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Original Article

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

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Abstract

We investigated the viability of particle bound 1-nitropyrene (1-NP) air concentration measurements as a surrogate of diesel exhaust (DE) exposure, as compared with industry-standard elemental carbon (EC) and total carbon (TC) measurements. Personal exposures are reported for 18 employees at a large underground metal mine during four different monitoring campaigns. Full-shift personal air exposure sampling was conducted using a Mine Safety and Health Administration (MSHA) compliant diesel particulate matter (DPM) impactor cassette downstream of a GS-1 cyclone pre-selector. Each DPM filter element was analyzed for EC and organic carbon (OC) using NIOSH Method 5040. After EC and OC analysis, the remaining portion of each DPM filter was analyzed for 1-NP using liquid chromatography tandem mass spectrometry (LC/MS/MS). We observed high correlations between the quantiles of 1-NP and EC exposures across 10 different work shift task groups ($r = 0.87$ to 0.96), and a linear relationship with a slope between 6.0 to 6.9 pg 1-NP per μg EC. However, correlation between 1-NP and EC was weak ($r = 0.34$) for the 91 individual sample pairs due to low EC concentrations and possible heterogeneity of DE composition. While both 1-NP and EC differentiated between high and low exposure groups categorized by job location, measurements of 1-NP, but not EC further differentiated between specific job activities. Repeated measurements on individual subjects verified the relationship between 1-NP and EC and demonstrated substantial within-subject variability in exposure. The detection limit of TC air concentration ranged between 18 and 28 μg m^{-3} and was limited by OC contamination of the quartz filters in the MSHA compliant DPM samplers.

Keywords: elemental carbon; exposure assessment; organic carbon; particulate matter; total carbon

Introduction

Occupational exposure to high levels of diesel exhaust (DE) is associated with adverse health effects including asthma, respiratory disease, cardiovascular disease, allergic sensitization, and lung cancer (USEPA, 2002; Attfield *et al.*, 2012; Silverman *et al.*, 2012). In 2012, the International Agency for Research on Cancer classified DE as carcinogenic to humans (Group 1), basing its decision in part on recent studies of underground miners which reported that exposure to DE carries an increased risk for lung cancer (Attfield *et al.*, 2012; IARC, 2014). However, as diesel engine technology continues to evolve, the actual health risk to workers associated with contemporary DE exposures will vary with the choice and rate of implementation of new technologies. DE is a complex mixture of chemicals and the specific components responsible for toxicity have not been fully characterized. In addition, exposure to DE typically occurs in conjunction with exposures to other agents such as gasoline exhaust and industrial sources of ambient pollution, which are also complex mixtures. It is common to measure surrogate markers to indicate exposure to health-relevant components of DE including nitrogen oxides (NOx), sub-micron particulate matter (PM_1), and particulate carbon including elemental (EC) and total (TC) carbon (Schauer, 2003; Noll *et al.*, 2005, 2007).

In May, 2006, the Mining Safety and Health Administration (MSHA) issued a final ruling for diesel particulate matter (DPM) in metal/nonmetal mines, including a permissible exposure limit (PEL) for TC of 160 μg m^{-3} per cubic meter of air (30 CFR Part 57, 2006). Compliance with the PEL is determined based on personal EC and TC measurements, with adjustment for non-diesel sources of organic carbon (OC) where necessary based on area samples (MSHA, 2008). However, some concerns have been raised as to the feasibility of EC and TC monitoring for DE quantification especially regarding variations in analytical methods, non-diesel sources of carbon components, and variability in the ratio of EC to DPM (Schauer, 2003). In other occupational settings, EC from non-diesel sources may contribute to atmospheric concentrations of EC and lead to misclassification of a worker's DE exposure (Schauer, 2003). Therefore, we sought to evaluate an alternate surrogate measure of DPM.

1-Nitropyrene (1-NP) is one of several nitrated polycyclic aromatic hydrocarbons (NPAHs) formed in the combustion chamber of a diesel engine, due to high temperatures and excess air supply (IARC, 2014). 1-NP has been proposed as a marker for DE because it is enriched in DE relative to other combustion sources; it is present in DE at higher levels than other particle-associated NPAHs; and it is not formed to a significant extent through atmospheric photochemical reactions (Bamford *et al.*, 2003; Toriba *et al.*, 2007). 1-NP can be measured in DE particulate matter and its specificity for DE may aid in distinguishing between DE exposures and other combustion sources (Scheepers *et al.*, 1995; IARC, 2014; Schulte *et al.*, 2015). Additionally, many NPAHs are mutagenic or carcinogenic and 1-NP has been implicated as one of the primary compounds contributing to the direct-acting mutagenicity of DE (Lewtas, 1988; Purohit and Basu, 2000; Kim *et al.*, 2005; Andersson *et al.*, 2009). While no single analyte can be a perfect surrogate for a complex and variable mixture such as DPM under all conditions, use of 1-NP could not only provide a more specific measure of DE exposure compared to TC and EC, but may more accurately reflect the carcinogenic properties of DE as well (Scheepers *et al.*, 1995).

