FINAL REPORT

Evaluation of Biodiesel Fuel and a Diesel Oxidation Catalyst in an Underground Metal Mine

Part 3 – Biological and Chemical Characterization

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EXECUTIVE SUMMARY

This study was conducted as part of a collaborative multidisciplinary, international effort to evaluate the effects of using a 50% biodiesel fuel blend (hereafter referred to as the blend fuel) and an advanced-type diesel oxidation catalyst (DOC) on underground metal mine air quality. The primary objective of this portion of the study was to evaluate the effects of using the 50% biodiesel fuel blend (with an advanced-type DOC) on potentially health-related DPM components, particularly polynuclear aromatic hydrocarbons (PAH), nitro-PAH, and mutagenic activity. This was accomplished by examining high-volume sampler filters containing the submicrometer particles, which are considered to be diesel in origin. Comparisons were also made for DPM and DPM component concentrations, such as the soluble organic fraction (SOF) and solids (SOL). All samples were collected at Inco, Ltd.'s Creighton Mine #3 in Sudbury, Ontario. The procedures employed for sampling and analyses were generally the same as used in previous underground mine studies.

With use of the blend biodiesel fuel as compared to a D2 fuel with an advanced-type DOC, there was a general trend of at least 20% reductions in downwind concentrations of DPM and most DPM-components, based on data obtained using high-volume samplers. The reductions in SOL (up to 30%) and mutagenic activity (about 75%) concentrations were found to be significant, whether or not the data were normalized on a brake-specific basis. The reductions in DPM (about 20%) were found to be significant only when normalized on a brake-specific basis. Although large reductions (up to 75%) were also present for DPM-associated PAH concentrations, these reductions were significant only for PYR due to the relatively high variability between sampling dates associated with the measurements. However, analysis of the PAH data without one set of the D2 fuel plus DOC data (having unexplainably low PAH values) resulted in significantly lower concentrations for FLU, BbF, and BkF, in addition to the lower PYR concentrations.

Based on the results of this study, use of this blend fuel plus an advanced-type DOC in an underground mine environment should result in significant reductions in those DPM components potentially of human health concern.

INTRODUCTION

Diesel exhaust is a complex mixture of organic and inorganic compounds and gas, liquid, and solid phase materials (as reviewed by Johnson et al., 1994). The organic compounds originate primarily from the unburned fuel and lubricating oil, although some may be formed during the combustion process and/or reaction with catalysts. Some of the hydrocarbons may be found adsorbed onto the solids (often termed the soluble organic fraction or SOF) and some may be in the gas phase. The solid phase emissions, by number count, are primarily nuclei mode (10-80 nm), spherical carbon particles. These are variously termed the solids, solid particulate matter or soot. The particles typically display a bi-modal distribution, with most of the particles (by mass) in the larger accumulation mode ($\geq$0.056 µm) but by number count more particles are in the smaller nuclei mode (<0.056 µm). DPM is composed of organic and elemental carbon, adsorbed and condensed hydrocarbons, and sulfate. The proportion of organic to inorganic carbon varies depending upon a number of factors, which include fuel, engine type, duty cycle, engine maintenance, operator habits, use of emission control devices and lube oil consumption.

Most of the health-related attention for diesel exhaust has focused on the carcinogenic potential of inhaled exhaust components, particularly the highly respirable diesel particulate matter (DPM). About 90 % of DPM are present in a size range from 0.0075 to 1.0 µm (Johnson et al., 1994); these particles are important in terms of potential health impacts due to the ability of particles to be inhaled and deposited in the bronchial passages and alveoli of the lungs. Although direct extrapolations to humans cannot be made and epidemiological studies to date have not been highly conclusive (e.g., as reviewed by Stober and Abel, 1996), the available data have raised the question whether or not the primary carbon particles in diesel exhaust could be potentially carcinogenic even in the absence of any adsorbed organics which may be carcinogens. Several epidemiological studies in the U.S. have found some correlations between levels of ambient fine particles, defined as <2.5 µm in diameter, and increased incidences of respiratory-, cardiovascular-, and cancer-related deaths, as well as pneumonia, asthma, and other respiratory problems (as reviewed by Reichardt, 1995). Many of the recommendations from a recent workshop on the Formation and Characterization of Particles (Health Effects Institute, 1997) related to the need for detailed information on both the physical and chemical character of ambient particles, including the impact of emission controls, alternative fuels, and new technologies.

One type of alternative fuel that has been proposed for use in reducing DPM emissions is soy methyl ester or biodiesel fuel, a fuel having no aromatic or sulfur content. Laboratory studies conducted with the University of Minnesota's Diesel Research Center have indicated that use of 100% biodiesel fuel with an advanced-type DOC could have some benefits in reducing the potentially health-related components of DPM emissions (Bagley et a., 1995a, 1998). Compared to emissions with a No. 2 diesel (D2) fuel without the DOC, use of the (low sulfur) D2 and 100% biodiesel fuels with the DOC resulted in similar, large (50 to 80%) reductions in DPM, including associated organics (SOF), and vapor phase organics levels. The solid component portion (SOL) of the DPM was also lowered with the 100% biodiesel fuel. Particle-associated polynuclear aromatic hydrocarbon (PAH) and 1-nitropyrene emissions were lower with use of the 100% biodiesel fuel (which had no measurable aromatic content) as compared to the D2 fuel, whether or not the DOC was used. Vapor phase PAH emissions were also similarly reduced (up to 90%)

when the DOC was used with either the D2 or 100 % biodiesel fuel. Use of the DOC resulted in over 50% reductions in both particle and vapor phase-associated biological (mutagenic) activity with both fuels. No vapor phase-associated mutagenic activity was detected with the 100% biodiesel fuel; only very low levels were detected with the D2 fuel and the DOC. Overall, use of this DOC for the engine conditions tested with the 100% biodiesel fuel, in particular, resulted in generally similar or greater reductions in emissions than for use of the D2 fuel. Based on these laboratory studies, use of the 100% biodiesel fuel was not expected to increase any of the potentially toxic, health-related emissions (over the D2 fuel levels without a DOC) that were monitored as part of this laboratory study. In tests with 100% biodiesel vs. No. 2 diesel fuel (with a modern DOC) in an underground metal mine, DPM reductions of up to 75% were found (McDonald et al., 1997). However, no other DPM-related measurements were made as part of this study.

This study was conducted as part of a collaborative multidisciplinary, international effort to evaluate the effects of using a 50% biodiesel fuel blend (hereafter referred to as the blend fuel) and an advanced-type diesel oxidation catalyst (DOC) on underground metal mine air quality. The primary focus of the overall study was on diesel particulate matter (DPM) emissions. As has been done with several other recent in-mine studies on effects of control devices on diesel-related emissions (Bagley et al., 1995b, Carlson et al., 1996, Johnson et al., 1996), information specifically related to not only DPM concentrations but levels of key DPM components could add to the evaluations of the effectiveness of using the 50% biodiesel fuel blend in place of low sulfur, D2 fuel (both with a DOC). For general comparison purposes, a few samples were also obtained from one day's engine operation with the 50% biodiesel fuel blend without the DOC.

OBJECTIVES

The primary objective of this portion of the study was to evaluate the effects of using the 50% biodiesel fuel blend (with an advanced-type DOC) on potentially health-related DPM components, particularly PAH, nitro-PAH, and mutagenic activity. This was accomplished by examining the high-volume sampler filters containing the submicrometer particles, which are considered to be diesel in origin. A limited number of filters containing the supermicrometer particles was also examined to evaluate the potential contributions of these emissions to the health-related parameters. It was expected that substantial reductions DPM-associated PAH, nitro-PAH, and mutagenic activity would be observed.

