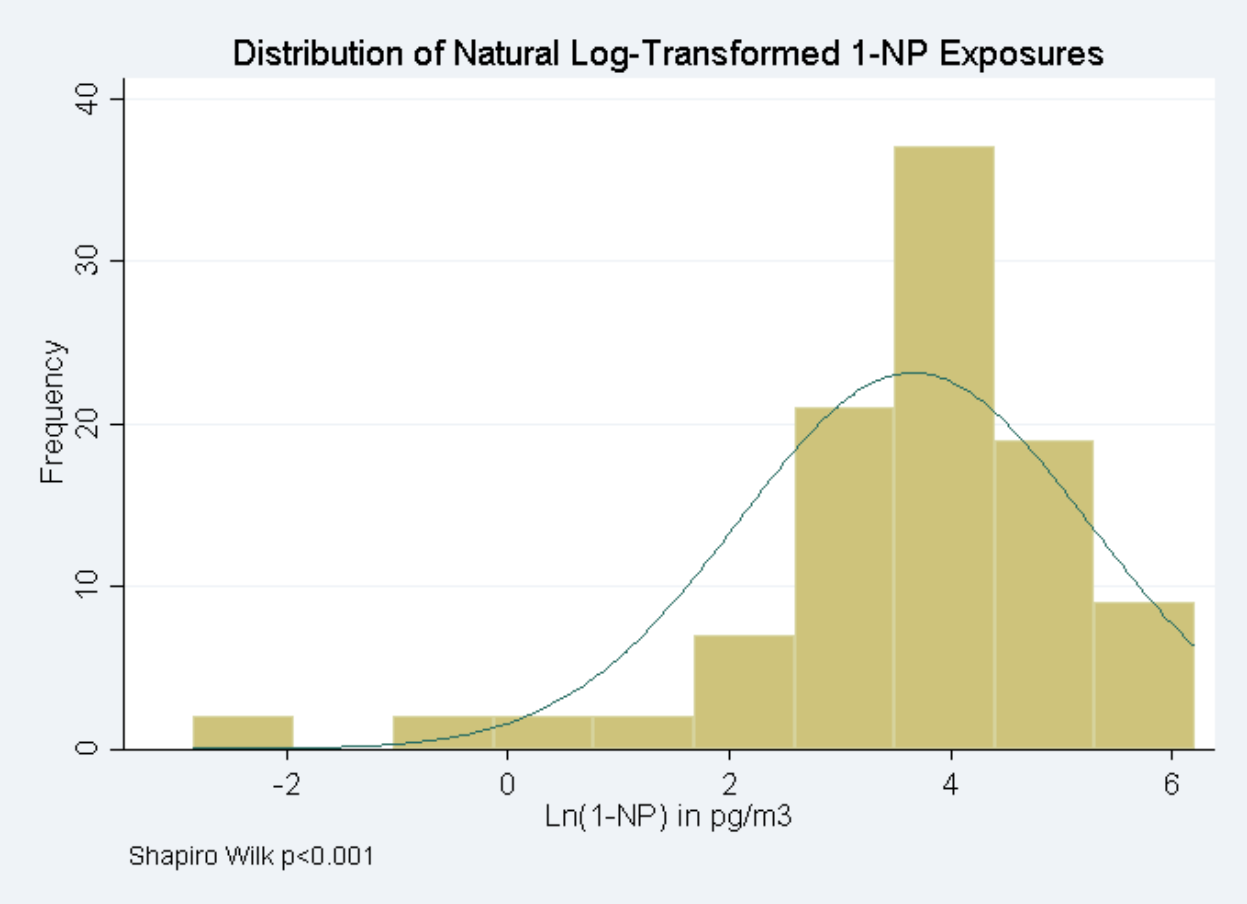
Task Reporting Matrix

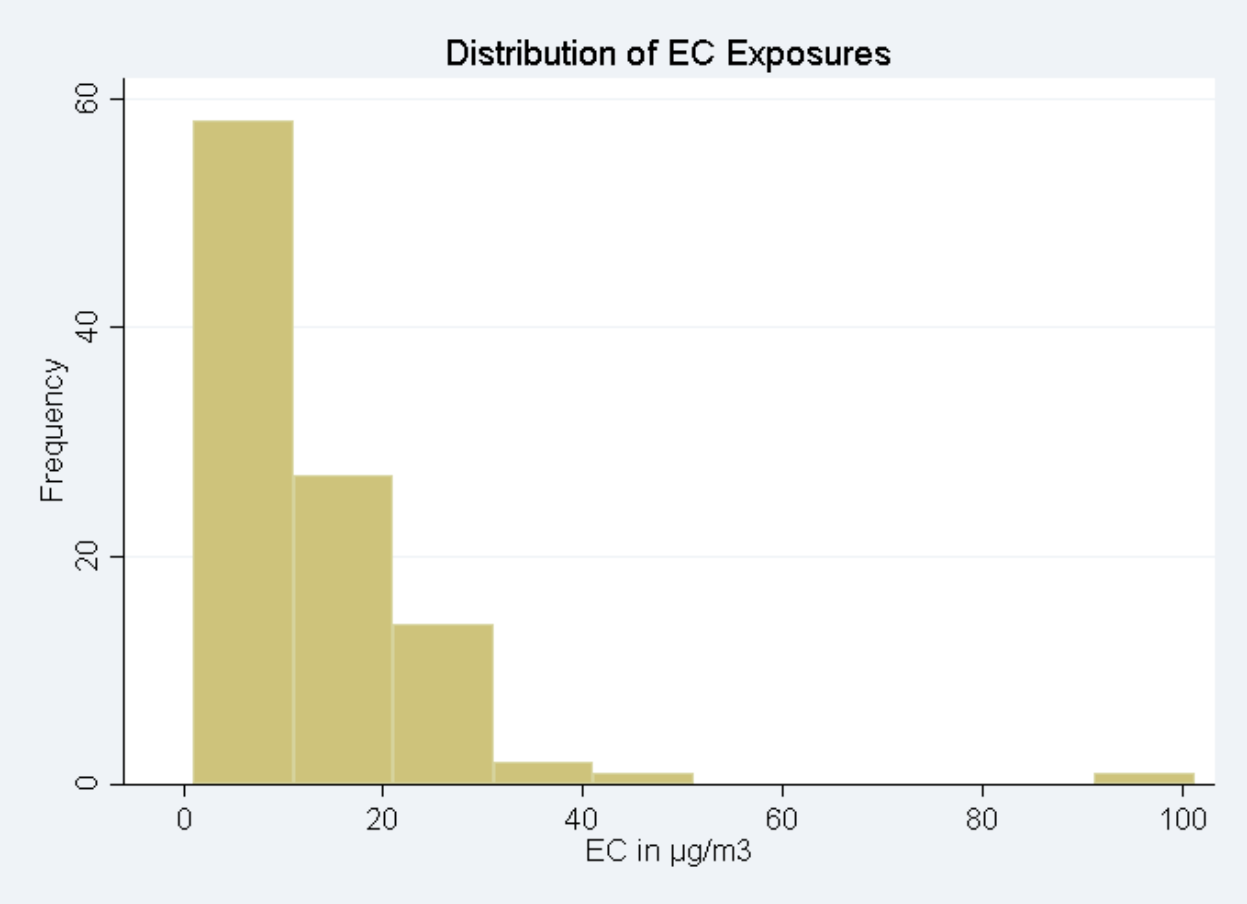
Frequency that tasks were reported together (diagonal is number of times task was reported alone)																														
	n	Ore Channeling	Jack leg drill operator	Load-Haul-Dump operation	Cage Tending	Diesel Engine Repair	Above ground office work	Sandplant	Geology	Electrician	Utilities	Maintenance & Repair	Pumphouse/Mill (surface)	Bolting, loading, & drilling	Pasteplant (Surface)	Training	Testing New Equipment	Mechanic	Equipment Haulage	Sand filling	Travel in Mine	Prepping Face to Drill	Jumbo Drill	Prepping to Shotcrete	Shotcrete	Clean-up	Mucking	Rise bore pilot	Out of Mine
n		72	47	76	4	30	21	12	27	10	15	10	12	9	2	1	1	9	1	1	1	1	1	1	2	1	8	3	1
Ore Channeling	72	11	35	32	4	1	13				1			1							1	1							
Jack leg drill operator	47	35	2	12	3	2	6														1						6		1
Load-Haul-Dump operation	76	32	12	30	4		18				2			1					1										
Cage Tending	4	4	3	4	0		4																						
Diesel Engine Repair	30	1	2			28																					1		
Above ground office work	21	13	6	18	4		1																						
Sandplant	12							12																					
Geology	27								27																				
Electrician	10									10																			
Utilities	15	1		2							12																		
Maintenance & Repair	10											9																1	
Pumphouse/Mill (surface)	12												12																
Bolting, loading, & drilling	9	1		1										7															
Pasteplant (Surface)	2														2														
Training	1															1													
Testing New Equipment	1																1												
Mechanic	9																	9											
Equipment Haulage	1			1															0										
Sand filling	1																			1									
Travel in Mine	1	1	1																		0								
Prepping Face to Drill	1	1																				0							
Jumbo Drill	1																						1						
Prepping to Shotcrete	1																							1					
Shotcrete	2																								2				
Clean-up	1																									1			
Mucking	8		6			1																					2		
Rise bore pilot	3											1																2	
Out of Mine	1		1																										0