In the current study, we assess the feasibility of using 1-NP as a surrogate measure of exposure to DE by comparing measurements of personal exposures to 1-NP and EC in a cohort of miners from an underground metal mine. Occupational exposures to DE in underground mines are often highly elevated compared to ambient settings (Stephensen *et al.*, 2006; Noll *et al.*, 2007). This setting permits a more direct examination of the hypothesized association between 1-NP and EC or TC specifically for DE because there are few confounding sources of EC and TC that might otherwise be present in other occupational settings (such as coal and shale dust or gasoline exhaust particulate matter). Furthermore, DE exposures in mines have been found to be associated with work location within the mine and at the mine surface (Scheepers *et al.*, 2003; Stewart *et al.*, 2010). In the current study, we use a detailed self-reported activity survey to investigate the relationship between specific job tasks and potential exposures to 1-NP and EC.

Materials and Methods

Study setting and subjects

We studied exposures to DE among workers at a large underground metal mine employing about 1300 miners. Twenty mine workers of various job titles were recruited and followed for four work weeks during 7–10 March; 11–14 June; 14–17 August; and 1–4 October in 2014. Subjects were assigned *a priori* to one of three exposure groups (low, medium, and high) by mine health and safety staff, based on job titles and typical work location. At this mine site, workers typically work a schedule of four 10-hour shifts followed by 4 days off. Further, successive work weeks alternate between day-shift and night-shift. To simplify study logistics, all study participants were members of "B crew" - one of four rotating shifts of workers at the mine, and sample collection only took place when B crew were on the day shift.

This mine operates ~370 diesel-powered pieces of equipment including ~110 utility vehicles, ~130 load/haul/dump vehicles, a 30 unit muckhaul fleet, and assorted other vehicles. Most of the engines have been fitted with flow-through diesel-particulate filters that also catalyze oxidation of the DPM (DCL Mine-X soot filter and DCL Mine-XpDPF flow-through filter). This mine uses exclusively a B70 blend biodiesel fuel (prepared by blending B99 FAME Biodiesel (Archer Daniels Midland, Chicago IL) with #2 Ultra Low sulfur diesel in a 70:30 ratio. Due to the seasonal temperature differences affecting fuel performance, the mine uses a different fuel additive for diesel-powered vehicles in the summer months (including June and August) compared to the winter months (including March and October).

Intake questionnaire and surveys

At the commencement of the project, we administered an intake questionnaire to study subjects to obtain demographic information including date of birth, gender, years worked in mining, and years worked at the mine. Subjects also completed work-activity surveys at the conclusion of each monitored work shift, to obtain information about specific tasks performed during the work shift, respirator use, time spent underground, time spent in proximity to DE, and cigarette smoking.

The study was approved by the Institutional Review Board of Boise State University, with the concurrence of the Institutional Review Board of the University of Washington.

Personal exposure sampling

Data collection took place during four monitoring periods, each of 4 days duration. Due to logistical limitations, personal air samples were collected on the first 10 subjects on days 1 and 3 of the week, and the other 10 were monitored on days 2 and 4. The personal air exposure sampling train consisted of an MSHA compliant SKC DPM impactor downstream of a SKC Respirable GS-1 cyclone pre-selector attached in the breathing zone of each worker (SKC Inc., Eighty-Four, PA). The DPM impactor contains two 37-mm heat-treated quartz fiber filters with a cellulose support pad (part # 225–317, SKC Inc.). 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 l min^{-1} to provide a nominal cut point of 0.8 µm at the DPM impactor. The GS-1 Cyclone is used with the DPM Cassette as a pre-selector to remove large particles that might otherwise occlude the inlet to the DPM sub-micron impactor. The impactor has a median aerodynamic diameter cut point of 0.8 µm. About 80–90% of DPM is <1 µm by mass, so these samplers meet MSHA and NIOSH Method 5040 specifications for DPM collection and analysis through quantification of EC, OC, and TC (Noll *et al*., 2005; MSHA, 2016; NIOSH, 2016b, 2016a). Additionally, we conducted a supplementary analysis to examine the size distribution of 1-NP in area samples collected in the mine using a SKC Sioutas cascade impactor, and determined that overall >90% of the 1-NP is found 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 SKC drycal calibrator. Flow measurements were repeated at the end of each work shift. Air volume for each sample was calculated as the average of the pre- and post-shift flow, multiplied by the sample time; samples were invalidated if flow differences exceeded 10%.

For quality assurance purposes, two field blanks were deployed per day, for a total of 32 field blanks overall. Field blanks consisted of filters that were placed in a sampling train, but did not have air drawn through them. Field blanks were analyzed for EC/OC/TC and 1-NP in a manner identical to the personal filter samples.

Filter analysis

A portion (1.5 cm^2) of the DPM quartz filters was removed using a rectangular punch and analyzed for EC, OC, and TC using the NIOSH method 5040 (NIOSH, 2016b) by ALS Environmental (Salt Lake City, UT). This method has been thoroughly validated by NIOSH and MSHA who report an accuracy for EC of ±16.7% (NIOSH, 2016b) and precision (for EC) of 6–9% (MSHA, 2016; NIOSH, 2016a). The samples were submitted in four analytical batches, one per field campaign. Five of the collected field blanks were submitted for analysis in each batch, the average field blank OC mass (17.8 μg/sample) was subtracted from each reported sample OC mass, and for QC purposes the limit of detection (LOD) for OC was set as three times the standard deviation of the blank samples (20.2 μg/sample). ALS Environmental provided a reporting limit of 1.7 μg/sample for EC, and censored all EC values <1.7 μg/sample. Only two of the 20 field blanks returned EC values above the reporting limit. EC and OC concentrations were reported in units of μg/sample and added together to calculate TC. EC, OC, and TC values <LOD were substituted with $\mathrm{LOD}/\sqrt{2}$; all sample concentrations were converted to air concentrations (μg m^{-3}) using air volume data from field logs and flow rates. The LOD for TC was treated as additive, and is simply estimated as the sum of the LOD for EC plus the LOD for OC.