PROCEDURES

Overall Test Protocol – All samples were collected at Inco, Ltd.'s Creighton Mine #3 in Sudbury, Ontario, by personnel from the University of Minnesota's Diesel Research Center. Detailed descriptions of the mine test site, test vehicle, DOCs, and daily emission testing schemes are presented in the related report by Watts et al. (1998). The procedures employed for sampling and analyses were generally the same as used in previous underground mine studies (Bagley et al., 1995b, Carlson et al., 1996, Johnson et al., 1996). High-volume samplers were used to obtain sufficient particulate mass to allow for chemical quantification and mutagenicity determinations. As was done in the previous in-mine studies, these samplers were operated until the flow controllers could not be adjusted to meet the predetermined flow rate. The samplers at the upwind site could generally be operated throughout the entire shift. However, the samplers at the downwind site could be operated for much shorter times due to the higher levels of particulates in the air.

Sample Collection – The high-volume samplers were equipped with slotted inertial impactors and operated at a flow rate of 1.13 m^3/min were used to collect size differentiated particles from >3.5 to <1 µm in size. The slotted impactors have cut sizes of 3.5, 2.0, and 0.95 µm; particles below 1 µm in size were considered to be primarily diesel in origin and were obtained from the 20 x 25-cm Teflon-coated glass fiber (backup) filters (Pallflex Products Corp., Putnam, CN). After sample collection, the back-up and slotted filters were placed in paper folders, wrapped in aluminum foil, and kept refrigerated until delivered to MTU.

At the upwind site, two hi-volume samplers were operated side-by-side for the D2 fuel tests (two filters exposed/date). Due to the change in the sampling schedule (i.e., the addition of a sampling date with the blend fuel without the DOC), one hi-volume sampler was operated at the upwind site for all but the first sampling date with the blend fuel. The samplers at this upwind site were essentially operated for the entire shift (from 352 to 381 minutes/filter).

The original experimental plan called for two high-volume samplers to be operated side-by-side at the downwind site. Two filters were to be exposed with each sampler, for a total of four exposed filters per test date. This plan was followed in tests with the D2 fuel. Individual filters were exposed for 16 to 44 minutes before the sampling had to be stopped due to high filter loading (i.e., the flow controllers could no longer be adjusted to meet the predetermined rate). The consecutive minutes of sampler operation for the D2 fuel tests ranged from 43 to 70 minutes/day. Only one high-volume sampler was operated at a time for the blend fuel tests due to power fluctuations that occurred during the second week of testing. Four filters were still exposed per test date, but the filters were collected consecutively. Sampling times for individual filters were from 17 to 79 minutes. The consecutive minutes of sampler operation for the blend fuel tests ranged from 134 to 240 minutes/day.

DPM, SOF, Sulfate, and Solids Determinations – Before the sampling trip, the backup filters from the hi-volume samplers were equilibrated in a constant relative humidity (45 ± 2% R/H) and the pre-exposure weight was determined (to nearest 0.01 mg); this procedure was repeated after sample collection. The difference in mass before and after exposure gives the mass of DPM collected on the filter. After the second mass determination, the exposed filters were stored at -18±4°C until they were extracted (separately) for 3 h with dichloromethane (CH_2Cl_2) solvent in a Soxhlet apparatus to obtain the particle-associated soluble organic fraction (SOF). The extract was then concentrated using rotary evaporation under reduced pressure at 35°C. The mass of SOF was determined gravimetrically on a small (100 µL) aliquot of the total extract, which was brought to dryness. All extracts were stored frozen (-18 $\pm$ 4°C) until they were divided into aliquots for Ames assay and PAH quantification. For the Ames assay, PAH, and nitro-PAH determinations, the individual extracts from all upwind or downwind filters from the same sampling day were pooled to reflect average levels over each day's sampling period.

After SOF extraction, the filters were extracted by sonication in distilled, deionized water to obtain the sulfate fraction. Sulfate concentrations were determined on these extracts using an ion chromatograph with a conductance detector by comparison to an aqueous standard curve consisting of solutions having known concentrations of potassium sulfate. Because the filters could not be ammoniated after sampling to stabilize the sulfates (Society of Automotive Engineers Handbook, 1990), these resulting values only represent estimates. Solids (SOL) levels were estimated by subtracting the measured SOF and sulfate (SO_4) concentrations from the measured DPM concentration, as follows:

$$\mathrm{SOL} = \mathrm{DPM} - (\mathrm{SOF} + \mathrm{SO_4}) \tag{1}$$

Unexposed filters are also extracted and the masses of extractables were used to correct the exposed samples for background contributions on a mass basis.

Slotted filters from the downwind site for one sampling day with each fuel/DOC combination (D2 with DOC, blend with DOC, and blend without DOC) were also weighed and extracted to estimate levels of particles. The filters were separated by cut size and all filters of the same cut size/sampling date were placed together organics' extraction in order to obtain a larger and more accurately measured sample mass. After mass determinations, these extracts were prepared for use in Ames assays.

PAH and Nitro-PAH Determinations - The PAH quantified in this study were fluoranthene (FLU), pyrene (PYR), benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP) and benzo[*g,h,i*]perylene (BP). The PAH compounds chosen for quantification are all U.S. EPA priority pollutants. In addition, some of the compounds have been identified as animal carcinogens (BaP, Cook et al., 1932; BaA, Cavalieri et al., 1988 and Solt et al., 1982), co-carcinogens (FLU and PYR, Rice et al., 1984), or tumorigens (BbF, Amin et al., 1984). The nitropyrenes chosen for assay, i.e., 1-nitropyrene and 1,3- 1,6-, and 1,6-dinitropyrenes, are known animal carcinogens (1-nitropyrene, Ohgaki et al., 1984; 1,3- and 1,8-dinitropyrenes, Otofuji et al., 1987; 1,6-dinitropyrene, Ishizaka et al., 1982).

The SOF samples were first separated on a silica gel column to remove some of the interferences prior to analysis using high performance liquid chromatography (HPLC) with fluorescence detection. The silica gel column was first eluted with hexane to remove potentially interfering alkyl hydrocarbons. The PAH fraction was then collected using hexane (CH_2Cl_2; 80:20) as the eluant. The solvent was evaporated and the sample was reconstituted in acetonitrile (CH_3CN) containing the internal standard (2-chloroanthracene). A polymeric reverse phase C_{18} column was used for the HPLC separation. The procedural details for the separation and the wavelengths used for fluorescence detection are given in Table 1.

The quantification of nitro-PAH involved separation of nitro-PAH by reverse phase chromatography, reduction of the nitro compounds to fluorescent amines using a platinum catalyst, and detection using a fluorescence detector (FLD) (MacCrehan et al., 1988). The HPLC and FLD parameters are given in Table 2.

Although 1-nitropyrene and 1,3-dinitropyrene can be easily separated on the analytical column, these compounds coelute after elution through the catalyst column, where some resolution is lost. Therefore, the results are only semi-quantitative and given as 1-nitropyrene + 1,3-dinitropyrene. The nitro-PAH were present in the same silica gel fraction as the PAH; however, due to the different HPLC and detection conditions, the PAH compounds did not interfere with nitro-PAH .

Fuel Analysis – Analysis of the D2, blend, and 100% biodiesel fuels are presented in the related report by Watts et al. (1998). The D2 fuel had a Cetane Number of 44.2 and sulfur and aromatics contents of 0.0186 wt. % and 16.5 vol. %, respectively. The blend fuel had a Cetane Number of 48.9, and sulfur content of 0.0055 wt. %. The 100% biodiesel fuel had a Cetane Number of 53.5 and no detectable sulfur content. No analysis for aromatics content was conducted with either the blend or biodiesel fuel. Based on previous analyses (e.g., Bagley et al., 1998), the 100% biodiesel fuel would have had no detectable aromatics content. The aromatics content of the blend fuel would be expected to be roughly half that of the D2 fuel.

Samples of all three fuels were obtained for PAH analysis using techniques similar to those employed in previous studies (e.g., Bagley et al. 1995 a and b). However, the samples were obtained too late (March, 1998) to allow for quantitative comparisons between fuels to be made. No PAH were detected in the 100% biodiesel fuel; the relative amounts of PAH in the D2 and blend fuels could not conclusively be determined.