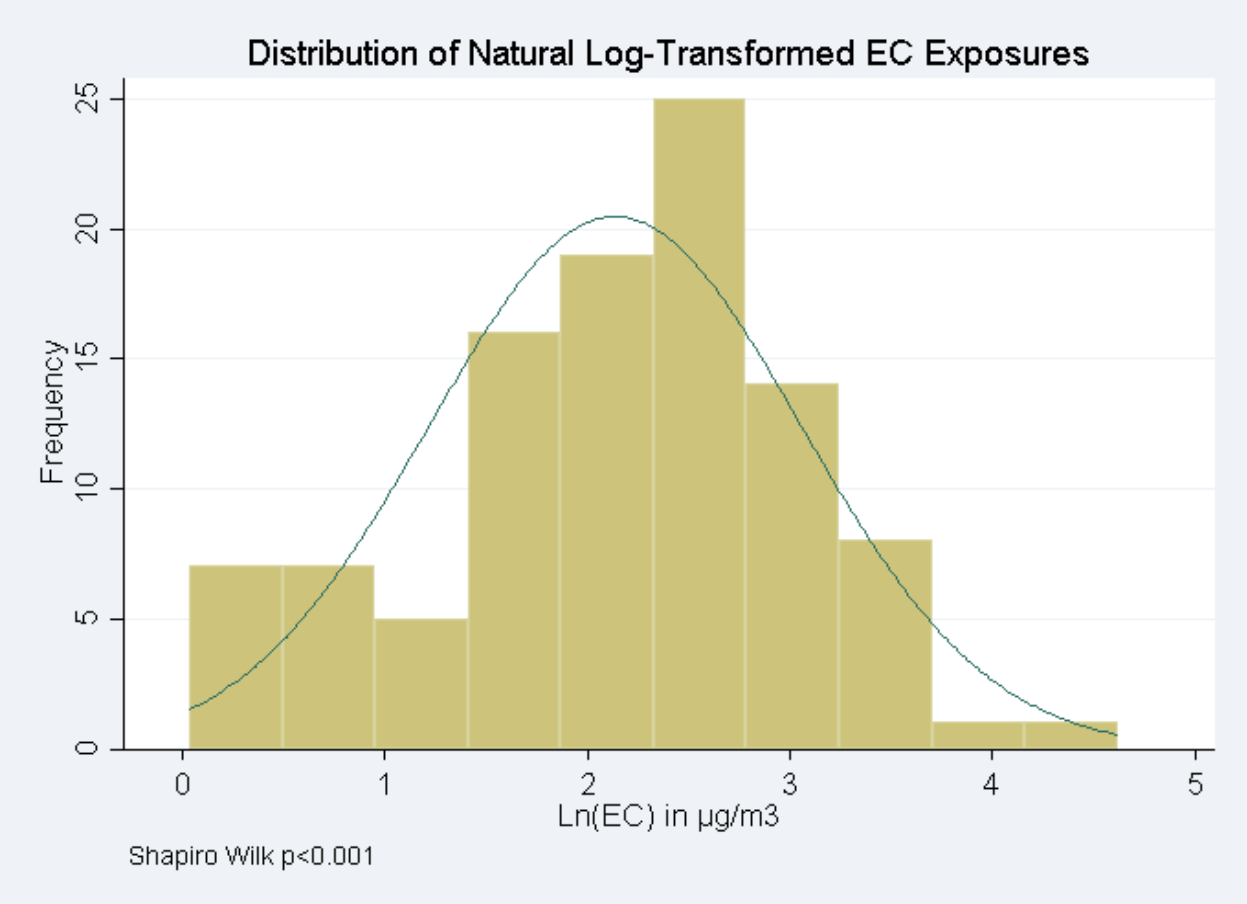
Appendix VII

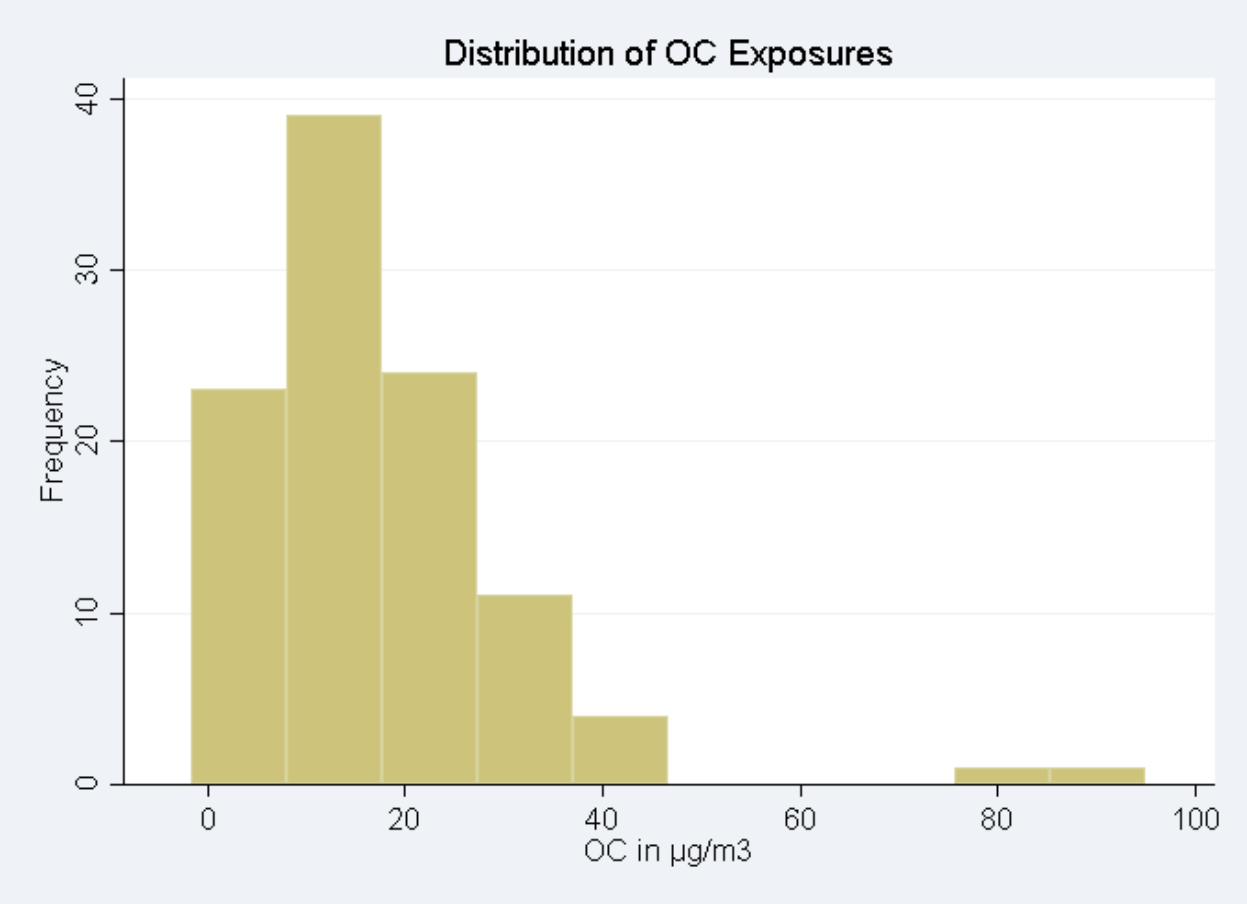
1-NP, EC, OC, and TC Lognormality Assessment