The remainder of each filter was analyzed for 1-NP at the University of Washington as described by Miller-Schulze *et al.* (2007). 1-NP determination including filter extraction and analysis by high-performance liquid chromatography tandem mass spectrometry (LC/MS/MS) occurred in three analytical batches. Of the 101 samples included in data analysis, 72 samples were analyzed in one batch containing filters from all campaigns, 22 samples from June were analyzed in a separate batch, and lastly 7 from August/October were analyzed in the third batch. In brief, filter samples were spiked with internal standard (d^9-1-NP) and extracted by ultrasonication in methylene chloride. Extracts were reduced to dryness under a stream of nitrogen and suspended in 3:1 ethanol: sodium acetate buffer (20 mM, pH 5.5), filtered, then analyzed using two-dimensional high-performance liquid chromatography tandem mass spectrometry (2D-HPLC-MS/MS). A detailed protocol is available in the Supplementary Material (available at *Annals of Work Exposures and Health*). The instrument LOD for 1-NP extract concentration was calculated by taking the average plus three times the standard deviation of 1-NP extract concentrations from field and process blank filters. Instrumental LODs (4–19 pg/sample) varied by sample batch, and were converted to concentration-equivalent LODs (units of fg m^{-3}) for each of the mine measurements by dividing by the air volume for each sample. Finally, measurements that were below the LOD were substituted with $\mathrm{LOD}/\sqrt{2}$. The accuracy and precision of the analysis was 87 ± 7% of the expected values as determined by measuring concentrations of 1-NP in spiked filters.

Data completeness

The study design consisted of enrolling 20 subjects, with personal air exposure samples collected on two work shifts per subject during each of the 4 weeks in the study (160 planned air samples). The largest single source of missing data was subject dropout (25 samples). During the study, two subjects dropped out after the first week of monitoring because they moved to one of the other work crews. Data from these two subjects were not included in the analyses. On several other occasions, individual study participants were not available for sampling, for example due to vacation or family/medical leave. Additional samples were invalidated due to technical problems with the pumps and analysis (34 samples), and were random in nature. Examples include pump failure, changes in airflow exceeding 10%, damage to filter cassettes, and interfering peaks in the LC/MS/MS chromatogram. The final number of samples was 101 out of 160 planned.

Data analysis

Many of the miners divide their work shift among a variety of mining tasks that can modify exposure by location and equipment use. In a previous mine study by Stewart *et al.* (2010), DE exposure groups were assigned based on location in the mine. We adapted this approach and assigned each work shift into one of three exposure categories or 'location groups': 'Face' represents work within or at the ends of mine tunnels such as drilling and haulage operation; 'Shop' represents work in well-ventilated underground workshops; and 'Surface' represents work done outside the mine or near the surface directly adjacent to fresh-air ventilation. 'Surface' was assigned to all work shifts where the subject reported being underground 5 hours or less (half the work shift).

Because not all tasks necessarily have the same exposure, we also examined the association between task and exposure for underground work shifts. The 'Shop' workers monitored always spent the entire work shift performing a single activity; however, many of the miners in the 'Face' category performed several different tasks in a work shift. Therefore, we simplified the reported time/task activity survey by first assigning a primary task for each work shift where a worker reported they spent >2/3 of the day performing a single activity. For work shifts in the 'Face' category where no single task was performed for >2/3 of the day, the work shifts were grouped into a "Various face" subcategory. Finally, mechanic 'Shop' tasks for which we had 1–2 measurements were combined to form a larger group 'maintenance repair/mechanic', and the 'Face' tasks of 'Jack leg drill operator' and 'Sand filling' were combined with 'Various face', because there were only three occasions that a subject performed these tasks the entire shift. For detailed information regarding subject activities see Table S1 in the Supplementary Material (available at *Annals of Work Exposures and Health*). Supplementary Table S1 (available at *Annals of Work Exposures and Health* online) also shows the breakdown of primary tasks amongst subjects and summaries of the 1-NP and EC air concentrations for those subject-task combinations.

Data management and quality control processes were performed using Stata version 13 (StataCorp, 2013) and statistical analyses were performed using the R-programming language.(R Core Team (2015))

Results

The cohort upon enrollment consisted of 20 subjects, two of whom were women. Average age was 41 years (range 27–58), and average years worked in mining was 11 years (range 0.75–34). After exclusions noted above, the final data set consisted of 101 personal air exposure samples from 18 subjects. A detailed tabulation of their job titles, and locations of job task on days with valid samples in each campaign, smoking status, and *a priori* exposure category (assigned by the mine health and safety staff) are provided in Table S2, see the Supplementary Material, available at Annals of Work Exposures and Health online (range 3–8 work shifts per person).

Table 1 reports the 1-NP, EC, OC, and TC concentrations for each of the exposure locations (Face, Surface, and Shop). For all analytes, the highest exposures occurred within the 'Face' location group. The mean TC exposure was 41.5 µg m^{-3} for the 'Face' location with a maximum of 135 µg m^{-3}; no single measurement exceeded the PEL for TC of 160 µg m^{-3}. Although EC typically comprises a majority of TC found in underground mine DE (e.g. an average EC/TC ratio of 0.68 reported by Stephensen *et al.*, 2006), both the minimum and mean concentration of OC for each location group is higher than that of EC. In this study, the detection limit for OC was 10-fold higher than that of EC (see 'filter analysis' in the methods section). Therefore, replacement of measurements below LOD with $\mathrm{LOD}/\sqrt{2}$ resulted in a higher minimum concentration for OC compared to EC. Further analysis of OC and TC was not performed due to the majority of OC sample concentrations being less than LOD.

