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Title: Comparison of Diesel and Biodiesel Emissions and Health Effects in Underground Mining

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List of Terms and Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
ADOT	Arizona Department of Transportation
ASTM	American Society for Testing and Materials
B10	ten percent biodiesel to ninety percent diesel fuel blend
B20	twenty percent biodiesel to eighty percent diesel fuel blend
B50	fifty percent biodiesel to fifty percent diesel fuel blend
B75	seventy-five percent biodiesel to twenty-five percent diesel fuel blend
B80	eighty percent biodiesel to twenty percent diesel fuel blend
CO	carbon monoxide
D	diesel fuel
DOC	diesel oxidative catalyst
DPF	diesel particulate filter
DPM	diesel particulate matter (also known as total carbon)
EPA	Environmental Protection Agency
FENO	fraction of exhaled nitric oxide
FEV_1	forced expiratory volume in one second
FMD	brachial artery flow-mediated dilation
FVC	forced vital capacity
GD	GDiesel®
IARC	International Agency for Research on Cancer
LHD	load-haul-dump
mg/m^3	milligrams per cubic meter
$\mu g/m^3$	micrograms per cubic meter
MPH	miles per hour
MSHA	Mine Safety and Health Administration
NIOSH	National Institute for Occupational Safety and Health
NO	nitric oxide
NO_2	nitrogen dioxide
NOx	oxides of nitrogen
PEL	permissible exposure limit
ppm	parts per million
rDPM	respirable diesel particulate matter
rEC	respirable elemental carbon
rOC	respirable organic carbon
SX	San Xavier Underground Mining Laboratory
tDPM	total diesel particulate matter
tEC	total elemental carbon
TLV	threshold limit value

tOC   total organic carbon

TWA_8 eight-hour time-weighted average exposure concentration

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Final Report Abstract:

Exposure to diesel exhaust is linked to a broad range of illnesses and is a known human carcinogen. Diesel engines are widely used in on- and off-road applications including personal vehicles, trucks, buses, trains, ships, underground mining, construction, and agriculture. Despite the Mine Safety and Health Administration attempts to limit miners' exposures to diesel exhaust, many operators continue to struggle to achieve compliance. As a result, some operators have turned to alternative fuels such as biodiesel and a natural gas/diesel blend, in an attempt to reduce exhaust exposures and associated health effects. There remains, however, a substantial gap in research regarding the effect of alternative fuels in mining settings.

Over a three-year period we investigated the differences in acute health effects and personal exposures associated with use of diesel fuel (D) and a 75% biodiesel/25% diesel blend (B75) in an underground mine with a load-haul-dump (LHD) vehicle. We also examined the effect on health exposures, compared to D, when using a natural gas/diesel blend (GD) in the same mine with two different LHD vehicles. Specific aims of the study were to 1) evaluate airborne exposures associated with use of D and alternative fuels in LHD equipment in an underground mine; and to 2) evaluate health effects and biomarkers of altered physiology associated with exposure to emissions generated using D and B75 in an underground mine. Our study measured lung function, lung and systemic inflammation, as well as particle and gas exposures associated with each fuel type.

Despite a significant reduction in particulate exposures when using B75, the only improved health effect was lung function. Use of B75 was also associated with decreases, no change, or increases in other exposures. GD resulted in significant reductions in particulate and gas emissions in one LHD with a DOC, but little difference in another fitted with a Purimuffler. This study helps inform policy- and decision-makers in government and industry alike and lays groundwork for future work to better understand the benefits and risks of using alternative diesel fuels.

Section 1

<u>Significant Findings</u>

In this project we evaluated multiple health parameters following acute exposure to diesel (D) and 75% biodiesel/25% diesel blend (B75) emissions. Most health endpoints, with the exception of changes in lung function, were similar when comparing the two fuel-types. This work demonstrates a setting in which use of B75 may not reduce acute health effects in underground miners. These results have been published (see Mehus et al 2015 in the Publications section).

Compared to diesel, use of B75 was associated with relative percent reductions of 22 and 28% in median respirable diesel particulate matter (rDPM) and nitrogen dioxide (NO_2) and 25 and 23% increases in median total diesel particulate matter (tDPM) and nitric oxide (NO) exposure concentrations, respectively. Diesel was associated with a slightly greater total geometric mean mass concentration and lower mean surface area concentration. This study provides insight into the mixed benefits of B75 use in reducing rDPM for compliance purposes in underground mines. These findings will be published, pending minor revisions (see Lutz et al in the Publications section).

In one load-haul-dump (LHD) vehicle with a diesel oxidative catalyst, the natural gas/diesel blend (GD) was associated with a 66% reduction in rDPM and a reduction in all other exposures except carbon monoxide (CO). In a second LHD vehicle outfitted with a Purimuffler, GD was associated with a reduction in NO_2 and increase in total elemental carbon (tEC) exposure concentrations, with no change in the remaining contaminants. This work elucidates the variation observed across two underground mine vehicle types and pollution control configurations. The results for the first LHD, compared to the year one findings for D and B75 exposures, have been published (see Lutz et al 2015 in the Publications section).

<u>Translation of Findings</u>

The significant findings of this project were included in two published and one pending manuscript (see Publications below). In addition, study findings have been disseminated via conference presentations at the following conferences or meetings: Institute of Occupational Medicine (2014), Society of Toxicology (2014 & 2015), International Society of Exposure Science (2014), Mine Safety and Health Conference (2014), American Industrial Hygiene Conference and Exposition (2015), Nevada Mining Association – Health and Safety (2015), American Industrial Hygiene Association Arizona Local Section – Fall PDC (2015), and Society for Mining, Metallurgy and Exploration (2016). It has also been presented to over ten mine operators, to the Arizona State Mine Inspector's office, and to the University of Arizona Mining Safety and Health Program's Technical Advisory Committee on multiple occasions.

The results of this study can be used to help prevent occupational disease and reduce exposures associated with diesel exhaust. The Mine Safety and Health Administration (MSHA) has begun

gathering information regarding diesel exhaust exposures and controls, and some anticipate that a new standard will be proposed in the coming years. Despite this potential shift, many mine operators continue to struggle to achieve compliance with current regulation. This research helps to inform government policy- and industry decision-makers regarding potential control options. For example, underground mine operators for whom purchasing new equipment and/or upgrading pollution control systems is cost prohibitive should consider the potential benefits of using biodiesel blends and GD in their underground diesel equipment. Depending on the supplier and site location, biodiesel or GD may be purchased at a price similar to that of D.

Additionally, this work establishes a foundation upon which future occupational exposure and health effect research will build to identify the vehicles, pollution control configurations, and environments with which alternative fuels can be used to protect human health.