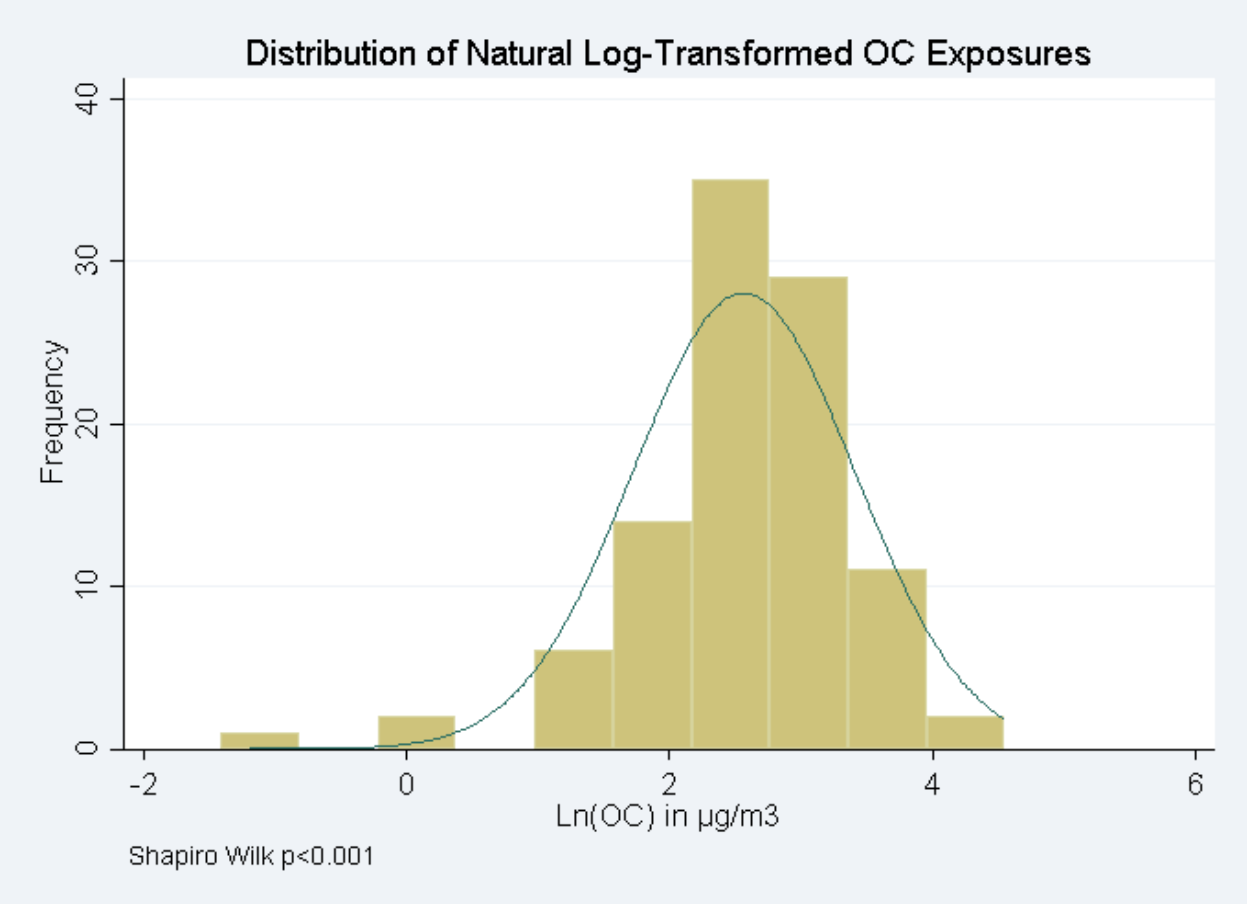


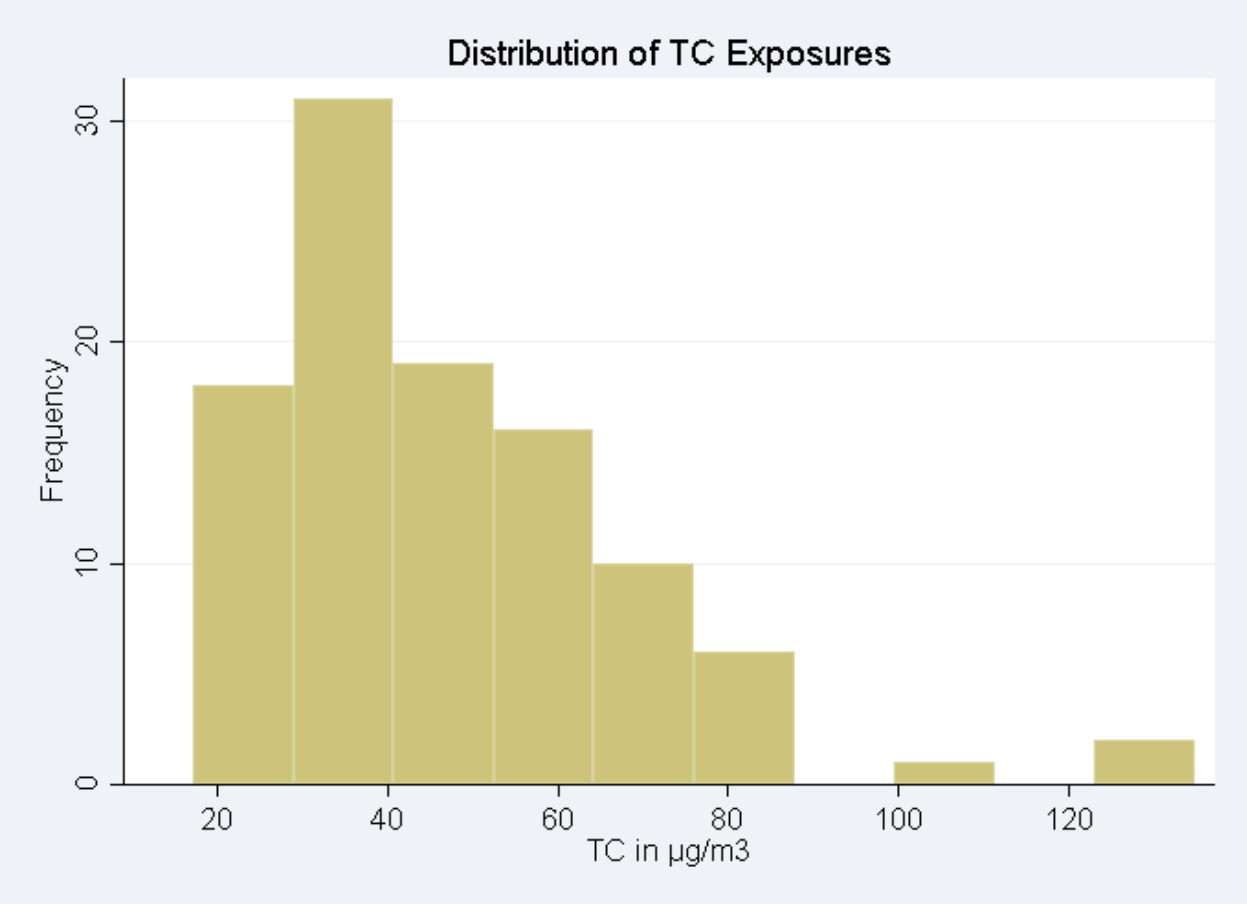

Distribution of Natural Log-Transformed 1-NP Exposures
Frequency
0
10
20
30
40
-2
0
2
4
6
Ln(1-NP) in pg/m3
Shapiro Wilk p<0.001