Dot plots of 1-NP and EC measurements as a function of job task and location are presented in Fig. 1. The largest concentrations of both EC and 1-NP occur at the 'Face' location. Within the 'Face' location, 'Ore-Channeling/drilling' stands out as a task with elevated air concentrations compared to 'Load-Haul Dump Operations', and 'Utilities' for both 1-NP and EC. Many of the Load-Haul-Dump vehicles in this mine were equipped with enclosed cabs and HEPA filtration, which may account for the lower EC and 1-NP exposures associated with this task, relative to other 'Face' tasks.

Table 1. Summary statistics for 1-NP, EC, OC, and TC (*N* = 101).

Analyte	Location	Mean (SD)	GM (GSD)	Median (IQR)	Min	Max	*N* (*N* < LOD)
1-NP	Face	97 (93)	64 (2.8)	65 (93)	2.1	500	66 (2)
	Shop	46 (77)	25 (2.9)	26 (24)	2.8	390	25 (1)
	Surface	18 (29)	4.4 (6.3)	2.5 (17)	0.53	80	10 (6)
	All	77 (90)	39 (4)	49 (65)	0.53	500	101 (9)
EC	Face	14 (8.1)	12 (1.8)	13 (11)	2.4	35	66 (0)
	Shop	6.5 (5.7)	4.8 (2.2)	5.3 (3.9)	1.3	25	25 (2)
	Surface	5.0 (6.4)	2.7 (3)	1.5 (5.6)	1.0	19	10 (5)
	All	11 (8.3)	8.1 (2.4)	9.1 (9.4)	1.0	35	101 (7)
OC	Face	21 (15)	18 (1.6)	15 (8.9)	12	95	66 (45)
	Shop	15 (5.4)	15 (1.3)	14 (1.6)	13	37	25 (23)
	Surface	15 (1.6)	15 (1.1)	15 (2.3)	12	17	10 (10)
	All	19 (12)	17 (1.5)	14 (4.2)	12	95	101 (78)
TC	Face	40 (25)	35 (1.7)	27 (35)	15	140	66 (15)
	Shop	23 (13)	21 (1.5)	19 (4.4)	15	64	25 (19)
	Surface	20 (7.5)	19 (1.4)	17 (7.4)	13	36	10 (8)
	All	34 (23)	29 (1.7)	25 (22)	13	140	101 (42)

GM (GSD) = geometric mean (geometric standard deviation); IQR = interquartile range; *N* < LOD, samples below LOD; SD = standard deviation. 1-NP in pg m^{-3}; EC, OC, and TC in µg m^{-3}.

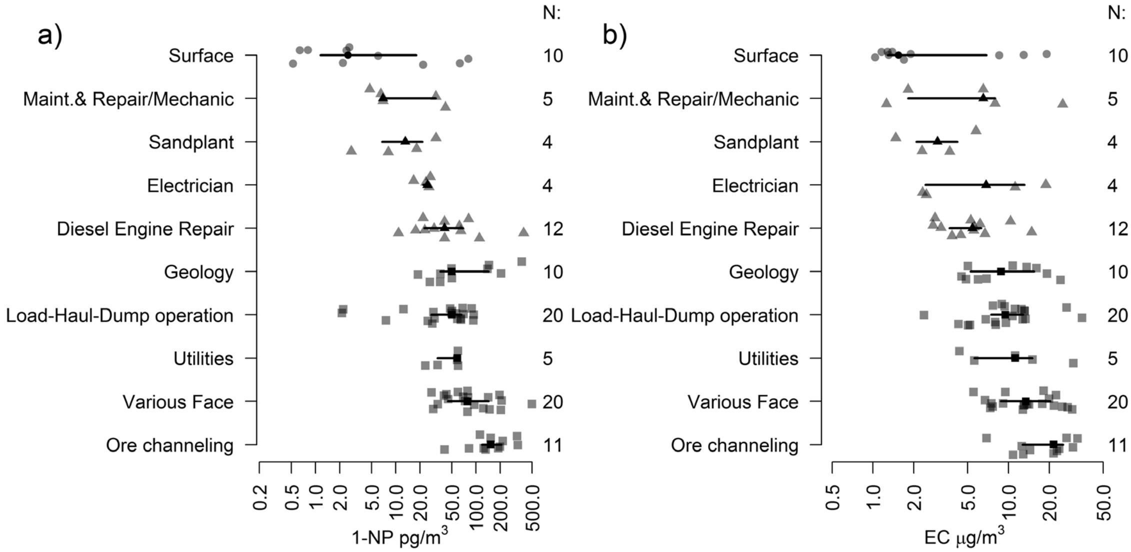

Figure 1. Air concentrations of 1-NP (left, **a**) and EC (right, **b**) from subjects grouped by tasks. Data presented on logarithmic scale. Black symbols indicate median concentrations, and the black line spans the 25th–75th percentiles. Gray symbols are individual measurements. Squares indicate tasks located at the mine 'Face', triangles are tasks located within underground 'Shops' and circles are at the 'Surface'. The number of measurements within each task group are provided at the right of each figure.

The dot plots show that both the median and variability of air concentrations for EC and 1-NP change with job task.