Research Outcomes/Impact
Our project has demonstrated that use of biodiesel in an underground mine can result in variable changes in health effect outcomes as compared with diesel fuel. However, we have identified a promising alternative fuel (GD) for reducing workplace exposures associated with diesel exhaust. It has also helped to demonstrate the variability in exposures based on vehicle type and pollution control configuration. Future studies are needed to evaluate the potential differential health effects from emissions of these fuels at both lower concentrations and for more chronic exposure periods, as well as for a larger selection of biodiesel fuel sources, blend concentrations, and pollution control devices. The present study also highlights the need to further evaluate the health effects associated with alternative fuels, and not assume that reduction in rDPM alone will lead to reduced health effects.

Section 2 - Scientific Report

Background

Diesel engines are widely used in on- and off-road applications including personal vehicles, trucks, buses, trains, ships, underground mining, construction, and agriculture. Exposure to diesel engine emissions is associated with adverse health effects including: chronic bronchitis, respiratory tract infections, asthma exacerbation, and increased cardiovascular morbidity and mortality [1-4], and in 2012, diesel emissions were classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen in humans [5]. Given the many health effects[6-8] of diesel emissions and the nature of exposures in the mining industry, reducing engine emissions has become an occupational health priority.

In the US, there are nearly 14,000 mine sites that employ over 210,000 individuals in the workforce[9,10]. Both underground and surface mines predominantly use diesel powered vehicles at their operations to perform tasks such as drilling, mucking, and hauling. Miners are regularly exposed to diesel fuel emissions during their shift, especially in areas where there is limited ventilation.

In an effort to control exposures to workers, the Mine Safety and Health Administration (MSHA) created standards for the allowable concentration of respirable (<1.0 µm with impactor) diesel particulate matter (rDPM) in diesel engine emissions. The permissible exposure limit (PEL)[11] of 160 µg/m^3 as total (combined inorganic and organic) carbon is monitored through air sampling and processing per the National Institute for Occupational Safety and Health (NIOSH) analytical method 5040. Despite the installation of controls such as mine ventilation, this underground rDPM exposure standard is frequently exceeded.

An alternative control measure that has been explored to reduce rDPM exposures to underground mine workers is the use of biodiesel/diesel fuel blends, ranging from 20/80% (B20) to 80/20% (B80). Studies on the biodiesel emission profile report a decrease in total carbon output but an increase in organic carbon, aldehydes, and nitrogen dioxide[12-16]. One study in an underground mine showed a reduction in elemental carbon emissions by 14 and 31% at idle and during operation, respectively, for B20 and a reduction of 38 and 45% for B50, respectively[17]. However, other research demonstrated an increase in aldehyde, nitrogen dioxide (NO_2), and organic carbon fraction concentrations with use of biodiesel mixtures[15,18]. Despite the increasing usage of biodiesel, there is a lack of information on the human health effects of exposure to these emissions and recent *in vitro* and *in vivo* studies suggest that exposure to biodiesel particulates may be more toxic than diesel particulates at equivalent concentrations [19-23].

Additionally, in 2011 an EPA-approved natural gas/diesel fuel mixture, GDiesel® (GD), became commercially available. GD is prepared by combining diesel with natural gas, using a proprietary charged-catalytic reaction. The end product is an American Society for Testing and Materials (ASTM)-designated diesel fuel with purportedly reduced tailpipe emissions of diesel

particulate matter (DPM) and oxides of nitrogen (NOx) compounds[24,25]. There was, however, no third-party research to support or refute these findings.

The objectives of this research were three-fold: 1) to compare the airborne exposures associated with use of D, B75 and GD in a non-operational underground mine using Wagner load-haul-dump (LHD); 2) to compare the acute human health effects related to exposures to emissions from D and B75 at said mine using the Wagner LHD; and 3) to compare the airborne exposures associated with use of D and GD in said mine using a JCI LHD. Our null hypothesis was that, compared to D, no change in exposures would be observed using the alternative fuels and that no reduction in adverse health effects would be found. Our alternative hypothesis was that use of the alternative fuels would be associated with a decrease in rDPM, NO and naphthalene exposure concentrations but increase in aldehyde and NO_2 concentrations, as well as reductions in acute human health effects.

Specific Aims

Specific Aims (SA) 1 and 2 were modified to compare D to B75, rather than B50. This was done in order to magnify any observed differences between the two fuels. SA 1 was modified to include the additional alternative fuel GD, and because all analytes of the polycyclic aromatic hydrocarbon (PAH) analytical profile, except naphthalene, were not detected during the first year they were dropped for subsequent testing. SA 1 ultimately included 48 subjects, rather than the 36 initially proposed, and was also modified to include an additional piece of LHD equipment for GD experimentation. For SA 2, several issues prevented research staff from obtaining clear brachial artery flow-mediated dilation (FMD) sonography. Sonographic image interpretation is still being attempted.

SA 1: Evaluate airborne exposures associated with use of diesel and biodiesel-fueled load-haul-dump (LHD) equipment during mucking operations in an underground mine. We measured personal and area exposures to D and B75 emissions including total carbon, elemental carbon, organic carbon, aldehydes, nitrogen dioxide, nitric oxide, carbon monoxide, polycyclic aromatic hydrocarbons (PAHs) and particle size/surface area distributions. We measured personal and areas exposures to D and GD exhaust including DPM, NO, NO_2, and formaldehyde using two different LHDs with different pollution control technology.

SA 2: Evaluate health effects and biomarkers of altered physiology associated with exposure to emissions generated using D- and B75-powered equipment in an underground mine. We measured respiratory (spirometry; symptoms; sputum cell count, differential and inflammatory mediator concentration; and exhaled NO), cardiovascular (inflammatory mediator concentration), DNA damage (urinary 8-OHdg) and other exposure (exhaled CO) biomarkers pre- and post-exposure.

SA 3: Compare the differences in health effects and biomarkers of altered physiology associated with exposure to diesel and biodiesel blend emissions and model the difference from baseline measures for each biomarker. The paired differences in pre- and post-exposure biomarkers of effect were compared for exposures to D and B75. For each fuel type, regression analysis was used to model the effects of emissions contaminants on pre- to post-exposure changes in individual biomarkers.

Methodology

Overview: The primary body of the study (referred to as D vs B75 Study, or the 'main study') was used to compare D and B75 exposures and health effects in human subjects operating a Wagner LHD outfitted with a diesel oxidative catalyst. As an appendage to the main study, researcher staff operated the same LHD with GD (referred to as the GD Year 1 Pilot Study) using a similar procedure (details below) during year one of the study and compared the exposure concentrations to those of D from year one. A second LHD, made by JCI, with a Purimuffler was later borrowed and operated separately by research staff with D and GD (referred to as the GD Extension Pilot Study) in order to compare the exhaust exposures associated with use of each fuel (see below for detailed procedures). *Subjects*: Human subject recruitment and testing procedures were approved by the University of Arizona (UA) Institutional Review Board. Subjects were recruited from the UA campus for work performed using D and B75 in the Wagner LHD. Inclusion criteria included: at least 18 years of age; non-smoker. Exclusion criteria included a diagnosis of asthma, heart disease, diabetes, hypertension, renal or hepatic failure, a difference in blood pressure greater than 15 mmHg between the arms, baseline forced expiratory volume in one second (FEV_1) divided by forced vital capacity (FVC) <0.7, or current respiratory illness.