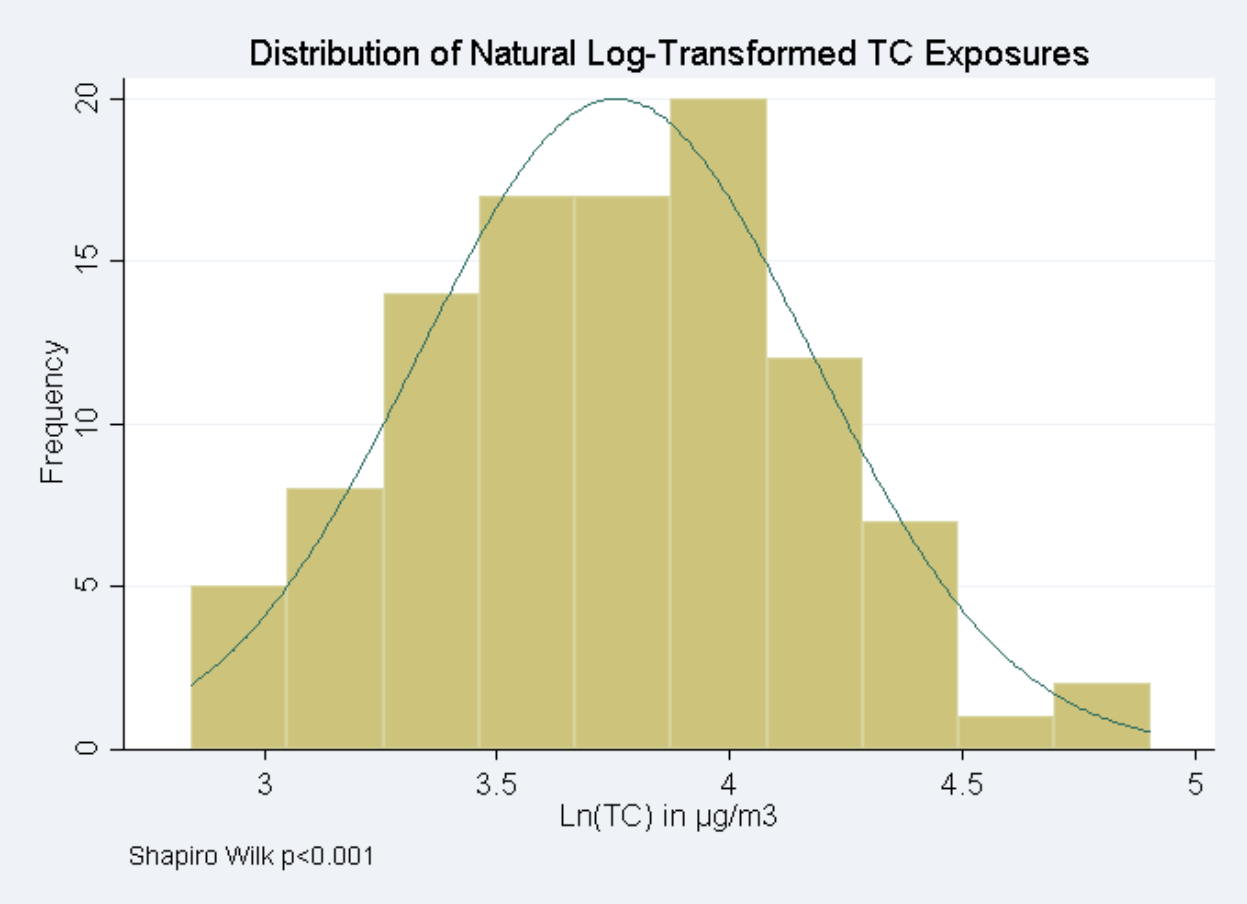

Distribution of EC Exposures
Frequency
0
20
40
60
0
20
40
60
80
100
EC in µg/m3


Distribution of Natural Log-Transformed EC Exposures
Frequency
0
5
10
15
20
25
0
1
2
3
4
5
Ln(EC) in µg/m3
Shapiro Wilk p<0.001


Distribution of OC Exposures
Frequency
0
10
20
30
40
0
20
40
60
80
100
OC in µg/m3


Distribution of Natural Log-Transformed OC Exposures
Frequency
0
10
20
30
40
-2
0
2
4
6
Ln(OC) in µg/m3
Shapiro Wilk p<0.001


Distribution of TC Exposures
Frequency
0
10
20
30
20
40
60
80
100
120
TC in µg/m3


Distribution of Natural Log-Transformed TC Exposures
Frequency
0
5
10
15
20
3
3.5
4
4.5
5
Ln(TC) in µg/m3
Shapiro Wilk p<0.001

Appendix VIII

Regression Output & Cross-Validation Plots for 10 Prediction Models

Preliminary Prediction Models Outputs

Model 1:	**ln(1NP) predicted by location group (face/shop/surface), with subject ID as a random effect**
Coding:	xi: xtmixed Dair1np i.locationgrpno || subjectid:, nolog reml *where locationgrpno is coded: 0/1/2, with 0=surface, 1=face, 2=shop* *& Dair1np is natural log-transformed*
Equation:	ln(1NP) = ß0 + ß1*face + ß2*shop + ßsubj.

Output:	Coef.	Std. Err.	z	P-value	95% CI	
ß1	1.085	0.307	3.540	0.000	0.483	1.686
ß2	0.420	0.381	1.100	0.269	-0.326	1.166
ß0	-10.752	0.284	-37.830	0.000	-11.309	-10.195

Model 2:	**ln(1NP) predicted by primary task group (1-8), with subject ID as a random effect**
Coding:	xi: xtmixed Dair1np i.primarytaskgrp || subjectid:, nolog reml *where primarytaskgrp is coded: 0-8; other surface/ diesel engine repair/ other shop/ ore channeling/jack leg drill operation/ load-haul-dump operation/ other face/ face geology* *& Dair1np is natural log-transformed*
Equation:	ln(1NP) = ß0 + ß1*[diesel engine repair] + ß2*[other shop] + ß3*[ore channeling] + ß4*[jack leg drill operation] + ß5*[load-haul-dump operation] + ß6*[other face] + ß7*[face geology] + ßsubj

Output:	Coef.	Std. Err.	z	P-value	95% CI	
ß1	0.578	0.455	1.270	0.204	-0.314	1.471
ß2	-0.409	0.450	-0.910	0.363	-1.291	0.472
ß3	1.649	0.405	4.080	0.000	0.857	2.442
ß4	1.034	0.421	2.460	0.014	0.209	1.859
