

## COMPARISON OF IN-MINE AND LABORATORY-GENERATED DIESEL PARTICULATE MATTER, BIOLOGICAL ACTIVITY, AND POLYNUCLEAR AROMATIC HYDROCARBON LEVELS

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### ABSTRACT

The extensive use of heavy-duty diesel engines in underground mines has raised concerns about the potential adverse health effects to miners exposed to diesel particulate matter (DPM) which contains mutagenic and carcinogenic organic compounds. The purpose of this study was to compare the levels of DPM, biological activity, and polynuclear aromatic hydrocarbons (PAH) attributable to diesel engines in underground coal mines and similar data collected in engine laboratory facilities to assess whether laboratory data could be used to predict in-mine levels of diesel emissions. Mean in-mine DPM and SOF concentrations varied from 0.90 to 1.9 mg/m<sup>3</sup> and 0.08 to 0.40 mg/m<sup>3</sup>, respectively. DPM-associated mutagenicity (mean) levels at two of the mines varied from 400 to 970 revertants/m<sup>3</sup>. Concentrations of three PAH differed by up to four-fold between two of the mines, largely reflecting differences in DPM and SOF levels. The laboratory raw exhaust data were divided by 100 to obtain expected or predicted values for in-mine concentrations. The predicted DPM, SOF, mutagenicity, and PAH values based on laboratory samples were not significantly different from many of the in-mine values. Using a potential in-mine dilution ratio range of 20 to 200:1, virtually all in-mine data fell within the predicted range. Therefore, an estimate of in-mine concentrations can be determined by evaluating laboratory data. This will allow for extrapolations from laboratory tests with particulate emission control devices to assess potential health-related benefits due to their in-mine use.

### INTRODUCTION

Use of diesel equipment in underground mines, particularly coal mines, has increased by more than 10-fold since 1974 (1). This has led to concern about increased exposure to diesel-related emissions in the underground mine environment. The International Agency for Research on Cancer (IARC) has classified diesel engine exhaust as a "probable human carcinogen" (2) and the National Institute for Occupational Safety and Health (NIOSH) has recommended that whole diesel exhaust be considered as a "potential occupational carcinogen" (3).

Much of the health-related concern has focused on diesel particulate matter (DPM). These particles are composed of solid and liquid compounds formed during the combustion process. The solid fraction is composed mainly of small

spherical carbon particles between 10 to 80 nm in size which agglomerate to form the core of the DPM (4). Liquid hydrocarbons and sulfates, in particular, adsorb and/or condense onto the surface of the solid core. The liquid hydrocarbon component is termed the soluble organic fraction (SOF) due to its solubility in solvents such as dichloromethane. Unlike other aerosol particles found in underground mines, DPM is almost entirely respirable, with 90% by mass being 1  $\mu$ m or less (5,6). Due to their small size, the DPM may reach and be retained by the deepest recesses of the lungs (7), thus potentially contributing to a variety of lung diseases (8). It has been well established that the SOF contains mutagenic and carcinogenic compounds such as polynuclear aromatic hydrocarbons (PAH; 2, 4, 9). Diesel SOF has been well documented to have wide ranging biological effects in assays indicating mutagenicity to potential carcinogenicity (2, 10 - 12).

Because of the extensive health-related data base on DPM and various gaseous exhaust components, NIOSH has further stated that "employers should assess the conditions under which workers may be exposed to diesel exhaust and reduce exposures to the lowest feasible limit" (3). At approximately the same time, the Mine Safety and Health Administration (MSHA) was also directed by an advisory committee to establish both a DPM standard and regulations to minimize exposure to all diesel pollutants, particularly in underground coal mines (1).

There currently is some information on DPM levels found in underground mines, but little information specifically on the composition or biological activity of DPM found in underground coal mines, particularly in areas with heavy diesel activity. Due to difficulties inherent in either scheduling or conducting in-mine tests, studies on emissions from diesels with and without DPM control devices for use in underground mines have typically been conducted under controlled engine laboratory conditions (13-15). Only Mogan et al. (16) have attempted to compare in-mine with laboratory DPM biological activity (mutagenicity) values; these comparisons were for hard rock mines without size-selective sampling to segregate the DPM from other in-mine particle aerosols.

This study was conducted 1) to determine a range of DPM, biological activity (as mutagenicity), and PAH levels found over a period of several working days in underground coal mines, 2) to calculate a DPM dilution ratio based on actual in-mine data, and 3) to compare engine laboratory-generated versus in-mine values to determine how well the

laboratory data predicted actual in-mine DPM-related levels. If a good relationship were found, then laboratory studies could more readily be used to evaluate how DPM emission control devices might aid in reducing DPM emissions and related potential health risks in the underground mine environment. This is also important because the lowest feasible concentration or limit could then be predicted for use in regulatory proceedings.