Mutagenic Activity Determination- Mutagenic activity of the SOF samples was determined using a version of the microsuspension (micropreincubation) modification of the *Salmonella typhimurium*/microsome mutagenicity bioassay (or Ames assay). After solvent removal from the filter extracts, dimethyl sulfoxide (DMSO) was added to an aliquot of known mass for use in this assay. Tester strain TA98 (provided by Dr. B. Ames, University of California, Berkeley) was used for all assays as it has consistently been shown the highest level responses to diesel emission organic components. If sufficient mass was available, assays were conducted with and without the S9 metabolic activation system to assess the significance of compounds requiring enzymatic transformation before displaying biological activity. Rat liver S9 from Arochlor 1254-induced male Sprague-Dawley rats was purchased from Molecular Toxicology, Inc. (Annapolis, MD).

The modified microsuspension assay was conducted as follows. The tester strain was grown in Nephelo flasks containing Oxoid broth No. 2 (Unipath Co., Ogdensburg, NY) with 25 μg/mL ampicillin at 37°C and 125 rpm for approximately 10 hours on a gyratory shaker. The incubation period was ended when culture growth reached an absorbance corresponding to approximately 1.0 x 10^9 bacteria/mL (late log phase). The bacteria are then separated by centrifugation (10,000 x g for 10 minutes at 4°C) and concentrated 10-fold in phosphate-buffered saline (0.015 M, pH 7.4). For these assays, 100 μl of the same phosphate buffer, 5 μl of sample in DMSO, and 100 μl of concentrated bacteria (about 1.0 x 10^{10} cells/mL) are transferred to 12 x 75 mm test tubes. If the S9 mixture was used to assess whether indirect-acting compounds requiring metabolic activation are present, 100 μl of a 2% mixture is added in place of the buffer. These tubes were capped and placed on a gyratory shaker in the dark at 37°C and 180 to 200 rpm for 90 minutes. Top agar (7 mL) was added to each tube and the contents mixed and poured onto the bottom agar dishes (60 x 15 mm containing 10 mL of bottom agar). All tests were conducted using duplicate dishes per dilution. Test dishes were counted after 63 hours incubation at 37 ± 2°C using an automatic colony counter. Appropriate controls were used with all tests. The mean (and Coefficient of Variation, CV) spontaneous revertant levels (revertants/plate) with and without S9 were 40.5 (12%) and 34.8 (22%), respectively. TA98-S9 mean (and CV) revertant levels with 2-nitrofluorene (400 ng/dish) and 1-nitropyrene (12.5 ng/dish) were 833 (6.3%) and

Table 1. HPLC Separation and Fluorescence Detection of PAH in SOF.

Column: Vydac 52TP201 C_{18}
Mobile Phase: H_2O / CH_3CN gradient as follows:

Run time, min.	% H_2O	% CH_3CN
0.0	35	65
15	35	65
35	0	100[1]
40	0	100

Fluorescence Detector Wavelengths (λ) and Expected Relative Retention Times (RTR):

Compound	λ, excitation/emission, nm	Expected RTR[2]
Fluoranthene	232/440	0.63
Pyrene	232/440	0.72
Benz[*a*]anthracene	267/389	1.2
Benzo[*b*]fluoranthene	247/418	1.7
Benzo[*k*]fluoranthene	247/418	1.8
Benzo[*a*]pyrene	247/418	1.9
Benzo[*g,h,i*]perylene	247/418	2.1

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[1] The gradient from 65 to 100% CH_3CN is linear over the 20 min. interval.

[2] Relative to 2-chloroanthracene, the internal standard, which elutes at approximately 17.1 min.

Table 2. HPLC Separation and Fluorescence Detection of Nitropyrenes in SOF.

Column: Zorbax ODS C_{18} , 4.6 mm x 25 cm
Reduction Column: 10% Pt on alumina, 2mm x 15 mm
Mobile Phase: Aqueous acetic acid (0.1% v:v) / CH_3OH gradient with flow rate programming as follows[1]:

Run time, min.	Flow rate, mL/min	% Acetic Acid	% CH_3OH
0.0	0.60	25	75
20		5	95
22	0.80		
35		0	95

Fluorescence Detector Wavelengths (λ) and Expected Relative Retention Times (RTR):

Compound	λ, excitation/emission, nm	Expected RTR [2]
1,6-Dinitropyrene	242/440	0.75
1,8-Dinitropyrene	242/440	0.78
1-Nitropyrene + 1,3-Dinitropyrene	242/440	0.86

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[1] The gradient from 75 to 95% CH_3OH is linear over the 0 - 20 min. interval; the change in flow rate is also linear over the interval 0 - 22 min..

[2] Relative to 2-chloroanthracene, the internal standard, which elutes at approximately 22.9 min.

714 (16%) revertants/dish, respectively. 40 (11) for TA98. TA98+S9 revertant levels with 2-aminoanthracene (97.5 ng/dish) were 419 (6.3) revertants/dish.

Data Analysis - All upwind and downwind data were converted to a unit per volume of air concentration basis using the total volume flow (m^3) for each sample. A mean of daily means value was calculated from the DPM and SOF data and estimated SO_4 and SOL data from each sampling location for the D2 fuel plus DOC and blend fuel plus DOC conditions. A mean for the downwind values based on type of fuel being tested was calculated for the mutagenic activity, PAH, and nitro-PAH data, that had only one value per sampling day.

The power correction factors determined in as part of the University of Minnesota-led portion of this study (Tables 1 and 2, Watts et al., 1998) were also applied to the downwind data sets for comparisons between fuels based on an energy-specific (or brake specific) basis. For all but the slotted filter values, the summary data are presented both as normalized (on a brake-specific basis) and as non-normalized concentrations. These normalized values are only approximations, as the power correction factors were determined based on full-shift operation and the high-volume samplers in the downwind locations were operated for less than the full shift.

These data were analyzed statistically using the similar procedures to those described in the companion report by Watts et al. (1998). As reductions in values for SOL, mutagenic activity, PAH, and nitro-PAH, in particular, were expected based on previous studies (Bagley et al., 1995a and 1998), a one-tailed, pooled Student's *t*-test was used to test for significant differences between sample sets (comparing emissions with the DOC using either fuel). The null hypothesis is:

$$H_0: \mu_1 \geq \mu_2 \qquad (2)$$

where μ_1 is the population mean of the blend fuel distribution and μ_2 is the population mean of the D2 fuel distribution. If the calculated t value is greater than the t critical (t_c)value ($t > -t_c$), then the null hypothesis can not be rejected. If the calculated t value is greater than the t critical value ($t \leq -t_c$), than the null hypothesis is rejected; the alternative hypothesis is:

$$H_A: \mu_1 < \mu_2 \qquad (2)$$

As the blend fuel without the DOC was tested on only one day, these data were not included in any statistical analyses. The upwind data were not used to correct the downwind data and were not analyzed statistically due to the low number of samples (only one or two per day) and the low levels of mass typically found, which resulted in having to use the extractable masses for PAH and nitro-PAH or mutagenic activity analyses (not both). These data were used to evaluate background vehicle emissions and potential contributions to downwind concentrations.

RESULTS AND DISCUSSION

DPM and DPM-Component Concentrations - Summary data for DPM and DPM-component (SOF, SO_4, and SOL) data obtained using the high-volume samplers located at the upwind and downwind sampling sites are presented in Table 3. . The data are presented both normalized

($mg/m^3/MW$-h) and non-normalized (mg/m^3). The mean daily values are presented inTables A-1 and A-2 (in Appendix A) for the D2 and blend fuels, respectively. The data in these Appendix tables are corrected for filter background masses but have not been normalized for vehicle power output on each test date. As with the other, related studies (Johnson and Walker, 1998, Watts et al., 1998), the data from sampling dates 10/16/97 (D2 fuel with DOC) and 10/24/97 (blend fuel with DOC) were not included in these calculations.

The measured emissions are particularly notable for the low variability (either within or between sampling dates). The variations in the DPM levels from this study were all about 10% (Table 3). In contrast, the coefficients of variation (obtained by dividing the standard deviations by the mean of mean values) for downwind DPM levels in previous studies in underground metal mines (Bagley et al., 1995b, Carlson et al., 1996, Johnson et al., 1996) ranged from 25 to 70. This low variability is due in large part to the uniform equipment operation and lack of upwind diesel activity. The low variability with the non-normalized downwind data also indicates that the collected samples were fairly representative of full shift conditions. The normalized data should, therefore, also represent good approximations for comparison purposes.