ß5	0.809	0.379	2.140	0.033	0.067	1.551
ß6	0.920	0.410	2.240	0.025	0.116	1.725
ß7	0.877	0.410	2.140	0.033	0.073	1.681
ß0	-10.661	0.294	-36.290	0.000	-11.237	-10.085

Model 3:	**ln(1NP) predicted by location group (face/shop/surface) & fuel type (summer/winter), DOW (1-4), & tDE (continuous), with subject ID as a random effect**
Coding:	xi: xtmixed Dair1np i.locationgrpno i.fueltype i.DOW tDE || subjectid:, nolog reml *where locationgrpno is coded: 0/1/2, with 0=surface, 1=face, 2=shop* *& Dair1np is natural log-transformed*
Equation:	ln(1NP) = ß0 + ß1*[face] + ß2*[shop] + ß3*[winter (fueltype)] + ß4*[DOW 2] + ß5*[DOW 3] + ß6*[DOW 4] + ß7*tDE + ßsubj

Output:	Coef.	Std. Err.	z	P-value	95% CI	
ß1	0.948	0.327	2.900	0.004	0.308	1.588
ß2	0.343	0.392	0.880	0.382	-0.426	1.112
ß3	-0.011	0.160	-0.070	0.946	-0.325	0.304
ß4	-0.493	0.301	-1.640	0.101	-1.083	0.097
ß5	-0.361	0.213	-1.700	0.090	-0.779	0.056
ß6	-0.146	0.317	-0.460	0.646	-0.768	0.476
ß7	0.043	0.032	1.330	0.185	-0.020	0.106
ß0	-10.677	0.413	-25.860	0.000	-11.486	-9.868

Model 4:	**ln(1NP) predicted by primary task group (1-8), & fuel type (summer/winter), DOW (1-4), & tDE (continuous) with subject ID as a random effect**
Coding:	xi: xtmixed Dair1np i.primarytaskgrp i.fueltype i.DOW tDE || subjectid:, nolog reml *where primarytaskgrp is coded: 0-8; other surface/ diesel engine repair/ other shop/ ore channeling/jack leg drill operation/ load-haul-dump operation/ other face/ face geology* *& Dair1np is natural log-transformed*
Equation:	ln(1NP) = ß0 + ß1*[diesel engine repair] + ß2*[other shop] + ß3*[ore channeling] + ß4*[jack leg drill operation] + ß5*[load-haul-dump operation] + ß6*[other face] + ß7*[face geology] + ß8*[winter (fueltype)] + ß9*[DOW 2] + ß10*[DOW 3] + ß11*[DOW 4] + ß12*tDE + ßsubj

Output:	Coef.	Std. Err.	z	P-value	95% CI	
ß1	0.253	0.529	0.480	0.632	-0.784	1.291
ß2	-0.578	0.500	-1.160	0.248	-1.559	0.403
ß3	1.326	0.455	2.910	0.004	0.434	2.218
ß4	0.864	0.458	1.880	0.059	-0.034	1.762
ß5	0.474	0.444	1.070	0.286	-0.397	1.344
ß6	0.651	0.458	1.420	0.155	-0.247	1.550
ß7	0.740	0.430	1.720	0.085	-0.103	1.584