## MATERIALS AND METHODS

### Collection of In-mine Samples

Samples were collected from three underground coal mines, two in Utah (mines 1 and 2) and one in Kentucky (mine 3), using Hi-volume samplers operating at a flow rate of 1.13 m<sup>3</sup>/minute and equipped with inertial impactors to collect size-differentiated particles. The particles present on the back-up 20 x 25 cm Pallflex Corporation (Putnam, CT) TX40HI20-WW Teflon-coated woven glass fiber filters were less than 1 μm in size and were considered to be primarily diesel in origin (17,18). The Hi-volume samplers were placed in approximately the same location in each mine, in a haulageway one crosscut downwind from the feeder breaker and belt where diesel shuttle cars operated. For the most part, the samplers were operated only when coal was being mined, i.e., when there was diesel activity in the haulageway. From one to six samples were collected on each day for up to five days at each mine by Bureau of Mines (BOM) personnel. Mean sampling times at mines 1, 2, and 3 were 42, 38, and 19 minutes, respectively.

### Collection of Engine Laboratory Samples

The laboratory samples were collected from a Caterpillar 3304 heavy-duty diesel engine at the BOM Twin Cities Research Center diesel engine laboratory. Diluted DPM exhaust samples were collected on 51 x 51 cm filters of the same composition as those used in the mines. Samples were collected under both steady-state and transient operating conditions. The steady-state conditions were developed by researchers at Michigan Technological University (MTU; 19) and represent the six basic conditions a load-haul-dump vehicle experiences in the mine: 1) traveling in the drift with the bucket empty; 2) mucking at the face; 3) backing out of the drift loaded; 4) traveling to the dump site; 5) dumping the load; and 6) traveling back to the drift empty. By implementing a weighting factor for each condition, an estimate of average engine emissions was obtained. The transient cycle was developed by the BOM to represent many of the conditions a haulage vehicle may experience in a mine (15). An actual time trace of speed and load over the cycle is shown in Figure 1. A complete test consisted of running the engine through two cycles to obtain equilibrium conditions and then sampling DPM over the next nine cycles to obtain sufficient mass on the 51 x 51 cm filters for analysis. The sampling time for the transient samples was 24 minutes; sampling times for the various steady-state conditions ranged from 15 to 24 minutes.

### Determination of DPM and SOF Levels

Before laboratory sampling, all filters were conditioned in a humidity controlled room for 24 hours prior to weighing. After sampling, these filters were ammoniated for about one hour to stabilize sulfates (20), reweighed, frozen, and shipped to MTU on dry ice. The in-mine samples had to be handled somewhat differently. For the first mine, the samples were

conditioned and weighed at the BOM as above, collected in the field, shipped back to the BOM on dry ice, conditioned, reweighed, and then shipped to MTU on dry ice. For the second and third mines, both the pre and post weights were obtained at MTU; these filters were conditioned and weighed at MTU, shipped to the BOM, collected in the field, shipped back to MTU on dry ice, conditioned, and reweighed. This eliminated one step and reduced the amount of time the filter was at ambient conditions before filter extraction, thus minimizing the possibility of any chemical reactions that may occur on the filter.

At MTU, the filters were kept at -20°C until extracted. The SOF was removed by Soxhlet extraction (24 hours) with dichloromethane. The filters from mine 1 were reweighed before extraction; after extraction, the filters were weighed again to determine SOF levels gravimetrically. A different method was used with the remaining in-mine and laboratory samples. The extracted SOF was reduced in volume to approximately 5 ml using rotary evaporation under vacuum at 30°C. The concentrated extract was then rediluted with dichloromethane to a small, known volume (usually 10 ml). A 100 μl aliquot was then transferred to a pre-tared 16.5 mm diameter glass fiber filter. After evaporation of the solvent, the disk was reweighed and the SOF mass was calculated from the net mass on the disk and the total sample volume. The individual extracts from all haulageway filters from the same day from each mine were pooled to reflect average levels over each day's entire sampling period. Representative bulk coal samples were obtained from the coal face at each mine and subsequently ground to obtain particles of submicrometer size that were extracted using the above methods.

Dichloromethane was removed from the SOF samples via rotary evaporation (under vacuum) at 30°C and then under a stream of nitrogen at room temperature. Samples for mutagenicity assays were prepared by dissolving a known mass of SOF in a known volume of DMSO. These samples were stored in sealed glass or teflon vials at -15 to -20°C prior to analysis.

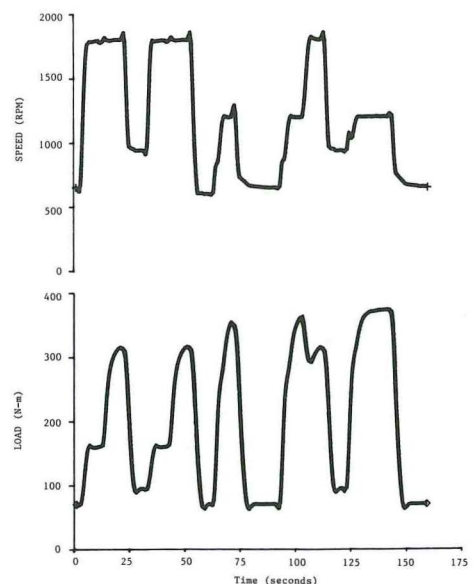


Figure 1. Time trace of transient cycle used in laboratory studies, showing variations in speed and load.