As was reported in the related reports (Johnson and Walker, 1998, Watts et al., 1998), the amount of material collected at the upwind sampling site was consistently very low. For the data presented in Tables A-1 and A-2, the upwind concentrations would have comprised ≤5% of the concentrations reported at the downwind site (using the high-volume samplers).

Although the primary intent of using the high-volume samplers was to obtain sufficient mass to conduct the PAH, nitro-PAH, and mutagenic activity analyses, some comparisons can be made between the different fuels and conditions tested for DPM, SOF, SOL, and SO_4 levels. As would be expected based on laboratory studies (Bagley et al., 1998, McDonald et al., 1995), use of the blend fuel without a DOC produced the highest DPM and SOF levels and the lowest SO_4 and SOL levels. In contrast, all of the DPM levels with the DOC are at the lower end of the range of downwind values found in two other metal mines (Bagley et al., 1995b, Carlson et al., 1996, Johnson et al., 1996). The emissions' levels obtained with the DOC are lower for SOF due to the DOC effects on hydrocarbon oxidation. As expected, all of the detected SO_4 levels are very low. None of the reported mean values are statistically significant due to the very low levels and high variability associated with these measurements.

DPM and SOL brake-specific levels were lower in tests with the DOC (by about 20 and 30%, respectively). As shown in Table 4, the reduction in SOL levels was found to be significant whether normalized or non-normalized data were used. The reduction in DPM concentrations was significant only for the brake-specific values. It is noteworthy that Watts et al. (1998) reported similar reductions in both total and elemental carbon levels. This indicates that the particulates collected over the shorter time intervals with the high-volume samplers were similar in composition to those collected by the full-shift samplers. The apparent increase in SOF levels (Table 3) may have been related in part to fuel spills on two of the biodiesel fuel sampling dates (as described by Watts et al., 1998). However, the brake-specific SOF concentrations were still about 10% higher when considering only data from the first two sampling dates with the blend fuel (plus DOC). Higher SOF levels with blends of D2 and biodiesel fuel, even with DOC use, have also been reported from laboratory studies (Last et al., 1995, McDonald et al., 1995).

Table 3. Summary of Downwind High-Volume Sampler Data.[1]

Fuel		DPM (mg/m^3)	SOF (mg/m^3)	SOF (%)	SO$_4$ (mg/m^3)	SO$_4$ (%)	SOL (mg/m^3)	SOL (%)
A. Not normalized on a brake-specific basis								
D2	Mean	0.58	0.10	21	0.024	4.1	0.45	78
(w/DOC)	[S.D.]	[0.09]	[0.02]	[6.4]	[0.008]	[4.5]	[0.08]	[3.9]
Blend	Mean	0.51	0.13	26	0.025	4.9	0.35	68
(w/DOC)	[S.D.]	[0.05]	[0.06]	[3.5]	[0.016]	[0.7]	[0.03]	[4.2]
Change (%)[2] (from D2)		-12	29	ND[5]	5.3	ND	-23	ND
Blend[3] (w/o DOC)		0.74	0.48	65	0.001	0.1	0.25	35
B. Normalized on a brake-specific basis[4]								
D2	Mean	1.1	0.20	NC[5]	0.047	NC	0.89	NC
(w/DOC)	(S.D.)	[0.11]	[0.07]		[0.054]		[0.05]	
Blend	Mean	0.91	0.24	NC	0.045	NC	0.63	NC
(w/DOC)	(S.D.)	[0.09]	[0.03]		[0.009]		[0.08]	
Change (%)[2] (from D2)		-20	19	ND	-2.8	ND	-30	ND
Biodiesel[3] (w/o DOC)		1.19	0.78	NC	0.002	NC	0.41	NC

1 Presented as mean of daily means (with S.D.) for data in Tables A-1 and A-2, not including data from test dates 10/16/97 (D2 w/DOC) and 10/24/97 (blend w/DOC).

[2] Calculated as [(Blend w/ DOC/D2 w/ DOC) –1] x 100.

[3] No mean or S.D. values as samples collected on only one date.

[4] Data normalized on a brake-specific basis are presented as mg/m^3/MW-h.

[5] ND = Not Determined; NC = Not calculated, as these data are not normalized (and, therefore, the values are the same as in part A of this table).

Table 4. Summary of Statistical Analysis of High-Volume Sampler DPM, SOF, SO_4 and SOL Data.

Parameter	Not Normalized[1]		Normalized[2]	
	t-value[3]	Significant[3]	t-value[3]	Significant[3]
DPM	-1.44	No	-3.12	Yes
SOF	0.50	No	1.04	No
SO_4	-0.30	No	-0.02	No
SOL	-2.41	Yes	-5.76	Yes

[1] Units of mg/m^3.

[2] Units of $mg/m^3/MW\text{-}h$.

[3] $t_c = 1.94$, $\alpha = 0.05$, 6 degrees of freedom (one-tailed test).

PAH and Nitro-PAH Concentrations - A summary of all of the PAH levels found in DPM extracts obtained from the downwind high-volume sampler filters is presented in Table 5 in units of ng PAH/m^3 (or ng PAH/m^3/MW-hr, when normalized on a brake-specific basis). The PAH levels obtained for both the upwind and downwind samples are presented for each test date in Tables A-3 and A-4 in Appendix A, in units of ng PAH/m^3 and μg PAH/g SOF, respectively. Summary data for PAH levels on a mass basis (μg PAH/g SOF) are presented in Table A-5. As with the other analyses, the levels obtained for 10/16/97 and 10/24/97 were not included in the summary tables. Compared to the ng PAH/m^3 units giving in-mine concentrations, the mass basis data provide a quantitative description of the nature of the extractable organic material associated with the particles, at least in terms of the relative concentrations of several specific PAH compounds. Comparing the two fuels, the data provide an indication of how the composition of the organic phase may be changing with fuel type.

There was too little mass for both Ames assay and PAH analysis for several of the upwind samples. Upwind samples from 10/20, 10/21, and 10/28/97 were used entirely for Ames assays. The upwind samples had lower particle-associated PAH concentrations (ng PAH/m^3) than the downwind samples from the same day but higher concentrations of PAH on a mass basis (μg PAH/g SOF). Similar results were reported in an earlier study (Bagley et al., 1995b), where particle samples were obtained under varying conditions from five different coal mines as well as a metal mine. As with the other measured concentrations, the upwind PAH values typically would have contributed ≤5% to the detected downwind PAH levels.

As shown in Table 5, the reductions in PAH concentrations (ng/m^3 or ng/m^3/MW-h) ranged from about 30 to 75% when using the blend fuel plus DOC to using the D2 fuel plus DOC. Similar reductions were found for comparisons on a mass basis (μg/g SOF; Table A-5). However, as indicated in Part A of Table 6, only the PYR concentrations were found to be significantly lower with the blend fuel plus DOC. Similar results were obtained for statistical analyses by PAH mass levels (Table A-6). An examination of the daily PAH data in Tables A-3 and A-4 indicates

Table 5. Summary of All Downwind PAH Concentrations (Mean and S.D.). [1]

Fuel	n	PAH Level [1]						
		FLU	PYR	BaA	BbF	BkF	BaP	BP
A. Not normalized on a brake-specific basis (ng PAH/m^3								
D2	4	12	15	0.92	0.69	0.26	0.54	1.1
(w/DOC)		[4.2]	[2.3]	[0.40]	[0.46]	[0.18]	[0.44]	[0.91]
Blend	4	7.3	5.6	0.63	0.32	0.11	0.20	0.29
(w/DOC)		[3.2]	[3.0]	[0.30]	[0.13]	[0.034]	[0.10]	[0.15]
Change (%) [2] (from D2)		-39	-63	-32	-54	-58	-63	-74
Blend (w/o DOC)	1	26	39	7.7	8.7	3.5	9.8	13
B. Normalized on a brake-specific basis (ng PAH/m^3/MW-hr)								
D2	4	24	29	1.8	1.4	0.50	1.1	2.1
(w/DOC)		[8.2]	[4.5]	[0.79]	[0.91]	[0.35]	[0.86]	[1.8]
Blend	4	13	11	1.2	0.59	0.20	0.36	0.54
(w/DOC)		[6.6]	[6.6]	[0.71]	[0.26]	[0.076]	[0.21]	[0.30]
Change (%) [2] (from D2)		-46	-62	-33	-58	-60	-67	-74
Blend (w/o DOC)	1	42	63	12	14	5.7	16	21

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[1] Based on data presented in Table A-3; data from test dates 10/16/97 (D2 w/DOC), and 10/24/97 (Blend with DOC) are not included. Standard deviations are given in brackets.