ß8	0.033	0.161	0.200	0.840	-0.284	0.349
ß9	-0.367	0.296	-1.240	0.215	-0.947	0.213
ß10	-0.405	0.213	-1.900	0.057	-0.823	0.012
ß11	-0.075	0.307	-0.240	0.808	-0.676	0.527
ß12	0.054	0.035	1.530	0.125	-0.015	0.122
ß0	-10.588	0.411	-25.750	0.000	-11.394	-9.782

Model 5: **ln(1NP) predicted by location group (face/shop/surface) with nested primary task groups, & fuel type (summer/winter), DOW (1-4), & tDE (continuous) with subject ID as a random effect**

Coding:

3 Models stratified by location group, with predictions later appended into one dataset:

xi: xtmixed Dair1np jldrillop lhdumpop orechan undgeo i.fueltype i.DOW tDE /// || subjectid: if locationgroup=="face", nolog reml

where jldrillop="jack leg drill operation (0/1); lhdumpop="load-haul-dump operation" (0/1), orechan="ore channeling" (0/1), undgeo="face geology" (0/1) & Dair1np is natural log-transformed

xi: xtmixed Dair1np derepair i.fueltype i.DOW tDE || subjectid: if /// locationgroup=="shop", nolog reml

where derepair="diesel engine repair" (0/1)
& Dair1np is natural log-transformed

xi: xtmixed Dair1np i.fueltype i.DOW tDE || subjectid: if /// locationgroup=="surface", nolog reml

where Dair1np is natural log-transformed

Equations:

ln(1NP), face = ß0 + ß1*[jack leg drill operation] + ß2*[load-haul-dump operation] + ß3*[ore channeling] + ß4*[face geology] + ß5*[winter (fueltype)] + ß6*[DOW 2] + ß7*[DOW 3] + ß8*[DOW 4] + ß9*tDE

ln(1NP), shop = ß0 + ß1*[diesel engine repair] +ß2*[winter (fueltype)] + ß3*[DOW 2] + ß4*[DOW 3] + ß5*[DOW 4] + ß6*tDE

ln(1NP), surface = ß0 + ß1*[winter (fueltype)] + ß2*[DOW 2] + ß3*[DOW 3] + ß4*[DOW 4] + ß5*tDE

Output (face):	Coef.	Std. Err.	z	P-value	95% CI	
ß1	0.097	0.400	0.240	0.809	-0.688	0.882
ß2	-0.204	0.355	-0.580	0.565	-0.900	0.492
ß3	0.550	0.387	1.420	0.155	-0.208	1.308
ß4	0.021	0.509	0.040	0.967	-0.977	1.019
ß5	-0.099	0.198	-0.500	0.618	-0.487	0.290
ß6	-0.428	0.353	-1.210	0.226	-1.120	0.265
ß7	-0.278	0.246	-1.130	0.258	-0.760	0.204
ß8	-0.121	0.363	-0.330	0.738	-0.833	0.590
ß9	-0.005	0.051	-0.090	0.927	-0.106	0.096

ß0	-9.359	0.569	-16.450	0.000	-10.475	-8.244
Output (shop):	Coef.	Std. Err.	z	P-value	95% CI	
ß1	0.755	0.591	1.280	0.202	-0.404	1.914
ß2	-0.564	0.424	-1.330	0.184	-1.395	0.267
ß3	-1.271	0.714	-1.780	0.075	-2.670	0.128
ß4	-0.383	0.529	-0.720	0.469	-1.419	0.653
ß5	-0.638	0.739	-0.860	0.388	-2.087	0.810
ß6	-0.065	0.093	-0.710	0.480	-0.247	0.116
ß0	-9.449	1.059	-8.920	0.000	-11.524	-7.374
Output (surface):	Coef.	Std. Err.	z	P-value	95% CI	
ß1	1.437	0.465	3.090	0.002	0.526	2.348
ß2	-0.206	0.748	-0.280	0.783	-1.673	1.261
ß3	-1.280	0.441	-2.900	0.004	-2.144	-0.416
ß4	-0.473	0.889	-0.530	0.594	-2.215	1.269
ß5	-0.060	0.062	-0.970	0.332	-0.182	0.061
ß0	-11.116	0.722	-15.400	0.000	-12.532	-9.701

Model 6:	**ln(1NP) predicted by time-weighted task group (1-8) with subject ID as a random effect**
Coding:	xi: xtmixed Dair1np timetask1 timetask2 timetask3 timetask4 timetask5 timetask6 timetask7 timetask8 || subjectid:, nolog reml *where timetask1-8 each have a value of 0.0-1.0, representing fraction of shift spent on each task category* *Dair1np is natural log-transformed*
Equation:	ln(1NP) = ß0 + ß1*[diesel engine repair] + ß2*[other shop] + ß3*[ore channeling] + ß4*[jack leg drill operation] + ß5*[load-haul-dump operation] + ß6*[other face] + ß7*[face geology] + ßsubj

Output:	Coef.	Std. Err.	z	P-value	95% CI	
ß1	-0.143	0.977	-0.150	0.884	-2.057	1.772
ß2	0.381	1.013	0.380	0.707	-1.604	2.365
ß3	-0.586	1.011	-0.580	0.562	-2.567	1.396
ß4	1.583	0.994	1.590	0.111	-0.365	3.532
ß5	0.644	1.082	0.600	0.552	-1.477	2.765
ß6	0.577	1.006	0.570	0.566	-1.394	2.549
ß7	0.559	0.989	0.570	0.572	-1.380	2.498
ß8	0.679	1.010	0.670	0.502	-1.301	2.658
ß0	-10.477	0.947	-11.070	0.000	-12.333	-8.621

Model 7: **ln(1NP) predicted by location group (face/shop/surface) & fuel type (summer/winter) & tDE (continuous), with subject ID as a random effect**

Coding:

xi: xtmixed Dair1np i.locationgrpno i.fueltype tDE || subjectid:, nolog reml
where locationgrpno is coded: 0/1/2, with 0=surface, 1=face, 2=shop
& Dair1np is natural log-transformed

Equation:

ln(1NP) = ß0 + ß1*[face] + ß2*[shop] + ß3*[winter (fueltype)] + ß4*tDE + ßsubj

Output:	Coef.	Std. Err.	z	P-value	95% CI	
ß1	0.986	0.320	3.080	0.002	0.358	1.613
ß2	0.326	0.392	0.830	0.406	-0.442	1.093
ß3	-0.038	0.162	-0.230	0.816	-0.355	0.279
ß4	0.035	0.031	1.100	0.272	-0.027	0.096
ß0	-10.870	0.335	-32.430	0.000	-11.526	-10.213

Model 8: **ln(1NP) predicted by location group (face/shop/surface) & tDE (continuous), with subject ID as a random effect**

Coding:

xi: xtmixed Dair1np i.locationgrpno tDE || subjectid:, nolog reml
where locationgrpno is coded: 0/1/2, with 0=surface, 1=face, 2=shop
& Dair1np is natural log-transformed