### Mutagenicity assays

Mutagenicity was determined using a modification of the microsuspension version of the *Salmonella typhimurium*/microsome mutagenicity bioassay or Ames assay (21, 22). Tester strain TA98 was provided by Dr. Bruce Ames (University of California, Berkeley). Rat liver S9 from Arochlor 1254-induced male Sprague-Dawley rats was purchased from Molecular Toxicology, Inc. (College Park, MD). Dimethyl sulfoxide was purchased from Sigma Chemical Co. (St. Louis, MO) and 2-nitrofluorene and 2-aminoanthracene were purchased from Aldrich Chemical Co. (Milwaukee, WI).

The TA98 tester strain was grown in a nephelo flask containing nutrient broth (Oxoid Broth No. 2, Oxoid USA, Columbus, MO) with 25 µg/ml ampicillin at 37°C and 125 rpm for approximately 10 hours on a gyrotary shaker. Growth was monitored at 540 nm using a Novaspec spectrophotometer (LKB Biochem Ltd., Cambridge, England) to an absorbance corresponding to approximately  $1.0 \times 10^9$  bacteria/ml (late log phase). The bacteria were separated by centrifugation (10,000 x g for 10 minutes at 4°C) and concentrated 10-fold in phosphate buffered saline (0.15 M, pH 7.4).

The microsuspension assay used was the same as the one developed by Kado et al. (21) except that 60 x 15 mm petri dishes containing 10 ml of bottom agar were used instead of 100 x 15 mm petri dishes (23, 24). For these assays, 100 µl of phosphate buffer (0.1 M, pH 7.4), 5 µl of sample in dimethyl sulfoxide, and 100 µl of concentrated bacteria (about  $1.0 \times 10^{10}$ /ml) were transferred to 12 x 75 mm test tubes. If the S9 mixture (22) was used to assess whether indirect-acting compounds requiring enzymatic metabolic activation were present, 100 µl of a 2% mixture was added in place of the buffer. These tubes were capped and placed on a gyrotary shaker in the dark at 37°C and 180 - 200 rpm for 90 minutes. Top agar (0.7 ml) was added to each tube and the contents mixed and poured onto the bottom agar dishes. All tests were conducted using duplicate dishes per dilution. Test dishes were counted after 63 hours incubation at  $37 \pm 2^\circ\text{C}$  (25) using an Artech Model 880 automatic colony counter (Artech Systems Corporation, Farmingdale, NY).

Controls were run on each test date, including solvent (dimethyl sulfoxide) controls for spontaneous revertant levels, genotypic checks (ampicillin resistance, crystal violet sensitivity, UV resistance, and histidine requirement), positive controls (2-nitrofluorene without S9 and 2-aminoanthracene with S9), and sterility checks on assay components (22). Spontaneous revertant levels (mean revertants/dish and coefficient of variation - C.V.) for TA 98 with and without S9 were 43 (11%) and 37 (7.5%), respectively. Mean responses with TA98 for 2-nitrofluorene without S9 (0.4/µg per dish) were 766 revertants per dish (8.2% C.V.). Mean responses with TA98 with S9 for 2-aminoanthracene (0.049 µg/dish) were 207 revertants per dish (11% C.V.).

### PAH Quantitation

The PAH compounds selected for these initial comparisons were fluoranthene, pyrene, and benzo[a]pyrene. The rationale for the choice of these compounds was based upon their known or suspected health effects (26) or their ability to act as precursors of PAH derivatives with known health effects (27, 28) and their known occurrence in diesel exhaust (9, 29).

Individual, crystalline compounds (fluoranthene, pyrene, benzo[a]pyrene, and 2-chloranthracene - the HPLC internal standard) were obtained from Aldrich Chemical Co. (Milwaukee, WI). The National Institute for Standards and Technology (Gaithersburg, MD) standard reference material (SRM) #1647a, a dilute acetonitrile solution of 16 priority pollutant PAH, was used as an HPLC calibration standard. Another SRM, #1650, which is a chemically characterized DPM reference standard, was used to determine recoveries during methods development and as a control sample. All solvents used for sample extraction, preparation, and HPLC analysis were distilled in glass (HPLC grade) obtained from Burdick and Jackson (Muskegon, MI).