[2] Calculated as [Blend with DOC / D2 with DOC) - 1] x 100%.

that the values for most of the PAH detected with the 10/21/97 D2 plus DOC downwind sample were noticeably lower than those detected on the other sampling dates. There is no discernable sampling or analytical reason to account for these differences in values. However, the PAH data were also analyzed without the inclusion of these data.

When the data from 10/21/97 were deleted, there was little change in the reductions observed at the downwind location with use of the blend fuel plus DOC for either PAH concentrations (ng/m^3 or mg/m^3/MW-h; Table 7) or masses (µg/g SOF; Table A-7). Removal of the 10/21/97 downwind PAH data had a major impact on the statistical analyses, probably due to reductions in the variability associated with the blend fuel plus DOC data set. As shown in Part B of Table 6, the FLU, PYR, BbF, and BkF concentrations with the blend fuel plus DOC were significantly lower than the D2 fuel plus DOC concentrations (with or without data normalization). The relatively high variabilities associated with the BaA (blend fuel plus DOC), BaP (both fuels), and BP (both fuels) concentrations (Table 7) probably accounted for the lack of significance associated with the calculated PAH concentration reductions. On a µg PAH/g SOF basis, all PAH levels were significantly reduced with the blend biodiesel fuel with DOC compared to D2 fuel with DOC (Table A-6). The reductions ranged from 57 to 83% (Table A-7).

Operating without the DOC resulted in generally higher PAH levels (except for FLU, which was unchanged) compared to the blend biodiesel fuel with the DOC. The increases in PAH concentrations were larger for the blend biodiesel without the DOC on a ng PAH/m^3 (or ng PAH/m^3/MW-hr) than on a µg PAH/g SOF basis. This was probably due to the increased SOF levels without the DOC (Table 3).

In a previous study (Bagley et al., 1995a and 1998) using a 100% soy-methyl-ester fuel and a DOC, most of the measured particle-associated PAH levels were also found to decrease compared to D2 fuel with a DOC. Without the DOC, however, some PAH levels were even lower than with the DOC (on a µg/g SOF basis). As well as a difference in fuels, the two studies used different engines, DOCs and test conditions (test cell vs. in-mine).

The particle-associated PAH levels measured at MTU using the high-volume filter samples were lower than those measured by ORTECH personnel as part of a related study (Johnson and Walker, 1998) using either the GAP sampler (with a denuder) or their reference method (without a denuder). The only compounds above the minimum detection limits in all cases were FLU and PYR, so these compounds form the basis of comparison between sampling and analytical methods. Walker and Johnson reported a positive sampling artifact for FLU and PYR, by comparing the particle sample levels with and without the denuder. A positive artifact was attributed to adsorption of vapor phase semi-volatile PAH onto particles already collected on the filter. Therefore, since the high-volume samplers did not employ upstream denuders, one might also expect to see higher levels (not lower) when compared to the GAP samplers. Another difference in the two sampling methods, however, was the flow rate. The flow rate and filter face velocity for the high-volume samplers was 1,130 L/min and 2 L/cm^2/min, respectively, compared to 16.7 L/min and 1 L/cm^2/min for the GAP sampler. A possible consequence of the higher filter face velocity and ΔP across the filter could be an increase in the revolatilization effect resulting in a net negative artifact, as observed for the PAH more volatile than FLU and PYR (e.g., fluorene) using the GAP and reference methods. It is likely that both effects (vapor

Table 6. Summary of Statistical Analysis of PAH.

PAH	Not Normalized[1]		Normalized[2]	
	t-value	Significant	t-value	Significant
A. Analyses with All PAH Data.[3]				
Fluoranthene	-0.91	No	-0.99	No
Pyrene	-2.87	Yes	-2.85	Yes
Benz[*a*]anthracene	-0.56	No	-0.61	No
Benzo[*b*]fluoranthene	-1.23	No	-0.69	No
Benzo[*k*]fluoranthene	-0.70	No	-0.72	No
Benzo[*a*]pyrene	-0.67	No	-0.65	No
Benzo[*g,h,i*]perylene	-0.69	No	-0.70	No
B. Analyses without the 10/21/97 D2 Fuel PAH Data.[4]				
Fluoranthene	-3.15	Yes	-3.09	Yes
Pyrene	-3.36	Yes	-3.18	Yes
Benz[*a*]anthracene	-1.17	No	-1.77	No
Benzo[*b*]fluoranthene	-2.60	Yes	-2.52	Yes
Benzo[*k*]fluoranthene	-2.48	Yes	-2.38	Yes
Benzo[*a*]pyrene	-1.61	No	-1.33	No
Benzo[*g,h,i*]perylene	-1.43	No	-1.40	No

[1] Units of mg/m^3.
[2] Units of mg/m^3/MW-h.
[3] Data included in Table 5. $t_c = 1.94$, $\alpha = 0.05$, 6 degrees of freedom (one-tailed test).
[4] Data included in Table 7. $t_c = 2.02$, $\alpha = 0.05$, 5 degrees of freedom (one-tailed test).

adsorption and revolatilization) interact to produce some net effect, which is a function of both the sampling conditions and the substances being sampled.

The only samples found to have detectable levels of 1-nitropyrene or dinitropyrenes were two of the blend biodiesel samples (with DOC). These results are given in Table 8. For the remaining samples, levels of these nitropyrenes were found to be less than detection limits, which were approximately 200 pg on column, which corresponded to approximately 0.2 ng/m^3 for most of the samples. The two samples for which levels were above the detection limits had the largest sample masses of all the SOF samples with either D2 fuel or blend biodiesel with the DOC. The largest mass sample analyzed was the SOF from the blend biodiesel without the DOC; however the 1-nitropyrene and dinitropyrene levels for this sample were all below the minimum detection. Two possible scenarios are that the presence of these compounds is either an artifact of sampling

Table 7. Summary of Downwind PAH Concentrations, without the 10/21/97 D2 Fuel Data (Mean and S.D.). [1]

Fuel	n	PAH Level [1]						
		FLU	PYR	BaA	BbF	BkF	BaP	BP
A. Not normalized on a brake-specific basis (ng PAH/m^3)								
D2 (w/DOC)	3	14 [1.1]	16 [2.2]	1.1 [0.16]	0.91 [0.20]	0.34 [0.084]	0.71 [0.35]	1.4 [0.72]
Blend (w/DOC)	4	7.3 [3.2]	5.6 [3.0]	0.63 [0.30]	0.32 [0.13]	0.11 [0.034]	0.20 [0.10]	0.29 [0.15]
Change (%) [2] (from D2)		-48	-65	-43	-65	-68	-72	-79
Blend (w/o DOC)	1	26	39	7.7	8.7	3.5	9.8	13
B. Normalized on a brake-specific basis (ng PAH/m^3/ MW-hr)								
D2 (w/DOC)	3	28 [2.4]	30 [4.5]	2.2 [0.35]	1.8 [0.41]	0.66 [0.18]	1.4 [0.71]	2.8 [1.5]
Blend (w/DOC)	4	13 [6.6]	11 [6.6]	1.2 [0.71]	0.59 [0.26]	0.20 [0.076]	0.36 [0.21]	0.54 [0.30]
Change (%) [2] (from D2)		-54	-63	-45	-67	-70	-74	-81
Blend (w/o DOC)	1	42	63	12	14	5.7	16	21

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[1] Based on data presented in Table A-3; data from test dates 10/16/97 and 10/21/97 (D2 w/DOC), and 10/24/97 (Blend with DOC) are not included. Standard deviations are given in brackets.