Fig. 1 and Table 1 demonstrate that the distribution of measurements is right skewed, or approximately lognormal. Although the groups in Fig. 1 are not balanced, an exploratory analysis of variance (ANOVA) was performed on the log-transformed data and we found strong significant differences between groups for both EC and 1-NP. The ANOVA results and mean difference plots are provided in Table S4 and Fig. S1 in the Supplementary Material (available at *Annals of Work Exposures and Health* online); briefly, the 1-NP and EC metrics performed similarly in ANOVA with slightly stronger significance for 1-NP. The 1-NP metric found that exposures were different between 11 pairs of tasks groups versus 10 for EC, and both metrics found significant differences between eight of the same pairs of task groups.

To quantify the association between EC and 1-NP, we examined both the individual samples and aggregates of the samples based on the task groups shown in Fig. 1. Fig. 2a–c is a plot of the 25th, 50th, and 75th percentiles of 1-NP versus EC for each task grouping from Fig. 1. The 25th and 75th percentiles provide the association between EC and 1-NP at the low-end and high-end of the measurement range. Fig. 2d is a plot of 1-NP versus EC for individual air samples, it is evident from the figure that the variability associated with individual samples is substantial in this concentration range. The ordinary-least-squares regressions were first fit to estimate a slope and intercept term. Since both 1-NP and EC are particle bound the 'true' intercept is zero; however, measurement errors may result in a systematic offset. We found that the intercept term was statistically insignificantly different from zero for all regressions except for the regression on individual measurements, which had an intercept of 40 pg m^{-3} ($P = 0.017$). Fitting the intercept did not substantially improve the residual standard error in any instance. The regression through the origin results for Fig. 2a–d is provided in Table 2. Table S3 in the Supplementary Material (available at *Annals of Work Exposures and Health* online) reports the results of the regressions with a variable intercept.

From Fig. 2d it is evident that there exists a large degree of variability in the 1-NP and EC relationship. On aggregate the 1-NP and EC concentrations are associated not only with task location, but also with task (see Fig. 1). To determine how well 1-NP characterizes exposures for different subjects across the workshifts where they reported performing the same kind of work, we compared the median and IQR of 1-NP and EC for subjects that performed the same task on three or more days (see Fig. 3). Despite the small number of samples for each subject-task, 1-NP appears to perform better than EC for differentiating specific subject-task combinations, and the subject-task IQR for 1-NP are typically smaller relative to the overall range especially for the samples

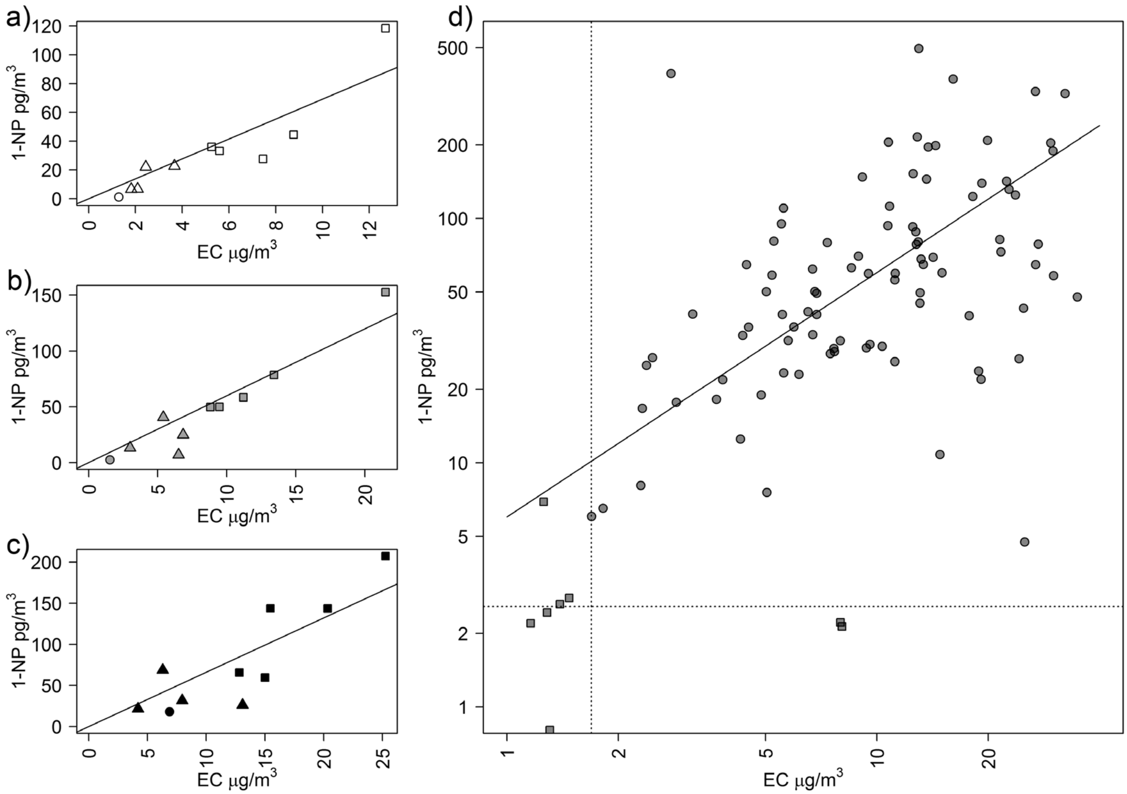

Figure 2. Dot plots of 1-NP versus EC air concentrations. The 25th, 50th and 75th percentiles calculated from the task groups as depicted in Figure 1 are plotted in figures **a**, **b** and **c**, respectively. Squares indicate tasks located at the mine face, triangles are tasks located in within-mine shops and circles are at the surface. (**d**) Dot plot of 1-NP and EC measurements for individual samples displayed on log-log axis. Vertical and horizontal dotted lines are the average LODs for EC and 1-NP, respectively. Square markers indicate measurement was below LOD for either 1-NP or EC (or both). Ordinary least squares regression through the origin shown for each sub-figure, regression coefficients are provided in Table 2.