The SOF samples for PAH analysis by HPLC were first separated into two fractions using column chromatography. The SOF was dried onto Florisil (1.0 g; Supelco, Bellefonte, PA) and packed into a 23 cm silanized glass Pasteur pipet which was plugged with silanized glass wool. This column was eluted first with hexane (10 ml) and then dichloromethane (8 ml) to elute the PAH + aliphatics and nitro-PAH fractions, respectively. The aliphatics were removed from the hexane fraction by a second column clean-up using a reverse-phase medium (octadecylsilane, C<sub>18</sub>, Supel-clean cartridges from Supelco). The aliphatics were retained on this column and the PAH eluted using acetonitrile (8 ml). After the internal standard was added and the acetonitrile fraction diluted to 10.0 ml, this fraction was quantitatively analyzed for the PAH of interest using reverse-phase HPLC and fluorescence detection.

The HPLC samples were eluted from a Vydac TP201 4.6 x 250 mm C<sub>18</sub> column (Alltech Assoc., Inc., Deerfield, IL), using a water/methanol gradient. After isocratic elution of 70% methanol for 30 minutes followed by a 30 minute linear gradient to 100% methanol, the final conditions were held constant for 15 minutes and then followed with a short reverse gradient and initial composition equilibration periods (5 minutes each). A Schoeffel Model FS 970 Fluorescence detector was used for this analysis with an excitation wavelength of 267 nm and a 389 nm emission cut-off filter. To decrease fluorescence quenching by dissolved oxygen in the mobile phase solvents, a platinum/alumina catalyst (Alfa Products, Ward Hill, MA) pre-column was used (30).

Quantitation was accomplished by internal standard method calculations using 2-chloroanthracene as the internal standard and SRM #1647a as the reference (calibration) standard.

### Data Analysis

Mutagenic activity (revertants/µg of SOF) was determined from the linear portion of the dose-response curves using a least squares regression (22). Activity or PAH levels on a volumetric (per m<sup>3</sup>) or mass of DPM basis were calculated using measured in-mine or laboratory values.

A mean of (daily) means value was calculated from the DPM and SOF data from each mine for use in the statistical analyses. An analysis of variance (ANOVA) was used to compare DPM, SOF, SOF (%), mutagenicity or PAH levels between each of the three mines and the laboratory-generated data (31). If significant differences were found for any of the comparisons, Tukey's studentized range test was then used to determine which specific data sets were or were not significantly different from each other (31). A significance level of 0.05 was used for all analyses. All these analyses were conducted using the SAS statistical package (32) on MTU's IBM 4381 mainframe computer.

## RESULTS

## Range of In-mine DPM, Activity, and PAH Levels

Levels of DPM and SOF found in each of the three mines when diesel activity was present in a haulageway are presented in Table 1. There were up to two and five-fold differences in DPM and SOF concentrations, respectively, between the three mines. Mean daily DPM concentrations ranged widely for mine 1 (0.98 to 3.26 mg/m<sup>3</sup>) but less so from mines 2 and 3 (0.85 to 0.94 mg/m<sup>3</sup> and 1.24 to 1.82 mg/m<sup>3</sup>, respectively). The variability was mainly attributable to fluctuations in coal production and thus vehicle operation during the sampling periods.

Biological activity determinations using the Ames assay were made only on DPM-extracts from mines 1 and 2 (Table 2). No assays were conducted with the extracts from mine 3. These masses were very low due to the low levels of extractable organics (see Table 1) and what mass was available was reserved for later analyses for levels of PAH (not reported on as part of this study).

As shown in Table 2, there was little difference in mean biological activity levels on a SOF or DPM mass basis between mines 1 and 2, but there was an over two-fold concentration difference when compared on a revertant/m<sup>3</sup> basis. Use of S9 metabolic activation had little effect on the observed mutagenic response of any sample. On a SOF mass

Table 1. Comparison of Measured and Estimated In-Mine DPM and SOF Levels.<sup>a</sup>

Sample <sup>b</sup>	n <sup>c</sup>	Parameter <sup>d</sup>		
		DPM (mg/m <sup>3</sup> )	SOF (mg/m <sup>3</sup> )	SOF (%)
Mine 1	4	1.9 (57)	0.40 (33)	24 (31)
Mine 2	3	0.90 (5.2)	0.17 (12)	19 (5.2)
Mine 3	5	1.4 (17)	0.08 (24)	5.7 (13)
Laboratory - Transient	1	0.52 (2.6)	0.15 (6.2)	29 (65)
Laboratory - Steady-State	1	0.60	0.27	44

<sup>a</sup>Reported laboratory diesel particulate matter (DPM) and soluble organic fraction (SOF) values reflect 100:1 dilution ratio expected in coal mines.

<sup>b</sup>Mine 1-3 samples collected at a similar haulageway site in each mine while diesels were operating.

<sup>c</sup>Number of days on which samples were collected.

<sup>d</sup>Reported as mean (C.V.,%) for in-mine samples and mean (C.V.,%) of 10 laboratory-transient samples; laboratory - steady state value represents a weighted average for replicate samples collected at six operating conditions.

Table 2. Comparison of Measured and Estimated In-Mine Mutagenicity Levels.