LHD Training and Baseline Testing: After written consent was obtained, subjects were scheduled for LHD vehicle driver's training a minimum of 96 hours prior to baseline testing, which was completed at least 72 hours prior to the first emissions exposure. Baseline testing consisted of blood pressure measurement, phlebotomy, pulmonary function testing, and sputum induction. Blood pressure was measured in both arms using an automated sphygmomanometer (OMRON, Bannockburn, IL). Blood samples were collected in serum clot activator, heparin, sodium citrate, and EDTA tubes. The serum tube was allowed to clot for 30 minutes at room temperature prior to centrifugation. All of the tubes were initially centrifuged at 1000 x g for 15 minutes. The heparin and sodium citrate tubes were decanted and a second 10-minute centrifugation step at 10,000 x g was added to obtain complete platelet removal. Serum and plasma were decanted and stored immediately at -80°C until assayed. Pulmonary function testing was performed following American Thoracic Society standards in a sitting position using an EasyOne spirometer (ndd Medical Technologies, Andover, MA). FEV_1, FVC and age- and height-adjusted percent predicted values were recorded. Sputum induction was performed using DeVilbiss Ultra-Neb 99HD ultrasonic nebulizers (Somerset, PA) with 3% saline for 30 minutes, as previously described [26]. Samples were diluted with 10% Sputolysin® (Calbiochem, San

Diego, CA) in phosphate buffered saline, and incubated at room temperature for 15 minutes with gentle mixing by inversion every 5 minutes. A 500 µl aliquot was removed prior to centrifugation; 50 µl of sample was mixed with 50 µl Trypan Blue stain (Sigma Chemical Co., St. Louis, MO) prior to total cell counting performed using a hemocytometer. The remaining aliquot was mixed with an equal amount (450 µl) of preservative (Histochoice, AMRESCO, Solon, OH) and 500 µl of the mixture was cytocentrifuged, stained with Diff-Quik® (Dade Behring AG, Switzerland), and analyzed using the first 100 white cells counted, excluding epithelial cells. The remaining sample was centrifuged at 1900 rpm for 20 minutes and the supernatant and cell pellet were stored at -80°C until assayed.

Pre-Exposure Testing on Emissions Exposure Day: On the emissions exposure day, subjects were instructed to fast for at least 6 hours prior to arriving at the UA San Xavier Underground Mining Laboratory (SX). First morning void urine samples and all subsequent voids continuing through the end of the day's testing were refrigerated until transported following Arizona Department of Transportation (ADOT) regulations to the UA for processing. Baseline exhaled carbon monoxide (CO) and baseline fraction of exhaled nitric oxide (FENO) testing was performed following American Thoracic Society recommendations and standards, respectively; using the microCO Breath Carbon Monoxide Monitor (Micro Direct, Lewiston, ME), and a NIOX MINO (Aerocrine, Inc., New Providence, NH). Following a 20-minute rest in a supine position and ultrasound testing for brachial artery FMD [27], subjects were supplied a carbohydrate meal (cereal, 2% milk, energy bars) and water.

Fuels, Equipment, and Procedure: Both ultra-low sulfur #2 diesel and biodiesel blend were obtained from a regional distributor (Arizona Petroleum, Tucson, AZ). The B75 was prepared by mixing the aforementioned diesel fuel at 25% by volume with a soy methyl ester (SME) biodiesel fuel (ASTM D6751-compliant). The GD fuel #2 was purchased directly from the producer (Advance Refining Concepts, LLC., Reno, NV).

The University of Arizona San Xavier Underground Mining Laboratory (SX) is a non-operational hard rock mine where University mine engineering and public health students perform laboratory work. Exposure to vehicle emissions was evaluated in the 'decline,' a naturally ventilated portion of the SX with sloping underground opening for rubber-tired vehicle access to the mine (see Figure 1 from Lutz et al 2015). Mucking activities (the removal of material created during the mining process) were performed by study subjects operating a University-owned 2005 Wagner B10-203 LHD vehicle with open cab and diesel oxidation catalyst (DOC), but no diesel particulate filter (DPF). The vehicle was operated on a 35-meter all-underground path with a 9.5% grade. The LHD idled in the decline for approximately 45 minutes before each exposure assessment. The decline's gate (position B) was covered with a canvas tarp and the metal door (position D) entering the 'adit' level was closed at the start of each exposure session to minimize airflow into and out of the decline. The vehicle's fuel tanks

were emptied between each fuel type. After changes of fuel, the LHD was operated for approximately one hour with the "new" fuel to ensure all remnants of the previous fuel were removed. The vehicle's fuel tank was used for diesel and B75, while a separate fuel tank and fuel line were used for GD, so as not to disrupt the larger ongoing study. Fuel tests occurred using the same LHD with no engine or operational changes between fuels. The mucking path and pile were sprayed with water during assessments in order to limit dust exposures. To ensure carbon monoxide (CO) levels in the decline remained below 35 ppm, real-time levels were monitored using the 4X Altair gas monitor (MSA Corporation, Cranberry Township, PA).

During each 200-minute exposure assessment, two research subjects alternately mucked (110 minutes) and closely observed (80 minutes) LHD operation, with a 10-minute break in the decline. Mucking activities included filling the LHD bucket at the muck pile (position A), driving to the decline gate (position B), returning to and unloading at the muck pile, driving once again to the decline gate, and then returning to the muck pile for loading. Subjects observing LHD operations stood at the decline 'rib', approximately three meters from the muck pile (position C). For GD with the Wagner LHD, research scientists mucked using the identical procedure, albeit for 100-minute total rotations (50 minute mucking/50 minute observation). Generally, after one pair of subjects finished a second pair replaced them, for a total of two exposure sessions (four subjects) per day. Subsequent exposure assessments typically occurred no sooner than one week following the prior day of testing, with seven total days for diesel, eight for B75 and two for GD in the Wagner LHD. For D and B75, 58% and 64% of exposure sessions were the first session of the day, respectively. There were four days during which only one D exposure session occurred, while there were eight for B75. In nine instances, four times for diesel and five for B75, two days of exposure assessments were performed on subsequent days. A cross-over study design was utilized, with 23 subjects completing first their diesel (early spring) then B75 (late spring) exposure rotations during the first year, and 25 subjects completing their B75 (early spring) exposure rotation first, followed by their diesel (late spring) rotation during the second year.