Table 2. Results for regression through the origin and Pearson correlation coefficients.

Data	Figure Ref.	β_1	Std. error of estimate	Residual Std. error	Adjusted R^2	Pearson *r*
25% By task	Fig. 2a	6.9	0.76	15	0.89	0.92
Median by task	Fig. 2b	6	0.47	15	0.94	0.96
75% By task	Fig. 2c	6.6	0.78	35	0.88	0.87
Median by subject & task	Fig. 3	6.5	0.8	40	0.79	0.7
Individual measurements[a]	Fig. 2d	6	0.63	88	0.49	0.34

[a]Regression was performed on samples for which both 1 – NP and EC were above LOD (91 measurements). Regression model: 1 – NP (pg m^{-3}) = 0 + β_1*EC (µg m^{-3}). See Supplementary Table S3 (available at *Annals of Work Exposures and Health* online) for regression results with fitted intercept. The adjusted R^2 is reported for qualitative purposes, and should not be used to compare goodness of fit of the fitted intercept. Instead refer to the residual standard error.

<10 µg m^{-3} EC. A plot of the task-subject IQRs from Fig. 3 expressed as a percentage of the range is provided in Fig. S2 Supplementary Material (available at *Annals of Work Exposures and Health* online). In summary, all but four percent IQRs were lower for 1-NP than for EC, with eight IQRs below 10% for 1-NP versus three for EC; and only six IQRs were in excess of 20% of the range for 1-NP versus 11 for EC. The sample sizes per subject-task pairs were insufficient to explore the modification of exposure by task controlling for subject.

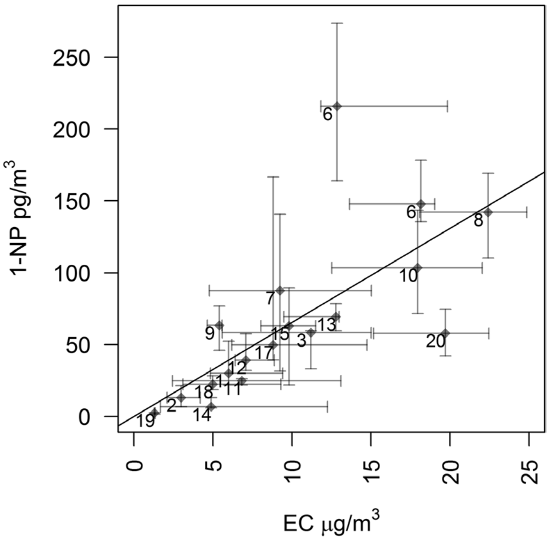

Figure 3. Median 1-NP versus EC air concentrations for subjects that performed the same task for multiple work shifts. Data shown are the median concentrations for $n \geq 3$ measurements for each task-subject combination (data provided in Table S1 in the Supplementary Material, available at *Annals of Work Exposures and Health* online, for all air measurements). Subject 6 is shown twice because they reported two tasks on at least three workshifts each, and subject 4 does not appear because they did not have at least three workshifts performing the same task. The error bars are the 25th and 75th percentiles. Overlaid is the ordinary-least-squared regression with intercept zero. ($1-\mathrm{NP}_{\mathrm{pg\,m^{-3}}} = 6.5(\pm 0.8)_{\mathrm{pg\,\mu g^{-1}}} \times \mathrm{EC}_{\mathrm{\mu g m^{-3}}}$; Pearson $r = 0.70$).

We additionally investigated whether an effect on 1-NP or EC concentrations could be seen for cigarette use, day of the work week, and the differing use of fuel additives in the June and August campaigns versus the March and October campaigns. We did not see any compelling modifications in EC or 1-NP concentrations at any of the location groupings ('Face', 'Shop', or 'Surface') for cigarette use (see Fig. S3 in the Supplementary Material, available at *Annals of Work Exposures and Health* online). We investigated day of work week for the 'Face' location (highest exposures) and did not find it significantly impacted concentrations, nor was there evidence that it modified the 1-NP and EC relationship (see Supplementary Fig. S4, available at *Annals of Work Exposures and Health* online). Differences in fuel additive between the cool and warm months may modify the concentrations of 1-NP relative to EC (see Fig. S5a–c in the Supplementary Material, available at *Annals of Work Exposures and Health* online). The 1-NP to EC ratio has a narrower distribution in the cool months than in the summer months, although the central tendencies are not significantly different. Dot plots of the EC versus 1-NP measurements confirm that the correlation between 1-NP and EC in the cool months of October and March ($r = 0.65$) is greater than in the warm months ($r = 0.30$) of June and August (Supplementary Fig. S5b and c, available at *Annals of Work Exposures and Health* online). The dot plots also revealed that the warm months are responsible for the non-zero intercept of the individual measurements in Fig. 2d, due to several points with large 1-NP concentrations at low to moderate EC concentrations. Direct emissions testing comparing the exhaust from the diesel equipment operating with the summer and winter fuel mixtures would be needed to verify this finding.