[2] Calculated as [Blend with DOC / D2 with DOC) - 1] x 100%.

Table 8. Nitropyrenes Detected in Downwind SOF. [1]

Fuel	Date	Level (ng/m^3) 1,6-DNP	1,8-DNP	1-NP + 1,3-DNP[2]
Blend	10/25/97	0.77	1.2	0.27
(w/DOC)	10/28/97	0.11	0.18	0.92

[1] Results for the remaining DW samples were all below minimum detection limits.
[2] Levels are approximate due to coelution of 1-nitropyrene and 1,3-dintropyrene.

or a product of reactions occurring in the DOC. There are not enough data, however, to establish statistically valid conclusions.

Mutagenic Activity Concentrations – A summary of the mutagenic activity values obtained from the downwind high-volume sampler filters is presented in Table 9. The mutagenic activity values determined from both the upwind and downwind samples obtained from each date are presented in Table A-8 in Appendix A. The activity detected was primarily direct-acting in nature, as the addition of the S9 mammalian metabolic activation system did not increase the responses. this decrease in activity with S9 use has been found in other in-mine studies (Bagley et al., 1995b, Carlson et al., 1996, Johnson et al., 1996).

Activity determinations were not made for several of the upwind sampling dates, as too little mass was obtained to allow for both mutagenic activity and PAH/nitro-PAH measurements. As was found in these previous in-mine studies, the upwind samples typically had higher activity on a mass basis (revertants/µg), probably due to the longer collection times. These samples but typically had much lower activity on a concentration basis (revertants/m^3) due to the lower amount of mass collected (as shown in Tables A-1 and A-2).

Use of the blend biodiesel fuel even without the DOC resulted in a decrease in mutagenic activity on a mass basis (revertants/µg SOF). Due to the increased amount of SOF with the DPM (Table 3), the blend fuel without the DOC activity levels (revertants/µg DPM and revertants/m^3 or /m^3/MW-h) were higher than obtained with the D2 fuel (Table 9). However, use of the DOC with the blend fuel resulted in at least 70% decreases in all of the mutagenic activity parameters listed in Table 9. All of these decreases were found to be significant (Table 10). These decreases were not unexpected, based on results of laboratory studies with a 100% biodiesel fuel and an advanced-type DOC where similar results were obtained (Bagley et al., 1995a and 1998).

Table 9. Summary of Downwind Mutagenic Activity Data (Mean and S.D.).[1]

Fuel	n	Mutagenic Activity SOF - S9 (rev/µg)	SOF +S9 (rev/µg)	DPM -S9 (rev/µg)	Activity -S9 (rev/m^3)[2]
A. Not normalized on a brake-specific basis					
D2 (w/DOC)	4	1.00 [0.30]	0.63 [0.19]	0.17 [0.03]	97.3 [22.2]
Blend (w/DOC)	4	0.19 [0.03]	0.14 [0.05]	0.05 [0.03]	24.6 [5.61]
Change (%)[3] (from D2)		-82	-79	-71	-75
Blend (w/o DOC)	1	0.30	0.22	0.19	140
B. Normalized on a brake-specific basis[2]					
D2 (w/OCC)	4	NC[4]	NC	NC	190 [44.9]
Blend (w/OCC)	4	NC	NC	NC	44.9 [13.6]
Change (%)[3] (from D2)		NC	NC	NC	-76.6
Blend (w/o OCC)	1	NC	NC	NC	230

[1] Based on data presented in Table A-7; data from test dates 10/16/97 (D2 w/DOC) and 10/24/97 (Blend w/DOC) are not included.

[2] Data normalized on a brake-specific basis are presented as rev/m^3/MW-h.

[3] Calculated as [(Blend w/ DOC/D2 w/ DOC) -1] x 100

[4] NC = Not calculated, as these data are not normalized (and, therefore, the values are the same as in part A of this table).

Table 10. Summary of Statistical Analysis of Mutagenic Activity Data.

Activity	t-value[1]	Significant[1]
Revertants/ µg SOF (– S9)	-7.47	Yes
Revertants/ µg SOF (+ S9)	-5.16	Yes
Revertants/ µg DPM (– S9)	-8.00	Yes
Revertants/m^3 (-S9)	-6.34	Yes
Revertants/m^3/MW-h (-S9)	-6.38	Yes

[1] $t_c = 1.94$, $\alpha = 0.05$, 6 degrees of freedom (one-tailed test).

Slotted Filter Studies – Three sets of slotted filters from three sampling dates (representing each of the tested fuel/DOC combinations) were analyzed for DPM, SOF, and mutagenic activity levels (Table 11) in order to assess whether biologically active organics associated with particles larger than 0.95 µm was present. No PAH or 1-NP determinations were made on these samples due to the low amount of extractable mass obtained/filter set. As indicated in Table 8, the top slotted filters (designated as A) should retain particles ≥3.5 µm, the middle slotted filters (designated as B) should retain particles should retain particles between 2.0 and 3.5 µm, and the bottom slotted filters (designated as C) should retain particles between 0.95 µm and 2.0 µm. The particle values could readily be corrected for background mass levels associated with unexposed filters (designated by "Corr." in Table 11). With several filter sets, very little extractable organic mass (SOF) was detected; when corrected for the mass of extractable organics from the unexposed filters, negative values were obtained for several of the samples for "Corr. SOF". The uncorrected SOF values (designated as "Uncorr. SOF") are therefore also reported in Table 11. No mutagenic activity was detected with the organics extracted from the unexposed filters. The particle-associated (revertants/µg Part.) and concentration (revertants/m^3) mutagenic activity values are based on the "Uncorr. SOF" values and therefore probably represent overestimates.

The results from this limited study indicate that the particles larger than 0.95 µm are both lower in extractable organics (SOF) and in mutagenic activity as compared to the submicrometer particles. With DOC use, relatively little particle or organic mass and no mutagenic activity was associated with the largest size particles (≥3.5 µm). Based on the data presented in Table 11, the highest levels of particles, extractable organics, and activity were typically associated with the 0.95 to 2.0 µm size range. Some of these particles could be diesel in origin, although they would represent a relatively small portion of the concentrations obtained with the submicrometer sampling stage (Tables 3 and 9).

Table 11. Summary of DPM, SOF, and Mutagenic Activity Obtained from Slotted Filters.[1]

Fuel	Date	Stage[2]	Corr. Part.[3] (mg/m³)	Uncorr. SOF[4] (mg/m³)	Corr. SOF[3] (mg/m³)	Uncorr. SOF[3] (%)	Mutagenic Activity SOF-S9 (rev/µg)	Mutagenic Activity DPM-S9 (rev/µg)	Mutagenic Activity Activity (rev/m³)
D2 (w/DOC)	10/21/97	A	0.07	0.01	0.002	13.9	NR[5]	NR	NR
		B	0.152	0.008	<-0.001	5.2	0.36	0.019	2.82
		C	0.26	0.02	0.015	8.9	0.17	0.015	3.95
Blend (w/DOC)	10/24/97	A	0.08	0.004	-0.007	4.8	NR	NR	NR
		B	0.14	0.01	0.001	8.3	NR	NR	NR
		C	0.15	0.06	0.05	38.5	0.14	0.05	8.04
Blend (w/o DOC)	10/30/97	A	0.20	0.01	0.004	8.1	NR	NR	NR
		B	0.17	0.02	0.01	9.7	0.20	0.02	3.22
		C	0.14	0.03	0.02	17.2	0.26	0.05	6.43

[1] Extractable mass from each of three samples per date was pooled.

[2] A = top slotted filter, with cut size of 3.5 µm; B = middle slotted filter, with cut size of 2.0 µm; and C = bottom slotted filter, with cut size of 0.95 µm.

[3] Data corrected for unexposed filter mass but not normalized on a brake-specific basis.

[4] Data not corrected for unexposed filter mass, as more mass on unexposed than on exposed filter; data also not normalized on a brake-specific basis.

[5] NR = no response above spontaneous revertant levels.