Sample <sup>a</sup>	n <sup>b</sup>	Parameter <sup>c</sup>					
		Revertants/ $\mu$ g SOF		Revertants/ $\mu$ g DPM		Revertants/m <sup>3</sup>	
		-S9	+S9	-S9	+S9	-S9	+S9
Mine 1	4	2.5 (51)	2.6 (45)	0.53 (22)	0.56 (12)	970 (60)	1100 (55)
Mine 2	3	2.6 (25)	2.4 (6.8)	0.50 (27)	0.45 (11)	440 (24)	440 (6.0)
Laboratory - Transient	1	2.6 (30)	2.7 (23)	0.74 (29)	0.78 (21)	380 (29) <sup>d</sup>	400 (21) <sup>d</sup>
Laboratory - Steady State	1	1.3	1.3	0.38	0.38	190 <sup>d</sup>	190 <sup>d</sup>

<sup>a</sup>Mine 1 and 2 samples collected at a similar haulageway site in each mine while diesels were operating.

<sup>b</sup>Number of days on which samples were collected.

<sup>c</sup>Reported as mean (C.V.,%) for in-mine (one sample per day) and laboratory-transient samples (10 replicates); laboratory - steady state value represents a weighted average for replicate samples collected at six operating conditions.

<sup>d</sup>Reported values reflect 100:1 dilution ratio expected in coal mines.

basis, activities without metabolic activation ranged from 1.9 to 4.3 revertants/ $\mu\text{g}$  and 1.9 to 3.0 revertants/ $\mu\text{g}$  at mines 1 and 2, respectively. Daily mutagenicity (without metabolic activation) on a DPM mass basis varied from 0.37 to 0.66 revertants/ $\mu\text{g}$  and 0.33 to 0.61 revertants/ $\mu\text{g}$  at mines 1 and 2, respectively. Concentration ranges for each sampling date for mines 1 and 2 were 0.56 to 1.8 revertants/ $\text{m}^3$  and 0.32 to 0.51 revertants/ $\text{m}^3$ , respectively.

The results for analyses for fluoranthene, pyrene, and benzo[a]pyrene at mines 1 and 2 are presented in Table 3. Data are available for only two of the four sampling days at mine 1. There was relatively low variability in the individual PAH levels on a mass (SOF or DPM) basis between days in each mine; if the variability increased for in-mine concentrations (based on C.V.), it was due to differences in DPM levels between sampling days. The range of concentrations ( $\text{ng}/\text{m}^3$ ) found for fluoranthene was 290 to 500 and 110 to 160 for mines 1 and 2, respectively. Pyrene concentrations ( $\text{ng}/\text{m}^3$ ) varied from 200 to 330 and 60 to 76 at mines 1 and 2, respectively. Benzo[a]pyrene concentrations ranged from 44 to 77 at mine 1 and 15 to 17 at mine 2. The consistently higher PAH concentrations detected at mine 1 may be related in large part to the higher DPM and SOF concentrations as compared to mine 2 (Table 1).

The coal samples from each mine had from 0.46 to 1.6% SOF, compared to 5.7 to 24% SOF for the haulage samples from the same mines (Table 1). None of the extracts had detectable mutagenicity, with or without metabolic activation. Levels of fluoranthene, pyrene, and benzo[a]pyrene were very low, comprising less than 0.3% to the total PAH collected with this size selective sampler. This appears to indicate that any submicrometer coal particles reaching the back-up filter would have contributed little in terms of either extractable organics, mutagenicity, or PAH to the in-mine concentrations considered to be DPM.

#### Estimation of In-mine Dilution Ratios

Concentrations of DPM and other diesel emissions obtained in the laboratory can be related to in-mine

concentrations if the in-mine dilution ratio can be determined. One method for estimating in-mine dilution ratios is by taking the ratio of carbon dioxide ( $\text{CO}_2$ ) emitted from the engine to the concentration measured underground and accounting for background concentrations (33). Carbon dioxide is a good tracer gas because it is fairly stable at atmospheric conditions, mixes well with air, and is easily measured with currently available instruments. Researchers have shown that when the engine is operated over simulated mine duty cycles, the average  $\text{CO}_2$  concentration ranges from 5.7 to 6.4% (19, 34; K.L. Bickel, BOM, 1990, Personal Communication). In-mine  $\text{CO}_2$  concentrations have been measured in nine underground dieselized coal mines. The concentrations ranged from near background levels (0.03%) to 0.144%, with an average of 0.084% (35; D.H. Carlson, MTU, 1990, Personal Communication). Using the range of engine  $\text{CO}_2$  values and the average in-mine concentrations, the average in-mine dilution ratio would vary from 105 to 118 or, for all practical purposes, an average dilution ratio of 100:1 could be assumed. Mogan et al. (16) estimated that dilution ratios in a dieselized nickel mine ranged from 54 to 119, averaging 92. Baumgard (33) has shown that dilution ratios in underground non-coal mines may vary anywhere between 20 to 200:1. However, for detailed comparison purposes, in-mine concentrations for this study were estimated by dividing the laboratory-generated data by 100.