After the main and pilot studies were concluded, a small JCI LHD with 48 kW Deutz AG (motor number 7534-462) engine with an Engine Control Systems Purimuffler (part number A17-0313) DOC, but no DPF, was borrowed from a local underground mine. Research staff operated the vehicle for five consecutive days using D fuel. Two 200-minute exposure sessions were completed each days, with one personal and one area sample was taken during each session. In order to change from D to GD fuel, the LHD fuel tank was emptied using a fuel pump, filled with GD, and then driven before beginning the first GD exposure session. The LHD was then operated for a total of four days with GD fuel. Due to water constraints in the mine, mucking was not performed to limit dust dispersion. The LHD was simply driven back and forth on the decline path by the same operator. The LHD was not idled before the first session of each day and, before beginning the second session, the tarp was removed for 30 minutes to allow natural

ventilation to disperse airborne contaminants. In addition, outdoor weather conditions were used for adjustment, rather than indoor wind speed (previous experience had demonstrated virtually no measurable wind speed at the rib of the decline). In virtually every other regard, the methodology was identical to the GD Year 1 pilot. For detailed exposure assessment methods, please reference those mentioned above.

Diesel and B75 Exposure Measurements: Exposure-related health effects to diesel and B75 fuel emissions were measured under the same engine operating conditions. Every 70 minutes during each session, wind speed measurements were taken using a Kestrel® 4500 Weather Meter (Nielsen-Kellerman Company, Boothwyn, PA) at the observer's location (position C). Universal PCXR 8 (SKC West, Inc., Fullerton, CA) and Escort ELF (Zefon International, Inc., Ocala, FL) air sampling pumps, as well as a 4X Altair gas monitor (MSA Corporation, Cranberry Township, PA), were placed in pockets of or clipped to a safety vest worn by subjects. Sampling media were placed in the subjects' breathing zone, clipped to the vest at shoulder level. The 4X gas monitor was clipped to the front of the vest at chest level. Additionally, assessment of the mean aerodynamic particle size distribution across the range of 500nm to 10 µm, including a per-size evaluation of airborne particulate concentration, surface area, and mass, was performed during exposure periods using an Aerodynamic Particle Sizer (APS) Spectrometer (TSI, Inc., Shoreview, MN) on a table three feet tall and directly behind the observer at position C.

Personal integrated sample collection and analysis was performed in accordance to NIOSH manual of analytical methods (NMAM). Specifically, a GS-1 Respirable Cyclone with 37 mm jeweled impactor and flow rate of 1.7 L/min (NMAM 5040)[28] were used in respirable (<1.0 µm) DPM (rDPM) sampling. A 37 mm open face quartz fiber filter with flow rate of 2.0 L/min (NMAM 5040)[28] was utilized to sample total (non-fractionated) DPM (tDPM). A tandem triethanolamine/oxidizer with flow rate 0.025 L/min (NMAM 6014)[29] was used to collect nitric oxide (NO) and NO_2 samples. Sorbent tubes containing silica gel and a flow rate of 0.1 L/min (NMAM 2016)[30] were used in formaldehyde and acetaldehyde sample collection. Finally, a 37-mm teflon filter cartridge and sorbent tube set at a flow rate of 2.0 L/min (NMAM 5506)[31] were used to sample naphthalene. At the project's onset, a profile of 18 polycyclic aromatic hydrocarbons was sampled. However, after several exposure assessments, naphthalene was the only compound with concentrations above the limit of detection. Sampling media were produced by the same manufacturer (SKC West, Inc., Fullerton, CA). A Bios Drycal® Defender 520 calibrator (Mesa Labs, Inc., Butler, NJ) was used for pre- and post-sampling confirmation calibration at the University's Medical Research Laboratory. An independent AIHA-accredited industrial hygiene laboratory performed laboratory analysis.

Post-Diesel and -B75 Emission Exposure Testing: At the end of the 200 minutes, the subjects repeated the exhaled CO monitoring immediately after exiting the mine. FENO measurement was repeated approximately 5 minutes after exiting the mine. Subjects repeated the 20-minute

rest prior to the measurement of post-exposure FMD. The subjects were then supplied a small lunch consisting of a sandwich, fruit, and water; and returning to the UA campus for a blood draw at 2 hours after exiting the mine (~5.5 hours after initial exposure). At 5 hours after exiting the mine (~8.5 hours after initial exposure), the subjects returned again to the UA campus for the remainder of the biological sample testing, including blood pressure, spirometry, sputum induction, and urine collection, conducted in the same fashion as during baseline and/or pre-exposure testing.

Biological Assays: Urine samples were allowed to return to room temperature, mixed well, and an aliquot of urine was transferred to a 15ml tube, centrifuged at 1000 x g for 10 minutes, and stored at -80°C until assayed. Specific gravity was measured on the unspun urine (10SG Urine Reagent Strips, Fisherbrand, Hanover, Germany). First catch (baseline) and last catch of the day (generally 8-10 hours post-exposure) urine was used for 8-OHdG analysis, which was measured only in the first year of our study. All sputum and plasma samples were allowed to thaw to room temperature, vortexed, and then briefly centrifuged to pellet any precipitates prior to assays. Endothelin-1 (ET-1), soluble P-selectin, and tenascin-C (TN-C) were assayed with heparin plasma samples, while high sensitivity interleukin-6 (IL-6), high sensitivity interleukin-8 (IL-8), matrix metalloproteinase-9 (MMP-9), myeloperoxidase (MPO), growth-regulated alpha protein (GRO-α), and matrix metalloproteinase-8 (MMP-8) were assayed with sputum samples, and creatinine and urinary 8-OHdG were assayed with urine samples. The level of each sputum analyte was adjusted to total protein for normalization. Standards, controls, and samples were assayed in duplicate using the enzyme-linked immunosorbent (ELISA) assay kits developed by R&D Systems, Inc. (Minneapolis, MN) with two exceptions: 8-OHdG was assayed in triplicate using the ELISA kit supplied by Japan Institute for the Control of Aging (Fukuroi, Japan) and TN-C high molecular weight variants were assayed in duplicate using the ELISA kit supplied by IBL America, Inc. (Minneapolis, MN). Absorbance for standards, controls, and samples were obtained using an automated microplate reader (Model ILx808, BioTek Instruments, Inc., Winooski, VT) and concentrations determined from the standard curve using a four-parameter algorithm for best fit, as determined by the BioTek KC4 automated software program (Winooski, VT).

LC-MS/MS Analysis: See Supplemental Material for detailed methods. Sputum and plasma samples from six subjects (3 males and 3 females) were analyzed for potential novel biomarkers of effect following diesel and B75 exposure. Proteins were extracted, reduced with dithiothreitol (DTT), alkylated with iodoacetamide, and then digested with Lys-C/trypsin mix (Promega, Madison, WI) following manufacturers recommendations. The resulting peptide samples were desalted using solid-phase extraction (SPE), and concentrated to dryness prior to analysis by high resolution LC-MS/MS for protein identification using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an Advion nanomate ESI source (Advion, Ithaca, NY). Three proteins (GRO-α, MMP-8, and TN-C) considered prime candidate

biomarkers were further validated in the entirety of the sputum and plasma samples using ELISA analysis.