We conducted a preliminary analysis comparing the reproducibility of 1-NP and EC measurements for fixed samplers deployed in duplicate at one underground location. The sampling train for area measurements was identical to that for personal measurements. The percent difference between the duplicates was calculated from the nine available instrument pairs. 1-NP typically outperformed EC with four of the duplicates having percent differences close to zero compared to one for EC (Fig. S6 in the Supplementary Material, available at *Annals of Work Exposures and Health* online). In addition, six of the nine duplicates had a lower percentage difference for 1-NP. Three percent differences exceeded 50% for 1-NP; however, these were correlated with similar percent difference to EC suggesting that for these duplicate samples with high variability in both EC and 1-NP, the amount of DPM collected by the collocated samples differed overall.

Discussion

Underground mines have historically been workplaces with high occupational exposures to DE, and have provided much of the data supporting IARC's determination that DE is a class-1 human carcinogen (Silverman *et al.*, 2012; IARC, 2014). In the current study, no samples yielded a TC air concentration value in excess of the MSHA DPM PEL of 160 μg m^{-3}. The median TC concentration from miners located at the mine 'Face' was 27 (35, IQR) μg m^{-3}. This location is where the highest exposures were anticipated to occur, consistent with our findings. In comparison, an earlier study conducted at this facility in 2003 reported TC concentrations as high as 490 μg m^{-3} (Stephensen *et al.*, 2006), whereas the maximum TC concentration in this study was 140 μg m^{-3}. Since the 2003 report the mine operators instituted multiple strategies to reduce DE concentrations. In 2007, the mine increased ventilation capacity from 1.6 million SCFM to 2.3 million SCFM. The mine also installed diesel particulate filters on almost all of their fleet of diesel-powered equipment and switched from a standard #1 diesel fuel to a B70 blend biodiesel fuel. Combined, these interventions appear to have been effective in reducing TC air concentrations by >3-fold.

The air concentrations of 1-NP from personal air measurements from this study were also lower than what has been previously reported in mine environments. Our measurements had 1-NP geometric means of 65, 24, and 4.4 pg m^{-3} for the 'Face', 'Shop', and 'Surface' locations, respectively. In another study that compared underground mine 1-NP air concentrations to 'Surface' workers, Scheepers *et al.* (2003) found 1-NP geometric means of 197–2483 pg m^{-3} (personal samples from underground workers) and 8–110 pg m^{-3} (indoor surface workplace). The lower concentrations of 1-NP in our study may be attributable to the various measures instituted by the mine to reduce TC concentrations below the MSHA standard. For example, Bagley *et al.* (1998) and Sharp *et al.* (2000) both reported dramatic reductions in NPAH emissions for biodiesel and biodiesel blend fuels relative to conventional diesel fuel. In general, diesel particulate filters and diesel oxidation catalysts also substantially reduce PAH and NPAH emissions (Heeb *et al.*, 2008, 2010; Hu *et al.*, 2013; Khalek *et al.*, 2015). However, there have been examples where specific diesel oxidation catalysts actually increased specific NPAH compounds (Sharp *et al.*, 2000; Heeb *et al.*, 2008, 2010). Nevertheless, 1-NP concentrations underground within the mine remain substantially elevated compared to ambient air concentrations at the surface, especially at the 'Face' locations where active ore extraction tasks are taking place and ventilation is limited.

Despite the low concentrations of both EC and 1-NP, we observed a clear association between 1-NP and EC air concentrations as demonstrated in Figs. 1–3. First, we note that the tasks that the miners performed generated unique exposure groups with different medians and variability in exposure as seen in Fig. 1. Tasks at the 'Face' were expected to have the highest exposures; however, we additionally see that job tasks within this location modify the exposures of the workers to DE. Second, the distributions of measurements by task track well between 1-NP and EC as shown in Figs. 1 and 3. This indicates that both EC and 1-NP are measuring the same source (which by design is DPM). Fig. 2a–c show a robust linear relationship for each quantile of the task groups in Fig. 1, which is summarized in Table 2 where we show that 1-NP is predicted to increase ~6 pg m^{-3} per 1 µg m^{-3} increase in EC. The fit performance decreases with the 75th percentile, indicating more variability in the 1-NP to EC relationship as concentrations increase. This is also seen in Fig. 2d, where a fanning in the individual sample dot plot with increasing concentrations is observed. This variability contributes to a low correlation between EC and 1-NP ($r = 0.34$) for the individual samples. However, examination of Fig. 1 reveals that 1-NP is actually more self-consistent within a task group than is EC. A similar pattern is shown in Fig. 3, where 1-NP shows promise for improving differentiation between exposures for subject-task combinations relative to EC. The analysis of duplicate measurements from co-located sampling instruments also suggests that 1-NP measurements may have less error than EC measurements; however, future studies should include a more detailed comparison of the sources of measurement error. For example, the composition of diesel engine exhaust varies by engine type and condition, and by engine operating conditions. Schuetzle and Perez (1983) reported that the emission rate of multiple NPAHs was higher under low- versus high-load conditions. Similarly, Fox *et al.* (2015) reported a higher mass fraction of 1-NP in DPM formed under low- versus high-load conditions

This is a pilot study undertaken in a single underground mine using B70 biodiesel blend fuel and the EC concentrations we measured (GM = 8.1 µg m^{-3}) are much lower than the average EC values reported in compliance samples collected by MSHA (79 µg m^{-3} in 2015; MSHA 2017). To assess the generalizability of our observations, this work should be replicated in a larger study.