#### Comparison of In-mine and Test Cell Data

The laboratory-generated DPM, SOF, and SOF (%) values obtained during transient and steady-state operation are also presented in Table 1, divided by 100 to represent estimated in-mine values. Results of a one-way ANOVA using the in-mine and laboratory transient data showed significant differences in DPM ( $p = 0.0002$ ) and SOF ( $p = 0.0001$ ) concentrations and in SOF (%) levels ( $p = 0.0001$ ). However, the laboratory values were not significantly different from some of the in-mine data when the results of Tukey's studentized range test were examined (Table 4). The values calculated using the laboratory steady-state data were not included in these analyses as replicate values were not available. However, these DPM and SOF values were within the ranges found for the laboratory and in-mine samples.

Table 3. Comparison of Measured and Estimated PAH Levels.<sup>a</sup>

Sample <sup>b</sup>	n <sup>c</sup>	Fluoranthene			Pyrene			Benzo[a]pyrene		
		$\mu\text{g}/\text{g}$ SOF	$\mu\text{g}/\text{g}$ DPM	$\text{ng}/\text{m}^3$	$\mu\text{g}/\text{g}$ SOF	$\mu\text{g}/\text{g}$ DPM	$\text{ng}/\text{m}^3$	$\mu\text{g}/\text{g}$ SOF	$\mu\text{g}/\text{g}$ DPM	$\text{ng}/\text{m}^3$
Mine 1	2	900	250	390	620	170	270	140	38	61
		(4.2)	(1.4)	(38)	(8.6)	(3.3)	(34)	(3.1)	(2.2)	(39)
Mine 2	3	780	160	130	400	85	70	94	20	16
		(14)	(9.8)	(16)	(13)	(9.7)	(14)	(5.8)	(2.4)	(6.8)
Laboratory	1	520	150	76 <sup>d</sup>	190	55	28 <sup>d</sup>	25	7.3	3.7 <sup>d</sup>
Transient		(31)	(28)	(30)	(35)	(32)	(33)	(19)	(18)	(18)

<sup>a</sup>Reported as mean (C.V.,%) for in-mine (one sample per day) and laboratory samples (10 replicates).

<sup>b</sup>Mine 1 and 2 samples collected at a similar haulageway site in each mine while diesels were operating.

<sup>c</sup>Number of days on which samples were collected.

<sup>d</sup>Reported values reflect 100:1 dilution ratio expected in coal mines.

Table 4. Significant Differences Between Mean Concentrations of DPM, SOF, and SOF (%).<sup>a</sup>

Comparison <sup>b</sup>	Sample			
	Mine 1	Mine 2	Mine 3	Transient
DPM (mg/m <sup>3</sup> )	A	AB	A	B
SOF (mg/m <sup>3</sup> )	A	B	B	B
SOF (%)	A	B	C	A

<sup>a</sup>Based on results of Tukey's studentized range test; means (Table 1) of samples with the same letters are not significantly different from each other.

<sup>b</sup>DPM - diesel particulate matter; SOF - soluble organic fraction.

Mutagenicity data (divided by 100) for the laboratory-generated transient and steady-state samples are presented in Table 2. A two-way ANOVA was used to compare the in-mine and transient data with and without S9 metabolic activation. All in-mine and laboratory activities were direct-acting in nature as there was no significant difference in responses with or without metabolic activation based on revertants/ $\mu\text{g}$  SOF ( $p = 0.9977$ ), revertants/ $\mu\text{g}$  DPM ( $p = 0.8088$ ) or revertants/m<sup>3</sup> ( $p = 0.8792$ ). There was no significant difference in mutagenic responses between the in-mine and transient data on a SOF mass basis ( $p = 0.9409$ ), but there was on a DPM mass ( $p = 0.0114$ ) and a concentration (revertants/m<sup>3</sup>;  $p = 0.0001$ ) basis. The results of Tukey's studentized range test are presented in Table 5. The differences in activity based on DPM mass was likely related to the higher amount of SOF (%) found with the laboratory DPM (Table 1). The lack of difference in transient and mine 2 concentration activities was not unexpected as no differences were found with the DPM and SOF concentration data (Table 1). In contrast, the calculated steady-state mutagenicity values (divided by 100) were not within the range of values found for either the mines or the

Table 5. Significant Differences Between Mutagenicity Mean Values.<sup>a</sup>

Comparison <sup>b</sup>	Sample		
	Mine 1	Mine 2	Transient
Revertants/ $\mu\text{g}$ SOF	A	A	A
Revertants/ $\mu\text{g}$ DPM	A	B	A
Revertants/m <sup>3</sup>	A	B	B

<sup>a</sup>Based on results of Tukey's studentized range test; means (Table 2) of samples with the same letters are not significantly different from each other.

<sup>b</sup>SOF - soluble organic fraction; DPM- diesel particulate matter.

transient samples (Table 2). Activity values on a mass or concentration basis were consistently about 50% of the other responses.