Statistical Analysis: For the D v B75 and GD extension studies, all manual results were double entered into a spreadsheet and the results checked for accuracy. In order to compare exposure concentrations to their associated MSHA PEL and American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) concentrations and to each other, each individual sample's reported laboratory concentration was time-weighted (see Equation 1) over an 8-hour exposure period (TWA$_8$).

$$\frac{(C \times T)}{480 \; minutes}$$

Equation 1
Where C = Contaminant concentration in mg/m3 or ppm, and T = Sampling duration in minutes.

Four out of 43 acetaldehyde samples (9%) resulted in breakthrough while sampling diesel fuel exhaust. Analysis was performed with and without the breakthrough samples, with no significant change in our conclusion; the data containing the results have been included. When comparing the morning to the afternoon, the morning exposure rotation analyte TWA$_8$ concentrations tended to be higher than those of the afternoon, though not significantly so.

Exposure distributions were compared using the Wilcoxon rank-sum test (STATA 12.0, StataCorp, College Station, TX). Health data (exhaled CO and NO, cell counts, and ELISAs) were compared for statistical significance using the Wilcoxon signed-rank test. Spirometry data were normally distributed; therefore a paired t test was used for statistical analysis. Data were analyzed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). Statistical differences were measured for post-diesel or post-B75 compared to baseline values and also post-diesel compared to post-B75 values. For all analysis, statistical significance was taken as a two-tailed p-value of $p<0.05$. Data are expressed as median [interquartile range (IQR)] or mean $\pm$ standard deviation (SD), as appropriate. For the GD extension pilot study only, a multiple linear regression (MLS) was used to measure the relationship among rDPM exposure concentrations and outdoor temperature, dew point, relative humidity, and wind speed (obtained from the Tucson Airport for the time period specific to each session). In the MLS model, only temperature was related at the $p<0.1$ level. Simple linear regression between temperature and rDPM demonstrated a significant relationship ($p=0.008$). Therefore, in rDPM hypothesis testing both an temperature-adjusted (ug/m^3*^{o}F) and unadjusted concentrations were used. Because adjustment did not change overall conclusions, the unadjusted results have been included here.

For the GD year 1 pilot study, a similar approach was used. However, TWA$_8$ exposure distributions were first compared across fuel types using the Kruskal-Wallis rank test with Bonferroni

correction. Those analytes with significant differences across all three fuel types were further analyzed using the Wilcoxon rank-sum test for un-paired analysis.

Results and Discussion
D vs B75 Health Effects Study: Study participants: Characteristics of the 48 study participants are summarized in Table 1 (see Mehus et al 2015). The average age was 25.7 years, approximately one third of the subjects were female, and three quarters were non-Hispanic white.

Diesel and B75 emission composition: rDPM sampling performed during mucking demonstrated a median of 336.4 µg/m^3 for diesel exposures and 270.4 µg/m^3 for B75 exposures, a 20% reduction (Table 2 from see Mehus et al 2015). There were no statistical differences between the fuel types when comparing total aldehydes, oxides of nitrogen (combined NO and nitrogen dioxide (NO_2)), or CO.

Lung function and exhaled CO and NO: FEV_1 was significantly reduced from both diesel and B75 exposures compared to baseline (Table 3 from Mehus et al 2015). Post-diesel FEV_1 was also significantly lower than post-B75 FEV_1. FVC was only significantly reduced following diesel exposures. Additionally, post-diesel FVC was significantly reduced compared to post-B75. FEV_1/FVC was significantly reduced after both diesel and B75 exposures. Both %COHb and FENO significantly increased in subjects following both exposures (Table 4 from Mehus et al 2015). There were no significant differences in the median levels of post-diesel %COHb, or FENO, compared to post-B75.

Lung inflammatory cell infiltration: Total white blood cell (WBC), neutrophil, and macrophage counts (~8.5 hours after initial exposure) increased following emission exposures to both fuels and lymphocytes increased following diesel, but not B75 exposures (Table 5 from Mehus et al 2015) in the sputum. When comparing post-diesel to post-B75 cell counts, there were no significant differences in any cell type.

Sputum and plasma inflammatory mediators: Sputum (~8.5 hours after initial exposure) and plasma (~5.5 hours after initial exposure) fluid-phase mediators selected *a priori* (IL-6, IL-8, MMP-9, MPO, and ET-1), increased from baseline following both exposures (Table 6 from Mehus et al 2015). P-selectin decreased in the plasma following both exposures. There were no significant differences found in these levels when comparing post-diesel to post-B75. Sputum levels of each analyte, not adjusted for total protein, are included in Supplemental Table S1 (see Mehus et al 2015).

Novel biomarker discovery: Following proteomic analysis, a total of 848 sputum and 407 plasma proteins were identified. Using label-free spectral counting for relative quantitation, 42 and 32 candidate protein markers in the sputum and plasma, respectively, were identified that met the previously defined criteria (see Supplemental Material Methods and Supplemental Tables S2 and

S3 from Mehus et al 2015). Two sputum (MMP-8 and GRO-α) and one plasma (TN-C) candidate markers were further validated in all samples using ELISA (Table 6 from Mehus et al 2015). Sputum MMP-8 significantly increased following both exposures. Sputum GRO-α was only significantly elevated in post-B75 exposures, although there were no significant differences when comparing post-diesel to post-B75 levels. Plasma TN-C was significantly increased following diesel exposure, and, albeit not quite significantly, in the post-B75 exposures. There were no significant differences in post-diesel and post-B75 TN-C levels.

Urinary 8-OHdG: The 8-OHdG levels were normalized to creatinine (ng 8-OHdG/mg creatinine, ng/mg). The urinary 8-OHdG levels increased significantly following diesel exposures only for this exposure period (8-10 hours after initial exposure). There were no significant changes in 8-OHdG when comparing post-diesel and post-B75 levels (Table 7 from Mehus et al 2015). The 8-OHdG levels were also normalized using specific gravity and no significant changes were observed following exposures to either fuel-type (data not shown).

In the current study, switching to B75 from diesel fuel led to a 20% reduction in exposure to rDPM. This was consistent with previous reports demonstrating a reduction in DPM using biodiesel blends [13,32,33], although at least one study reported no difference in DPM [34]. The mixed results can likely be attributed to the fuel blend, engine operating conditions, and pollution control devices [35,36]. This study did not find significant increases in aldehydes or decreases in oxides of nitrogen or CO levels by switching to B75 from diesel, consistent with previous reports [34,37].

The respirable particulate concentrations reported in this study represent high ambient exposures, such as those found most commonly in occupational settings [38-40]. At these elevated exposure concentrations, this study found significant reductions in lung function as well as increases in both respiratory and systemic inflammation. The marked inflammation observed is a likely pathway for lung cancer, other adverse respiratory effects, and cardiovascular disease; all of which are known sequelae of chronic DPM exposure [1-4,41]. The increased concentration seen post-exposure for most of the inflammatory biomarkers did not differ significantly between the two fuels, despite the 20% reduction in rDPM observed with B75 use. These findings bring into question the assumption that reductions in DPM concentrations from the use of alternative fuels will necessarily lead to decreased chronic toxicity.