The MSHA DPM regulation for underground metal/nonmetal mines relies on measurements of TC (EC + OC), and this analytical limitation presents a problem for measuring low concentrations of DE-derived TC owing to the higher LOD for OC. However, for full-shift personal samples, the mean OC LOD in our study was 20 µg m^{-3}, which is well below the MSHA PEL of 160 µg m^{-3}. The OC LOD in our study was higher than typical values for method 5040 reported by NIOSH and MSHA (MSHA, 2016; NIOSH, 2016a), and might be reduced by measuring OC on the back-up quartz filters (dynamic blanks), rather than using field blanks to define the LOD, as was done in the current study. Nevertheless, vapor phase adsorption of OC to the quartz fiber filters unavoidably contributes uncertainty to the OC analysis (NIOSH, 2016a).

While there is a clear association between EC and 1-NP for the measurements, the individual 1-NP:EC sample pair variability warrants further investigation to better understand the sources of this variability. In previous studies a variety of factors have been shown to influence emission rates of 1-NP from diesel engines. Higher engine and exhaust temperature were associated with higher 1-NP emissions (Scheepers *et al.*, 2001); higher 1-NP emissions were associated with lower engine loads (Draper, 1986; Schuetzle and Perez, 1983; Fox *et al.*, 2015); use of biodiesel fuels was associated with lower 1-NP emissions (Sharp *et al.*, 2000). Use of diesel particulate filters and/or diesel oxidation catalysts in general reduce NPAH emissions (Hu *et al.*, 2013; Khalek *et al.*, 2015), although there have been examples in which emissions of NPAHs, including 1-NP, increased (Heeb *et al.*, 2010). Many of these variables also affect the emission rate of EC. This is another reason our findings should be replicated in a larger study, with cohort members selected to provide sufficient representation of multiple job locations and tasks, and mines selected to provide a diversity of engine and exhaust-treatment technologies, in order to better assess potential effect modification by various factors upon the EC:1-NP relationship. In addition, chemical characterization of the emissions from in-use diesel equipment in the mine would help to determine what engine characteristics modify exposures and DE composition. For example, emission factors for 1-NP and EC on in-use equipment would help to determine the expected EC:1-NP association for personal samples, and establish the expected variability in this relationship. More measurements on individuals performing the same task would also help determine how much individual subjects contribute to the variability seen within task.

One potential advantage of 1-NP compared to EC as a surrogate measure of DPM, is the absence of confounding sources of 1-NP in a typical mine environment. For the current study we deliberately selected a workplace where DE was by far the predominant source of particulate carbon. To test the effectiveness of 1-NP in 'preferentially' detecting DE, future work should include sampling in workplaces where additional non-diesel sources of EC and TC are present. In particular, measurement of 1-NP may facilitate measurement of personal exposures to DPM in coal mines—a setting in which concerns over non-diesel sources have compromised the use of EC as a surrogate for DPM. In addition, the specificity of 1-NP as a surrogate for DPM would prove useful in non-occupational settings such as examining community exposures to DPM. Finally, the utility of 1-NP as a surrogate measure for DE would ultimately depend upon demonstrating health effects associated with this metric.

In addition to the value of direct measurement of 1-NP exposure in air as a surrogate measure of DE exposure, air measurements of 1-NP could be useful to validate biomarkers of exposure to DE including measurements of 1-NP metabolites in urine (a measure of dose aggregated across multiple exposure pathways) (Seidel *et al.*, 2002; Toriba *et al.*, 2007; Miller-Schulze *et al.*, 2013; Morgott, 2014) or measurements of hemoglobin adducts to 1-aminopyrene (a measure of bioactivation that may lead to genotoxic effects) (van-Bekkum *et al.*, 1997; Zwirner-Baier and Neumann, 1999). Moreover, as many of the nitro-arenes, including 1-NP, are mutagenic and carcinogenic (IARC, 2014), measurements of 1-NP and its metabolites may better reflect the carcinogenic properties of DE (Scheepers *et al.*, 1995) and personal susceptibility factors such as polymorphisms in enzymes affecting nitro-arene metabolism.

Conclusion

We measured 1-NP, EC, OC, and TC exposures in workers, and the associations between these parameters, in an underground metal mine. DPM exposures were well below the MSHA PEL, and we found 1-NP personal air concentrations were consistently and positively associated with EC personal air concentrations in a setting where few, if any, sources of interference with EC or 1-NP are present. We found that 1-NP measurements differentiated exposures associated with specific work tasks more effectively than EC, which suggests that 1-NP may be more sensitive to differences in DE composition in this concentration range. However, a larger study is needed to definitively characterize potential effect modification by potential covariates such as equipment type, age, operating conditions, and job task. In summary, our findings demonstrate the utility of 1-NP as a surrogate measure of personal exposure to DPM. Rulemaking for a DE PEL is a complex multifaceted process, and it would be premature to propose 1-NP as a replacement for TC and EC for monitoring compliance with the DPM PEL. Nevertheless, measurements of 1-NP may prove useful to help understand and quantify variability in DPM exposure in a diversity of mine environments, and may be particularly valuable in settings such as coal mines or the ambient environment, where other potential surrogate measures of DPM exposure such as EC, TC, and NO_x are confounded by non-diesel sources.

Supplementary Data

Supplementary data are available at *Annals of Work Exposures and Health* online.

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