Mean values for fluoranthene, pyrene, and benzo[a]pyrene from the laboratory transient samples are also presented in Table 3. One-way ANOVAs were again used to compare PAH levels and concentrations between the in-mine and laboratory samples (Table 6). Significant differences in levels and concentrations were found for all three PAH. The key similarity for all PAH was that the laboratory concentrations (ng/m<sup>3</sup>) were not significantly different from the mine 2 values, but both were significantly different from the mine 1 values. As indicated previously, this may largely be related to the differences in DPM and SOF levels noted between these samples (Table 1).

Table 6. Significant Differences Between PAH Mean Values.

Comparison <sup>a</sup>	P value <sup>b</sup>	Sample <sup>c</sup>		
		Mine 1	Mine 2	Transient
<u>Fluoranthene</u>				
$\mu\text{g/g}$ SOF	0.0059	A	A	B
$\mu\text{g/g}$ DPM	0.0159	A	AB	B
ng/m <sup>3</sup>	0.0001	A	B	B
<u>Pyrene</u>				
$\mu\text{g/g}$ SOF	0.0001	A	A	B
$\mu\text{g/g}$ DPM	0.0001	A	B	C
ng/m <sup>3</sup>	0.0001	A	B	B
<u>Benzo[a]pyrene</u>				
$\mu\text{g/g}$ SOF	0.0001	A	B	C
$\mu\text{g/g}$ DPM	0.0001	A	B	C
ng/m <sup>3</sup>	0.0001	A	B	B

<sup>a</sup>SOF - soluble organic fraction; DPM - diesel particulate matter.

<sup>b</sup>Based on one-way ANOVA.

<sup>c</sup>Based on results of Tukey's studentized range test; means (Table 3) of samples with the same letters are not significantly different from each other.

## DISCUSSION

### Comparison of In-mine Values to Previously Published Data

The mean DPM concentrations shown in Table 1 are comparable to previously published data for underground dieselized coal mines. McCawley and Cocalis (36) measured the DPM concentrations in two underground coal mines using an impactor with a 1  $\mu\text{m}$  separation point. The results indicated that the average submicrometer concentrations measured in the haulageways of each mine were 0.41 and

0.37 mg/m<sup>3</sup>. Watts et al. (15) evaluated a prototype personal DPM sampler in an underground coal mine and the average haulageway submicrometer concentration was 0.94 mg/m<sup>3</sup>. Haney (37) measured the DPM in the haulageway of three underground coal mines using single stage impactors; the concentrations varied from 0.14 to 0.95 mg/m<sup>3</sup>. In-mine DPM concentrations have also been determined using the Laser Raman Quantitative Analysis and the results indicated the concentrations measured on the actual haulage vehicle averaged 0.76 mg/m<sup>3</sup> (38). The samples reported on in Table 1 were obtained only when the diesel vehicles were operating and therefore probably represent the peak concentrations found in mines. The previously reported data were collected over an eight hour period and therefore included times when the diesels were not operating, thus resulting in lower values than reported in this study.

At least some of the differences observed in SOF concentrations between the three mines could be due to the specific mining practices during the times samples were being collected. The lowest SOF concentrations were found at mine 3 which had almost 3 times the coal production of the other two mines and therefore had the heaviest loads on the diesel engines. This would result in higher engine exhaust temperatures and proportionately lower amounts of SOF (39).

It is difficult to compare the mutagenicity results from this study to values reported from other underground, non-coal mines due to differences in how the Ames assays were conducted. The microsuspension version of the Ames assay is increasingly being used due to its increased sensitivity and lower mass requirements as compared to the "standard" plate incorporation assay. In-mine activity levels on a mass basis therefore were at least ten-fold higher than those reported previously (16, 40, 41). One other study assessing mutagenicity using the microsuspension assay has been conducted with extracts from particles collected in underground coal mines (42). However, the particle samples were separated into more size fractions using a different type of sampling system than in this study. The result was little detectable activity, perhaps due to the relatively small mass amounts available for testing.

As with the mutagenicity data, comparisons between PAH concentrations found in underground coal mines in this study and those reported in other types of mines (40, 41, 43) are made difficult due to differences in sampling locations within the mines and sampling and analytical procedures. The fluoranthene and pyrene concentrations found for both mines are generally within the broad ranges reported for dieselized underground hardrock, metal, and salt mines. However, the benzo[a]pyrene concentrations are two or more times the levels found in all but one sample from a metal mine (43). It is not known at this time if the presence of submicrometer coal dust could have contributed to these PAH values.

#### Similarities and Differences Between In-mine and Laboratory Data

The emissions from diesel engines are dependent on the type of engine, state of maintenance, fuel composition, and how the engine is operated (44). In the BOM engine laboratory, the engine was well maintained, the same fuel was used throughout the tests, and the engine operation was controlled by a computer. With this type of set-up, day-to-day variations in most engine emission parameters that do not require extensive sample manipulation were typically less

than 5%. (Some of the increased variability noted with the mutagenicity and PAH data were related to the more extensive sample manipulation required for these analyses.)