Lung function was one of the health endpoints for which a significant difference was found comparing post-diesel and post-B75 values. Although acute changes in spirometry are not typically reported in studies where subjects are exposed to diluted diesel exhaust using controlled exposure chambers [42-45], at least one previous study demonstrated exposure to diesel exhaust decreased peak expiratory flow [46]. In general, previous studies had a lower number of subjects, different exposure durations and environments, and lower particulate concentrations, all of which are plausible reasons for the observed variability.

Additionally, this study revealed that FENO, a marker of airway inflammation, increased significantly and to the same levels following emission exposures to both fuel-types, comparable with previous findings of FENO increasing following acute diesel exhaust exposures [42]. FENO has also been shown to increase in asthmatic children from urban air pollution with positive correlations between the concentration of pollutants and the level of airway inflammation [47].

Diesel exhaust has been shown to increase inflammatory cell recruitment into the airways [43-45]. These increases were typically measured 6–24 hours after initial diesel exposure, consistent with the approximately 8.5 hours after initial exposure to each fuel-type in this study. We observed similar increases in cell counts for neutrophils and macrophages following exposures to diesel and B75 and the lymphocytes increased significantly following diesel, but not B75 exposures.

IL-6 and IL-8 are cytokines released by several cell types during an inflammatory response. The mRNA and protein expression of these two cytokines has been studied in *in vitro* and *in vivo* studies [48-50] to measure the level of inflammation from exposures to diesel particulates and air pollution. Additionally, the increased airway release of IL-6 and IL-8 has been seen in human studies analyzing acute diesel exhaust exposures [51-53]. Accordingly, we observed a significant increased release of both IL-6 and IL-8 in the sputum following diesel and B75 exposures. The extent of IL-6 release was similar for both fuel-types and IL-8 was slightly (but not significantly) higher for B75 exposures.

MMP-8 (secreted exclusively by neutrophils) and MMP-9 (secreted by many cell types) are involved in the remodeling of the extracellular matrix under normal physiological processes and also inflammation and metastasis. MMP-8 has been used as a biomarker of inflammation and cardiovascular disease [54]. However, the acute elevation of MMP-8 observed in response to diesel and B75 emission exposures is a novel finding. The increased levels measured in this study are likely related to the neutrophil infiltration observed in the lung. Induced sputum MMP-9 levels have been shown to be correlated with lung function and airway inflammation, displaying an inverse relationship with FEV_1 and a significant correlation with total white cells, neutrophils and IL-8 [55]. This suggests MMP-9 may be a promising marker of airway effect for DPM exposure.

MPO is an enzyme that has potent pro-oxidative and pro-inflammatory properties and is typically released from activated neutrophils; accordingly its levels have been used as a marker of inflammation and cardiovascular disease. In a recent study analyzing mouse lung and liver toxicity following equivalent doses of diesel or biodiesel emissions, MPO levels displayed a greater dose-related increase following biodiesel-compared-to-diesel emission exposures [56]. In the current study, MPO levels in the sputum increased to similar levels following diesel and B75 exposures.

GRO-α preferentially chemoattracts and activates neutrophils. This protein is inducible by tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). Previous human studies have shown a non-significant increase of GRO-α following acute diesel exhaust exposures [51,57]. In the current study, GRO-α was identified in the sputum via proteomic strategies as a candidate biomarker being elevated following B75 exposures. When ELISA analysis was performed on all sputum samples, GRO-α was significantly elevated following only B75 emission exposures, also a novel finding.

ET-1 is an endothelin-derived vasoconstrictor peptide that is an inflammatory mediator. Increased levels of ET-1 have been reported in cardiovascular and inflammatory lung diseases [58,59]. ET-1 may play a role in the vascular dysfunction seen after exposures to diesel exhaust and traffic-related air pollution. A previous human study showed increases in plasma ET-1 levels after acute exposures to diesel exhaust [60], suggesting an early endothelial response and vasoconstriction. The current study demonstrated similar significant increases in plasma ET-1 levels after diesel and B75 exposures, suggesting that the level of vascular dysfunction is similar from exposures to both fuel-types.

P-selectin is responsible for mediating the rolling of leukocytes over vascular surfaces during early stages of inflammation. In exposure chamber studies of acute human exposure to filtered diesel exhaust (300 µg/m3 for 1 hour), plasma P-selectin levels were not significantly altered at 2 or 6 hours post-exposure [61], but increased significantly at 24 hours post-exposure [62]. These results are inconsistent with the current study findings where P-selectin decreased approximately 5.5 hours after initial exposure to both fuel-types. Different exposure environments, engine, and engine operating conditions may be plausible explanations for the inconsistencies between the studies. It has also been shown that exercise training can reduce plasma inflammatory mediators such as P-selectin [63-65]. While the study participants performed moderately physical demands during mucking, it is unclear yet if the short-term exertion the subjects in the current study experienced while driving the LHD caused the decrease in plasma P-selectin.

TN-C is an extracellular matrix protein important in tissue injury and repair, but also disease-states such as chronic inflammation and tumorigenesis. Additionally, TN-C is capable of inducing pro-inflammatory cytokines [66]. High circulating concentrations of plasma TN-C have been reported in association with mortality and cardiovascular disease in chronic kidney patients [67]. In the current study, TN-C was identified in the plasma via proteomic strategies as a novel biomarker candidate that increased following exposures to diesel and B75 emissions. These increased TN-C plasma levels may be due to lung and/or endothelial damage.

The current study is the first human study to use proteomic strategies to reveal novel biomarkers of emission exposures to both diesel and B75. The candidate biomarkers in the sputum and

plasma (MMP-8, GRO-α, and TN-C) were validated by ELISA. The most comparable study was performed on rat bronchoalveolar lavage fluid (BALF) after different exposure concentrations and durations to diesel exhaust particles (DEP) [68]; this study revealed a total of 65 proteins using LC/MS analysis on whole and weak cation exchange (WCX) extracted BALF, with two distinct proteins (anaphylatoxin C3a and calgranulin A) appearing post-exposure at all DEP doses.

The accumulation of rDPM in the lung leads to a large inflammatory response, as seen in this study, and many others. It is well established that inflammatory events leads to an increase in reactive oxygen species (ROS) that can damage proteins and DNA. A classic biomarker of oxidative stress to DNA is urinary 8-OHdG [69]. 8-OHdG is an oxidized DNA nucleoside and is eliminated in the urine after DNA repair enzymes excise damaged DNA. Increased levels of 8-OHdG have been measured following a minimum of three month's occupational exposures to ambient $PM_{2.5}$ in Taiwanese traffic conductors [70]. In the current study, urine 8-OHdG concentrations increased significantly following diesel exhaust exposures only, which was approximately 8-10 hours after initial exposure.