CONCLUSIONS

As expected, substantial reductions in DPM-associated PAH and mutagenic activity were observed with use of the blend fuel plus an advanced-type DOC as compared to use of D2 fuel with the same type of DOC. The following specific conclusions can be made based on the data collected using the high-volume samplers at the downwind sampling site.

1. There was a general trend of at least 20% reductions in concentrations of DPM and most DPM-components with use of the blend fuel plus DOC.

2. The reductions in SOL (up to 30%) and mutagenic activity (about 75%) concentrations were found to be significant, whether or not the data were normalized on a brake-specific basis.

3. The reductions in DPM (about 20%) were found to be significant only when normalized on a brake-specific basis.

4. Although large reductions (up to 75%) were also present for DPM-associated PAH concentrations, these reductions were significant only for PYR due to the relatively high variability between sampling dates associated with the measurements. However, analysis of the PAH data without one set of the D2 fuel plus DOC data resulted in significantly lower concentrations for FLU, BbF, and BkF, in addition to the lower PYR concentrations.

5. Based on the results of this study, use of this blend fuel plus an advanced-type DOC in an underground mine environment should result in significant reductions in those DPM components potentially of human health concern.

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APPENDIX A – ADDITIONAL DATA

Table A-1. High-Volume Sampler Data Obtained with Use of D2 Fuel (Mean and S.D.).[1]

Date	Site[2]	n[3]	DPM (mg/m^3)	SOF (mg/m^3)	SOF (%)	SO$_4$[4] (mg/m^3)	SO$_4$ (%)	SOL[5] (mg/m^3)	SOL (%)
10/16/97	UW	2	0.047	0.022	47.6	0.003	1.9	0.023	50.5
			[0.003]	[0.005]	[7.5]	[0.001]	[1.6]	[0.001]	[5.8]
	DW	1	0.19	0.033	17.6	0.002	0.81	0.155	81.6
10/17/97	UW	2	0.016	0.004	24.2	<0.001	0.8	0.012	75
			[<0.001]	[0.001]	[4.4]		[1.0]	[<0.001]	[3.5]
	DW	4	0.615	0.15	24.1	0.005	0.9	0.46	74.9
			[0.102]	[0.041]	[3.4]	[0.007]	[1.3]	[0.069]	[4.1]
10/20/97	UW	2	0.038	0.004	11.2	<0.001	0.5	0.034	88.3
			[0.001]	[0.001]	[3]		[0.1]	[0.001]	[3.1]
	DW	4	0.518	0.084	16.6	0.005	1	0.429	82.5
			[0.081]	[0.002]	[2.5]	[0.004]	[0.9]	[0.085]	[3.4]
10/21/97	UW	2	0.026	0.004	15.9	<0.001	0.9	0.022	83.2
			[<0.001]	[0.001]	[2.1]		[0.0]	[0.001]	[2.1]
	DW	3	0.552	0.083	15	0.022	3.9	0.447	81
			[0.042]	[0.002]	[1.6]	[0.007]	[1.1]	[0.042]	[1.8]
10/22/97	UW	2	0.025	0.006	23.3	<0.001	1.3	0.019	75.5
			[<0.001]	[<0.001]	[1.8]		[1.4]	[<0.001]	[0.3]
	DW	4	0.624	0.089	14.5	0.063	10.4	0.472	75.1
			[0.104]	[0.017]	[3.6]	[0.013]	[3.1]	[0.105]	[5.4]

[1] Corrected for unexposed filter values.
[2] UW = Upwind; DW = Downwind.
[3] n = Number of filters
[4] Estimated values, as filters were not ammoniated after exposure.
[5] Calculated values, as SOL = DPM – (SOF + SO$_4$).

Table A-2. High-Volume Sampler Data Obtained with Use of Blend Fuel (Mean and S.D.).[1]

Date	Site[2]	n[3]	DPM (mg/m^3)	SOF (mg/m^3)	SOF (%)	SO$_4$[4] (mg/m^3)	SO$_4$ (%)	SOL[5] (mg/m^3)	SOL (%)
A. DOC used									
10/24/97	UW	2	0.073	0.015	19.8	0.001	1.1	0.058	79.2
			[0.017]	[0.006]	[3.2]	[0.001]	[1.3]	[0.012]	[1.9]
	DW	4	0.67	0.145	21.7	0.005	0.8	0.521	77.5
			[0.195]	[0.038]	[0.9]	[0.005]	[1.0]	[0.158]	[1.0]
10/25/97	UW	1	0.007	<0.001	12.5	<0.001	<0.1	0.006	87.4
	DW	4	0.498	0.119	23.7	0.022	4.3	0.356	72
			[0.053]	[0.03]	[3.6]	[0.013]	[2.2]	[0.014]	[5.8]
10/27/97	UW	1	0.012	0.003	25.9	<0.001	5.9	0l0083	68.3
	DW	4	0.581	0.134	23.1	0.026	4.5	0.421	72.5
			[0.031]	[0.028]	[5.6]	[0.022]	[3.5]	[0.048]	[5.9]
10/28/97	UW	1	0.018	0.003	15.7	<0.001	3.2	0.0149	81.2
	DW	4	0.461	0.135	30.1	0.028	5.8	0.297	64.1
			[0.027]	[0.10]	[6.5]	[0.017]	[2.6]	[0.017]	[5.5]
10/29/97	UW	1	0.024	0.003	11	<0.001	2.6	0.021	86.4
	DW	4	0.487	0.141	28.7	0.024	5.1	0.321	66.2
			[0.065]	[0.037]	[5.0]	[0.005]	[1.4]	[0.041]	[5.0]
B. DOC not used									
10/30/97	UW	1	0.029	0.009	32.1	<0.001	2.6	0.0187	65.3
	DW	3	0.735	0.481	65.4	0.001	0.1	0.253	34.5
			[0.085]	[0.06]	[1.1]	[0.001]	[0.1]	[0.025]	[1.1]

[1] Corrected for unexposed filter values.
[2] UW = Upwind; DW = Downwind.
[3] n = Number of filters.
[4] Estimated values, as filters were not ammoniated after exposure.
[5] Calculated values, as SOL = DPM – (SOF + SO$_4$).

Table A-3. PAH Concentrations in DPM Extracts (SOF).

Fuel	Date	Site[1]	PAH Level (ng/m^3)						
			FLU	PYR	BaA	BbF	BkF	BaP	BP
D2 (w/DOC)	10/16/97	UW	2.5	3.5	0.19	<0.1	<0.03	0.082	<0.05
		DW	4.6	5.2	0.30	0.21	0.067	0.16	0.21
	10/17/97	UW	0.83	1.3	0.091	0.086	0.027	0.091	0.11
		DW	15	17	1.3	1.1	0.43	1.1	2.2
	10/20/97	UW	ND[2]						
		DW	14	16	1.0	0.84	0.30	0.62	1.0
	10/21/97	UW	ND						
		DW	6.1	13	0.35	<0.1	<0.03	0.070	<0.05
	10/22/97	UW	0.22	0.32	<0.02	<0.04	<0.01	0.0093	<0.02
		DW	13	13	1.0	0.76	0.28	0.42	0.95
Blend (w/DOC)	10/24/97	UW	6.3	6.6	1.0	0.57	0.21	0.42	0.36
		DW	18	16	1.3	0.52	0.32	0.58	0.58
	10/25/97	UW	ND						
		DW	3.0	1.9	0.34	0.15	0.063	0.078	0.13

[1] UW = Upwind; DW = Downwind.
[2] ND = Not determined due to small amount of mass available.

Table A-3. PAH Concentrations in DPM Extracts (SOF), Cont'd.

Fuel	Date	Site[1]	PAH Level (ng/m^3) FLU	PYR	BaA	BbF	BkF	BaP	BP
Blend	10/27/97	UW	0.18	0.15	0.026	<0.02	0.013	0.025	0.16
(w/DOC)		DW	10	5.0	0.59	0.45	0.13	0.24	0.43
	10/28/97	UW	ND						
		DW	9.5	9.2	1.1	0.38	0.13	0.030	0.40
	10/29/97	UW	0.38	0.47	0.16	<0.02	0.013	0.027	0.18
		DW	6.7	6.3	0.52	0.32	0.097	0.16	0.21
Blend	10/30/97	UW	1.4	1.9	0.55	0.65	0.29	0.75	0.95
(w/o DOC)		DW	26	39	7.7	8.7	3.5	9.8	13

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[1] UW = Upwind; DW = Downwind.