There is very little control of these parameters when testing in the field. The haulage equipment in mines 1 and 2 used the same make and model of engines while mine 3 used a different brand. The operation of the engine is also a major factor in determining the amount and composition of DPM emitted. The production from mines 1 and 2 was considerably less than from mine 3 on a tons per vehicle rate, leading to higher exhaust temperatures, and had little non-productive operating time. Mines 1 and 2 frequently needed repairs and during this time the diesel vehicles were left idling; in this condition, diesel engines produce a high percentage of SOF. Other variations in DPM emissions may be attributable to differences in the diesel fuel being used, level of maintenance of the engine, and changes in ambient conditions. All of these effects may account for the variations in DPM composition between the mines.

Variations may also be due to differences in sample collection. Laboratory sampling times and filter face temperatures were essentially all equal (24 minutes and 33°C, respectively) for the transient tests and consistent within a condition for the steady-state tests. In contrast, in-mine haulageway sample times varied from as little as eight minutes to over one hour; sampling temperatures did not vary greatly, but averaged 13 to 17°C. Laboratory samples were also handled using standard laboratory practices, while the in-mine samples were handled the best way possible considering the circumstances. The filters were changed underground and the ambient conditions varied from mine to mine. Typically, the in-mine samples were stored for longer time periods before extraction due to the extra shipping and filter conditioning required.

One previous study has compared in-mine versus engine laboratory-generated levels of DPM-associated mutagenicity (16). This study was conducted using various steady-state conditions including portions of the MTU load-haul-dump cycle in the laboratory compared to similar diesel engines operating under actual or experimental load-haul-dump conditions in a hardrock mine. The in-mine mutagenicity levels (revertants/ $\mu$ g) were two to three times lower than the laboratory data; the authors attributed the lower in-mine mutagenicity values to the presence of other soluble, organic contaminants which might have diluted the diesel-produced SOF. However, their in-mine vs. laboratory mutagenic concentration values were generally similar. In the present study, little difference was found between the in-mine and laboratory transient mutagenicity data, except where differences in DPM or SOF levels or concentrations had already been found.

The steady-state cycle was designed to represent the majority of engine conditions a load-haul-dump vehicle experiences in a non-coal mine. The operation of a haulage vehicle in an underground coal mine is somewhat different and is more represented by the transient cycle. This cycle includes 13 different engine conditions, takes into account the emissions emitted during ramping, and includes an idle condition. Observations have indicated that mine vehicles spend a considerable amount of time idling. In contrast, the steady-state cycle consists of six different engine conditions and does not include idling. Therefore, the transient data should be more representative of the real-world, underground coal mine situation. This is evident from the results from this study which indicate that the transient data

more closely agree with the in-mine data than do the steady-state data.

The observed differences between the laboratory transient and steady-state SOF and mutagenicity data may also be related to how the weighted average for the steady-state values was calculated. As also noted by Draper et al. (14) in laboratory studies with the same engine, the first three modes of the MTU cycle have lower SOF concentrations but higher activity than do the last three modes. However, the cycle weighting factors place heavier emphasis on the data from the last three modes resulting in calculated weighted averages with lower SOF and activity values. The increased activity during the transient operation may be due to the emissions formed during changes in engine speed and load. During this transient time, the engine has a high fuel to air ratio and poor mixing in the combustion chamber. Consequently, there may be unburned fuel in the exhaust system which would adsorb onto the DPM and may cause the higher activity.

Despite these potential differences between the laboratory-generated and in-mine samples, there were frequently no significant differences ( $p = 0.05$ ) between the measured parameters assuming a 100:1 in-mine dilution ratio. When the potential 20:1 to 200:1 dilution ratio range (33) was examined, both the transient and steady-state data bracketed virtually all of the observed in-mine data. This held true even when the mean values for each sampling date or each individual sample value were examined. Therefore, laboratory-generated data collected under controlled conditions can be used to estimate in-mine levels in underground dieselized coal mines. Emission data from laboratory studies with different types and combinations of particulate emission control devices, fuels or engines can then be used to assess potential reductions in particulate levels and potential health-related benefits in the underground mine environment.

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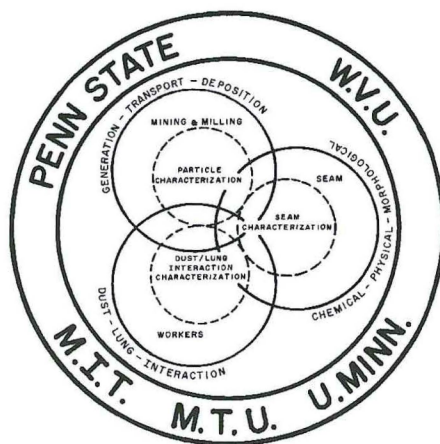
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