Equation:

ln(1NP) = ß0 + ß1*[face] + ß2*[shop] + ß4*tDE + ßsubj

Output:	Coef.	Std. Err.	z	P-value	95% CI	
ß1	0.994	0.316	3.140	0.002	0.374	1.614
ß2	0.332	0.389	0.850	0.394	-0.430	1.094
ß3	0.035	0.031	1.120	0.264	-0.026	0.096
ß0	-10.896	0.314	-34.730	0.000	-11.511	-10.281

Model 9: **ln(1NP) predicted by primary task group (1-8) & fueltype (summer/winter) & tDE (continuous), with subject ID as a random effect**

Coding:

xi: xtmixed Dair1np i.primarytaskgrp i.fueltype tDE || subjectid:, nolog reml
where primarytaskgrp is coded: 0-8; other surface/ diesel engine repair/ other shop/ ore channeling/jack leg drill operation/ load-haul-dump operation/ other face/ face geology
& Dair1np is natural log-transformed

Equation:

ln(1NP) = ß0 + ß1*[diesel engine repair] + ß2*[other shop] + ß3*[ore channeling] + ß4*[jack leg drill operation] + ß5*[load-haul-dump operation] + ß6*[other face] + ß7*[face geology] + ß8*[summer fuel] + ß9*[tDE] + ßsubj

Output:	Coef.	Std. Err.	z	P-value	95% CI	
ß1	0.331	0.505	0.650	0.513	-0.660	1.321
ß2	-0.528	0.471	-1.120	0.262	-1.451	0.395
ß3	1.456	0.439	3.320	0.001	0.596	2.317
ß4	0.888	0.443	2.000	0.045	0.020	1.756
ß5	0.579	0.428	1.350	0.176	-0.260	1.418
ß6	0.720	0.445	1.620	0.106	-0.153	1.593
ß7	0.833	0.419	1.990	0.047	0.012	1.655
ß8	0.015	0.163	0.090	0.926	-0.305	0.335
ß9	0.040	0.034	1.190	0.234	-0.026	0.106
ß0	-10.767	0.336	-32.070	0.000	-11.425	-10.109

Model 10: **ln(1NP) predicted by primary task group (1-8) & tDE (continuous), with subject ID as a random effect**

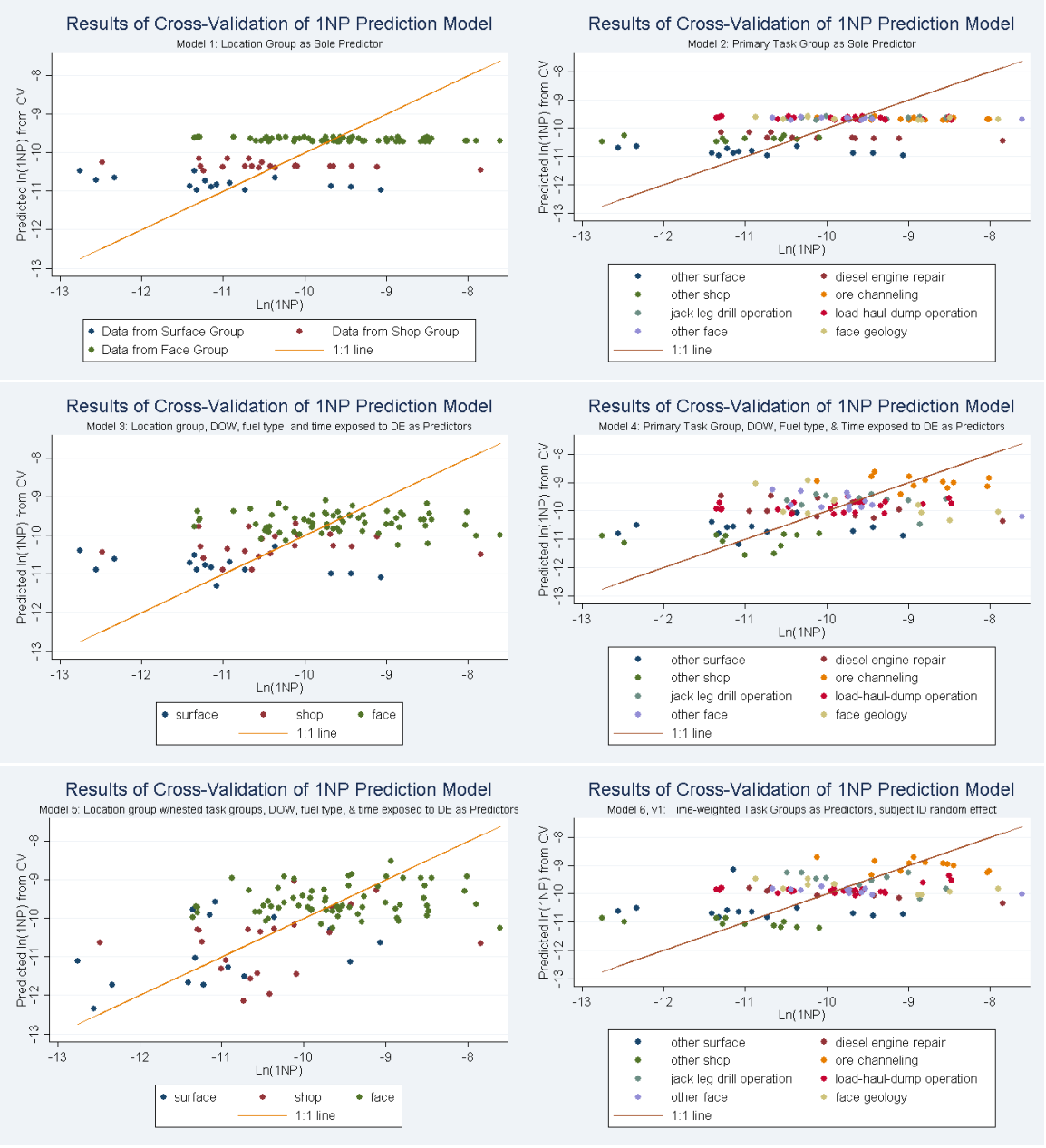
Coding:

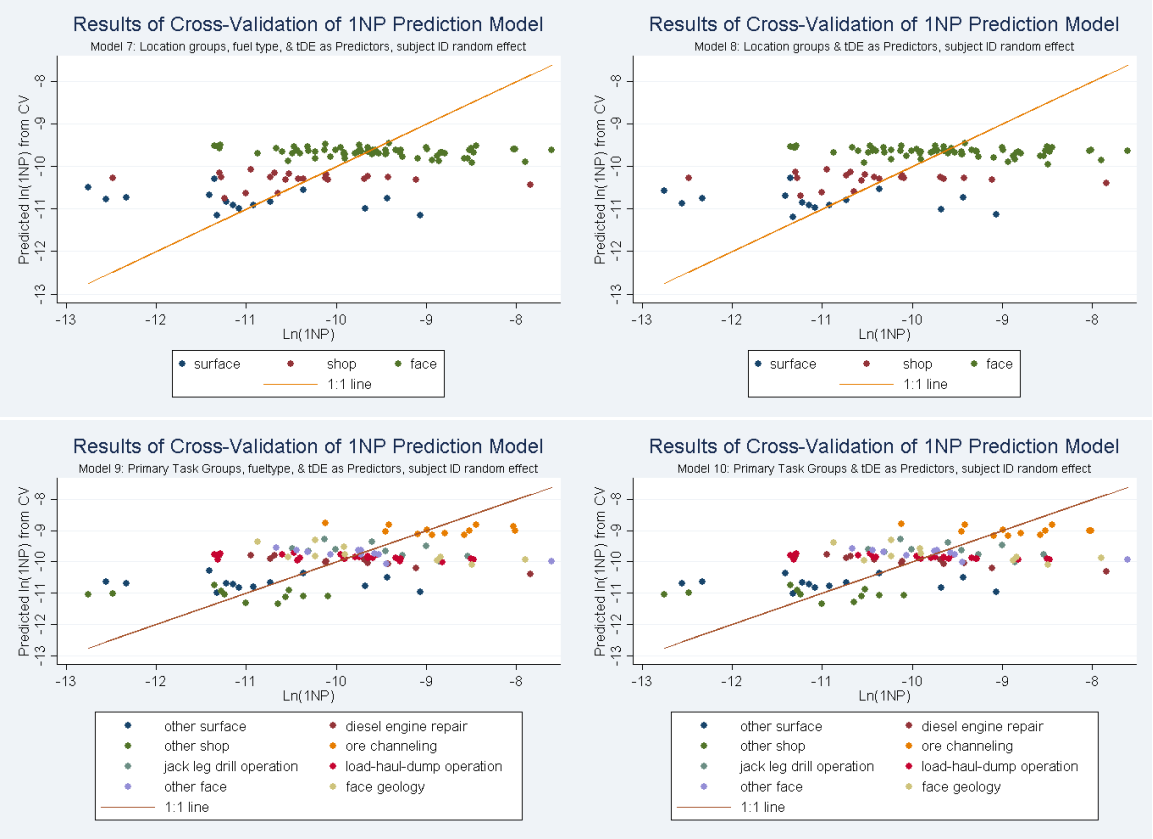
xi: xtmixed Dair1np i.primarytaskgrp tDE || subjectid:, nolog reml
where primarytaskgrp is coded: 0-8; other surface/ diesel engine repair/ other shop/ ore channeling/jack leg drill operation/ load-haul-dump operation/ other face/ face geology
& Dair1np is natural log-transformed

Equation:

ln(1NP) = ß0 + ß1*[diesel engine repair] + ß2*[other shop] + ß3*[ore channeling] + ß4*[jack leg drill operation] + ß5*[load-haul-dump operation] + ß6*[other face] + ß7*[face geology] + ß8*[tDE] + ßsubj

Output:	Coef.	Std. Err.	z	P-value	95% CI	
ß1	0.320	0.505	0.630	0.526	-0.669	1.310
ß2	-0.534	0.471	-1.130	0.257	-1.457	0.389
ß3	1.442	0.435	3.320	0.001	0.590	2.295
ß4	0.877	0.441	1.990	0.047	0.013	1.741
ß5	0.573	0.428	1.340	0.181	-0.266	1.411
ß6	0.711	0.444	1.600	0.109	-0.159	1.581
ß7	0.819	0.414	1.980	0.048	0.007	1.631
ß8	0.040	0.033	1.200	0.231	-0.025	0.105
ß0	-10.751	0.316	-34.070	0.000	-11.370	-10.133


Results of Cross-Validation of 1NP Prediction Model
Model 1: Location Group as Sole Predictor
Predicted ln(1NP) from CV
Ln(1NP)
-13
-12
-11
-10
-9
-8
Data from Surface Group
Data from Shop Group
Data from Face Group
1:1 line
Results of Cross-Validation of 1NP Prediction Model
Model 2: Primary Task Group as Sole Predictor
Predicted ln(1NP) from CV
Ln(1NP)
other surface
diesel engine repair
other shop
ore channeling
jack leg drill operation
load-haul-dump operation
other face
face geology
1:1 line
Results of Cross-Validation of 1NP Prediction Model
Model 3: Location group, DOW, fuel type, and time exposed to DE as Predictors
Predicted ln(1NP) from CV
Ln(1NP)
surface
shop
face
1:1 line
Results of Cross-Validation of 1NP Prediction Model
Model 4: Primary Task Group, DOW, Fuel type, & Time exposed to DE as Predictors
Predicted ln(1NP) from CV
Ln(1NP)
other surface
diesel engine repair
other shop
ore channeling
jack leg drill operation
load-haul-dump operation
other face
face geology
1:1 line
Results of Cross-Validation of 1NP Prediction Model
Model 5: Location group w/nested task groups, DOW, fuel type, & time exposed to DE as Predictors
Predicted ln(1NP) from CV
Ln(1NP)
surface
shop
face
1:1 line
Results of Cross-Validation of 1NP Prediction Model
Model 6, v1: Time-weighted Task Groups as Predictors, subject ID random effect
Predicted ln(1NP) from CV
Ln(1NP)
other surface
diesel engine repair
other shop
ore channeling
jack leg drill operation
load-haul-dump operation
other face
face geology
1:1 line



Appendix IX

Comparison of Predictive Models with and without Subjectid Random Effect Term

Model:	Covariates:	In-Sample Predictions:		Cross-Validated Predictions:	
		RMSE	MSE-based R^2	RMSE	MSE-based R^2
1	location group (1-3) + subjid random effect	0.904	0.251	0.939	0.191
1	location group (1-3)	0.896	0.264	0.929	0.209
2	primary task group (1-8) + subjid random effect	0.826	0.374	0.902	0.254
2	primary task group (1-8)	0.817	0.388	0.896	0.264
3	location group (1-3), fuel type, DOW (1-4), time exp. to DE + subjid random effect	0.882	0.287	0.959	0.156
3	location group (1-3), fuel type, DOW (1-4), time exp. to DE	0.870	0.306	0.951	0.171
4	primary task groups (1-8), fuel type, DOW (1-4), time exp. to DE + subjid random effect	0.814	0.393	0.936	0.197
4	primary task groups (1-8), fuel type, DOW (1-4), time exp. to DE	0.797	0.417	0.940	0.189

6	time-weighted task group (1-8), fuel type, DOW (1-4) , time exp. to DE + subjid random effect	0.829	0.370	0.921	0.222
6	time-weighted task group (1-8), fuel type, DOW (1-4) , time exp. to DE	0.818	0.386	0.946	0.179