The current study has several important limitations. It measured health effects reflecting the use of diesel and alternative (B75) fuel while operating the same vehicle, and therefore did not compare the toxicity of the same concentrations of rDPM from each fuel type. Multiple health parameters were assessed, but each at a single time point following an acute 200-minute exposure. More prolonged exposure periods, or the measurements of health parameters at other time points, would potentially produce different results. Additionally, only one blend of biodiesel (B75) and type (soy methyl ester) was evaluated in this study, while there are a variety of biodiesel blends in use. Also, the use of different pollution control devices and engine operating conditions will affect the overall toxicity of the exhaust produced, so it is important not to generalize the study findings beyond the use of diesel engines with only a DOC. Lastly, this study concentrated on markedly elevated exposure concentrations, and the effects of lower concentrations would be expected to result in less marked health effects, which could potentially vary to a greater or lesser degree by fuel type.

D vs B75 Exposure Study: Comparing D and B75 using the Wagner LHD with diesel oxidative catalyst, we found there to be significant differences between the exhaust profiles of D and B75, in both particulate and gas composition (Table 1). TWA_8 median values for rDPM, respirable elemental carbon (rEC), and and total elemental carbon (tEC) were statistically significantly lower in B75 than in D ($p<0.05$, $p<0.001$, and $p<0.001$, respectively), while concentrations of total diesel particulate matter (tDPM) and total organic carbon (tOC) were higher in B75 than in D ($p<0.01$ and $p<0.001$, respectively). The concentration of respirable organic carbon (rOC) was higher in D than in B75, but this difference was not significant. In analyzing the gas composition of the exhaust, we found there to be fewer significant differences, with B75 having a slightly higher concentration of formaldehyde and naphthalene. D exhaust contained a higher

median concentration of acetaldehyde and CO, but neither was a significant increase from that of B75. The concentration of NO_2 was significantly lower in B75 at 1.19ppm ($p<0.05$) compared to D, which had a median concentration of 1.58 ppm. However, the concentration of nitric oxide was higher in B75 (12.63 ppm) than in D (10.00 ppm, $p<0.05$).

Table 1. Analyte TWA_8 exposure concentrations by fuel type

| Analyte	D (n=43-49)		B75 (n=47-49)	
	Median	Range	Median	Range
rDPM (ug/m^3)	336.4	129.3 - 711.9	270.4*	68.7 - 643.8
rEC (ug/m^3)	125.1	42.4 - 360.1	52.8***	15.4 - 186.8
rOC (ug/m^3)	205.2	61 - 353.6	176.0	21 - 571.5
tDPM (ug/m^3)	608.2	36.3 - 1099.6	783.8**	250-1530
tEC (ug/m^3)	134.6	56.6 - 412.5	61.3***	12.5 - 280.3
tOC (ug/m^3)	459.5	197.4 - 733.1	640.6***	237.5 - 1445
Acetaldehyde (ppm)	0.035	0.0085 - 0.174	0.031	0.008 - 0.055
Formaldehyde (ppm)	0.094	0.0192 - 0.5317	0.099	0.028 - 0.161
Naphthalene (ppm)	0.0003	0.0001 - 0.0031	0.0004	0.0001 - 0.001
Nitrogen Dioxide (ppm)	1.58	0.46 - 7.28	1.19*	0.39 - 6.77
Nitric Oxide (ppm)	10.00	3.56 - 23.08	12.63*	1.63 - 25.93
Carbon Monoxide (ppm)	12.46	1.44 - 24.29	11.15	0.42 - 23.78

Abbreviations: Diesel (D), Biodiesel (B75), Respirable DPM (rDPM), respirable elemental carbon (rEC), respirable organic carbon (rOC), total DPM (tDPM), total elemental carbon (tEC), total organic carbon (tOC). The Wilcoxon rank-sum test was used to compare data for statistical differences from B75 to D. *$p<0.05$; **$p<0.01$; ***$p<0.001$. Sample size variation due to equipment failure or non-detectable concentrations.

APS-derived, size differentiated, mean, aerosol, particulate mass (dM/dlogDp) and surface area (dS/dlogDp) data collected during D and B75 fuel tests are summarized in Figures 2 and 3, respectively. The peak geometric mean particle mass across the size range of 500nm to 10 µm

for both D and B75 occurred at 10.37 µm, with D having a slightly higher total mean mass concentration than B75 at 46.608 µg/cm^3 versus 41.471 µg/cm^3. The geometric mean particle surface area followed the expected bimodal distribution, with the first peak of 0.777 µm and 0.542 µm for D and B75, respectively, and the second peak for D at 9.647 µm and B75 at 5.048 µm. However, unlike with the mean particle mass measures, total mean geometric mean surface area was higher for B75 at 53,317.37 nm^2/cm^3 than diesel at 47,253.23 nm^2/cm^3. Further as depicted in Figures 2 and 3, B75 was observed to have higher concentration values for both geometric mean particle mass and surface area at lower size fractions, decreasing below D concentration values for the same measures above aerodynamic diameters of approximately 6.732 µm. The total mean aerodynamic diameter (dN/dlogDp) particle count was 4,918.92 particles for diesel and 9,688.33 particles for B75.
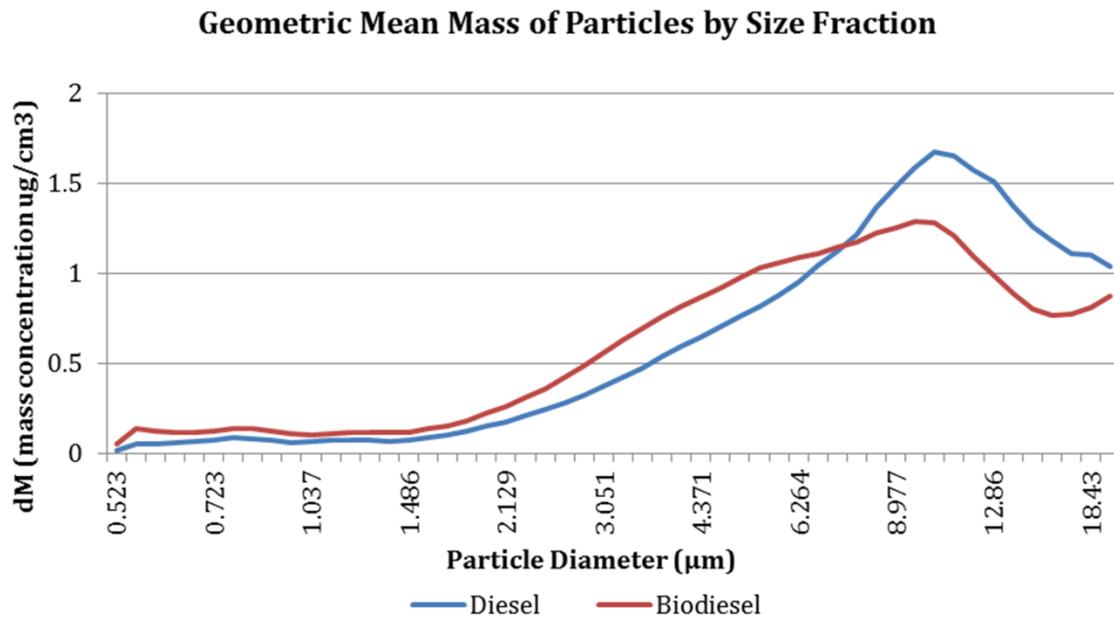
Figure 2: Average mass of particles based on size distribution, for diesel and biodiesel exhaust.