Table A-4. PAH Mass Levels in DPM Extracts (SOF).

			PAH Level (µg PAH/g SOF)						
Fuel	Date	Site[1]	FLU	PYR	BaA	BbF	BkF	BaP	BP
D2	10/16/97	UW	110	160	8.3	<4.7	<1.3	3.7	<2.2
(w/DOC)		DW	140	160	9.0	6.4	2.0	4.8	6.4
	10/17/97	UW	220	350	24	23	7.2	24	30
		DW	100	120	8.6	7.5	2.9	7.3	15
	10/20/97	UW	ND[2]	ND	ND	ND	ND	ND	ND
		DW	170	190	12	10	3.5	7.3	12
	10/21/97	UW	ND	ND	ND	ND	ND	ND	ND
		DW	73	150	4.2	<1.4	<0.39	0.84	<0.66
	10/22/97	UW	38	55	<3.2	<7.5	<2.1	1.6	<3.5
		DW	150	150	11	8.5	3.2	4.7	11
Blend	10/24/97	UW	430	450	70	39	14	29	24
(w/DOC)		DW	130	110	8.7	3.6	2.2	4.0	4.0
	10/25/97	UW	ND	ND	ND	ND	ND	ND	ND
		DW	25	16	2.9	1.2	0.53	0.65	1.1

[1] UW = Upwind; DW = Downwind.

[2] ND = Not determined due to small amount of mass available.

Table A-4. PAH Mass Levels in DPM Extracts (SOF), Cont'd.

			PAH Level (µg PAH/g SOF)						
Fuel	Date	Site[1]	FLU	PYR	BaA	BbF	BkF	BaP	BP
Blend	10/27/97	UW	57	48	8.2	<6.9	4.2	8.0	51.4
(w/DOC)		DW	75	37	4.4	3.4	1.0	1.8	3.3
	10/28/97	UW	ND[2]	ND	ND	ND	ND	ND	ND
		DW	70	68	7.8	2.8	1.0	2.2	2.9
	10/29/97	UW	140	180	60	<8	4.9	10	67
		DW	48	45	3.7	2.3	0.69	1.2	1.5
Blend	10/30/97	UW	150	210	60	71	31	81	100
(w/o DOC)		DW	54	82	16	18	7.4	20	27

[1] UW = Upwind; DW = Downwind.
[2] ND = Not determined due to small amount of mass available.

Table A-5. Summary of All Downwind PAH Mass Levels (Mean and S.D.). [1]

Fuel	n	PAH Level (µg PAH/ g SOF [1])						
		FLU	PYR	BaA	BbF	BkF	BaP	BP
D2 (w/DOC)	4	123 [44]	151 [30]	9.1 [3.6]	6.5 [4.4]	2.4 [1.6]	5.0 [3.1]	9.5 [6.5]
Blend (w/DOC)	4	54 [23]	42 [21]	4.7 [2.1]	2.4 [0.91]	0.80 [0.23]	1.5 [0.68]	2.2 [1.1]
Change (%) [2] (from D2)		-56	-72	-48	-63	-67	-70	-77
Blend (w/o DOC)	1	54	82	16	18	7.4	20	27

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[1] Based on data presented in Table A-4; data from test dates 10/16/97 (D2 w/DOC), and 10/24/97 (Blend with DOC) are not included. Standard deviations are given in brackets.

[2] Calculated as [Blend with DOC / D2 with DOC) - 1] x 100%.

Table A-7. Summary of Downwind PAH Mass Levels, without the 10/21/97 D2 Fuel Data (Mean and S.D.). [1]

Fuel	n	PAH Level (μg PAH/ g SOF) [1]						
		FLU	PYR	BaA	BbF	BkF	BaP	BP
D2 (w/DOC)	3	140 [34]	150 [37]	11 [1.9]	8.7 [1.2]	3.2 [0.32]	6.4 [1.5]	13 [2.1]
Blend (w/DOC)	4	54 [23]	42 [21]	4.7 [2.1]	2.4 [0.91]	0.80 [0.23]	1.5 [0.68]	2.2 [1.1]
Change (%) [2] (from D2)		-61	-72	-57	-72	-75	-76	-83
Blend (w/o DOC)	1	54	82	16	18	7.4	20	27

[1] Based on data presented in Table A-4; data from test dates 10/16/97 and 10/21/97 (D2 w/DOC), and 10/24/97 (Blend with DOC) are not included. Standard deviations are given in brackets.

[2] Calculated as [Blend with DOC / D2 with DOC) - 1] x 100%.

Table A-6. Summary of Statistical Analysis of PAH by Mass (µg PAH/g SOF).

PAH	t-value	Significant
A. Analyses with All PAH Data[1]		
Fluoranthene	-1.26	No
Pyrene	-2.88	Yes
Benz[*a*]anthracene	-0.97	No
Benzo[*b*]fluoranthene	-1.19	No
Benzo[*k*]fluoranthene	-0.81	No
Benzo[*a*]pyrene	-0.95	No
Benzo[*g,h,i*]perylene	-0.91	No
B. Analyses without the 10/21/97 D2 Fuel PAH Data[2]		
Fluoranthene	-2.16	Yes
Pyrene	-2.64	Yes
Benz[*a*]anthracene	-2.55	No
Benzo[*b*]fluoranthene	-4.26	Yes
Benzo[*k*]fluoranthene	-6.76	Yes
Benzo[*a*]pyrene	-2.97	No
Benzo[*g,h,i*]perylene	-4.48	No

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[1] Data included in Table A-5. $t_c = 1.94$, $\alpha = 0.05$, 6 degrees of freedom (one-tailed test).

[2] Data included in Table A-7. $t_c = 2.02$, $\alpha = 0.05$, 5 degrees of freedom (one-tailed test).

Table A-8. Mutagenic Activity Associated with DPM Samples.

Fuel	Date	Site[1]	Mutagenic Activity			
			SOF - S9 (rev/μg)	SOF +S9 (rev/μg)	DPM -S9 (rev/μg)	Activity -S9 (rev/m^3)
D2 (w/DOC)	10/16/97	UW	0.99	0.71	0.47	21.7
		DW	0.54	0.56	0.10	17.8
	10/17/97	UW	0.67	0.55	0.16	2.68
		DW	0.70	0.38	0.17	105
	10/20/97	UW	ND[2]	ND	ND	ND
		DW	0.83	0.61	0.14	70.1
	10/21/97	UW	ND	ND	ND	ND
		DW	1.10	0.78	0.17	91.5
	10/22/97	UW	1.08	0.99	0.25	6.49
		DW	1.38	0.77	0.20	123
Blend (w/DOC)	10/24/97	UW	0.82	0.66	0.16	12.3
		DW	0.298	0.255	0.065	43.2
	10/25/97	UW	ND	ND	ND	ND
		DW	0.158	0.107	0.039	18.8
	10/27/97	UW	ND	ND	ND	ND
		DW	0.155	0.089	0.036	20.8
	10/28/97	UW	24.4	ND	3.83	73.2
		DW	0.217	0.143	0.065	29.3

[1] UW = Upwind; DW = Downwind.

[2] ND = Not determined, due to small amount of mass available.

Table A-8. Mutagenic Activity Associated with DPM Samples, Cont'd.

Fuel	Date	Site[1]	Mutagenic Activity SOF - S9 (rev/μg)	SOF +S9 (rev/μg)	DPM -S9 (rev/μg)	Activity -S9 (rev/m^3)
Blend (w/ DOC)	10/29/97	UW	ND[2]	ND	ND	ND
		DW	0.21	0.20	0.06	29.5
Blend (w/o DOC)	10/30/97	UW	1.17	1.09	0.38	10.5
		DW	0.30	0.22	0.19	140

[1] UW = Upwind; DW = Downwind.

[2] ND = Not determined, due to small amount of mass available.