Geometric Mean Surface Area of Particles by Size Fraction
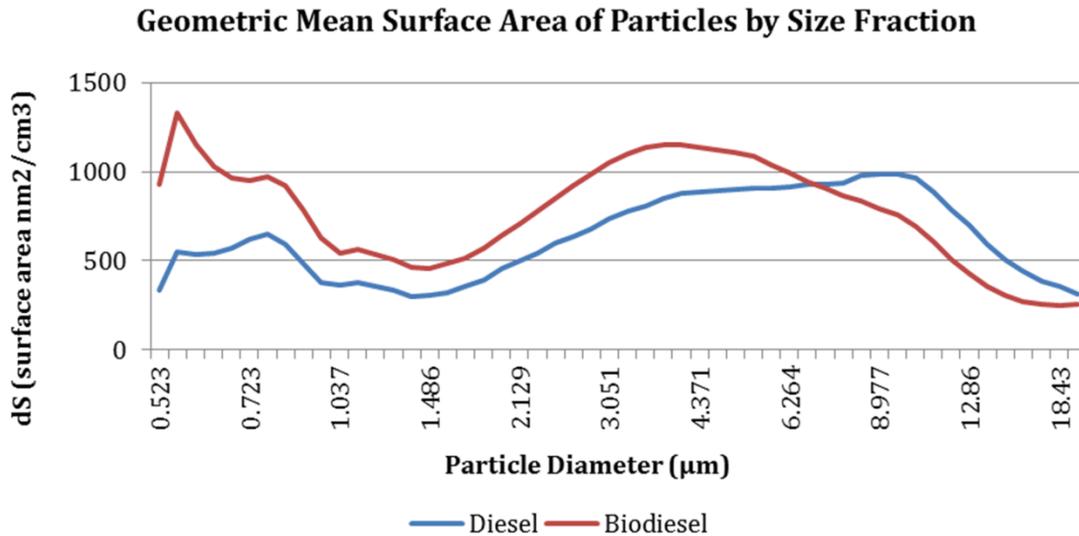
Figure 3: Average surface area of particles based on size distribution, for diesel and biodiesel exhaust.

Our study demonstrates that reductions in median rDPM can be achieved with a 75/25 biodiesel blend. Use of B75 would likely increase compliance with the MSHA federal standard (30 CFR 57.5060), as the regulation specifically targets rDPM exposures. However, use of B75 should be carefully considered as many components of vehicle exhaust contribute to adverse health effects and our particle mass and surface area measures indicate greater concentrations of B75 to diesel in the inhalable size fractions less than 6.7 µm. Likewise, we observed increases in certain analyte TWA_8 exposure concentrations associated with B75.

Relevant literature (from Table 2 of pending journal submission) has produced mixed results when comparing particulate exposures and DPM between or among diesel and biodiesel blends, with some finding no difference[12,17], others showing significant reductions[13,14,16,71-74], or a small increase[37]. Consistent with other studies[15,17,37,72,75], our work demonstrated increasing tOC and decreasing tEC concentrations associated with B75 use. Previous studies used a range of biodiesel blends, engine configurations, pollution control devices (or lack thereof), and loading procedures – all of which may influence vehicle emissions.

Comparable to prior research[12,17,18,37], our study failed to show a significant difference in CO exposures between D and B75. However, we did observe significant differences in NOx concentrations – unlike previous work[12,17,18,37]. Similarly, inconsistent, other studies have demonstrated a reduction in CO Biodiesel emission concentrations compared to diesel fuel[15,37]. Likewise, prior studies observing increased aldehyde concentrations with biodiesel use[12,18], compared to the lack of difference we observed. Unlike our study, however, the engines used in these studies[12,18] did not utilize a DOC. Dissimilar to some studies[12,73,74], we found increasing

naphthalene concentrations with use of B75 – though these results were not statistically significant.

For B75, our study showed a greater aerosolized particle peak count, geometric mass and geometric surface area concentration, particularly at lower size fractions, compared to diesel. While the mean mass concentration for B75 was less than that of diesel across all sizes, B75 size mass concentrations exceeded diesel's at all sizes less than approximately 6.732 µm. Several previous studies[17,73,76-79] have shown that some biodiesel blends under specific loading conditions also demonstrate greater particle concentrations at smaller fractions, compared to diesel. Others[73,79,80], however, have found that greater loads and/or moderate biodiesel blends, such as B10 or B20, can produce lower particle concentrations than diesel fuel. This may partially explain our observation of higher particle concentration for B75.

The type of equipment, load, and pollution control equipment, including catalytic converters and particle traps, have been observed as factors that influence diesel emissions[15,17,18,37,74,81]. Because it is frequently used in underground mine settings, and can be associated with high exposures to diesel exhaust[17,81], we selected the LHD vehicle for our study. Pollution control devices present on the LHD included a DOC, but no DPF. Other studies used neither DOC nor DPF[12,18,74], only a DOC[15], or combination of pollution control configurations[17,37,81]. Some evidence suggests that DOCs may have little effect on NOx exposures[82], but may reduce biodiesel's CO and hydrocarbon emissions. Our LHD is representative of the types of equipment commonly found in current use, despite it not representing the newest or most advanced vehicles available.

GD Year 1 Pilot Study: Our pilot study from year one, comparing exposures to emissions from use of D, B75 and GD in the SX mine, identified significant differences across the three fuels for all analytes tested except for CO (Kruskal-Wallis, p<0.001; Tables 1-2, and Figure 2 from Lutz et al 2015). For pairwise comparisons, B75 rDPM exposures were lower than with diesel exposures (p=0.009). Overall, mean B75 results were mixed, as emissions were significantly higher than diesel for tDPM and tOC, significantly lower for rDPM, tEC, rOC, and naphthalene; and not significantly different for rEC, NO, NO_2, formaldehyde and acetaldehyde. Mean rDPM exposures for GD were significantly lower than with D (p<0.001) and B75 (p=0.003) exposures and significantly lower than both D and B75 for all other analytes except CO. While the inclusion of acetaldehyde breakthrough samples in our analysis resulted in a higher mean TWA_8 exposure concentration (0.04 [±0.042] ppm) for D fuel, its ranked sum remained similar to that of B75.

Overall, the median TWA_8 exposure concentrations for tDPM (p=0.006), NO (p=0.025), NO_2 (p=0.045) and CO (p=0.038) were higher during the first exposure sessions than those of the second. While there were no differences in average exposures between first and second sessions

for D, tDPM (p=0.005), NO (p=0.024), and CO (p=0.012), exposure concentrations were higher during the first B75 sessions, and rDPM (p=0.025), tDPM (p=0.016), and formaldehyde (p=0.035) were higher during the first GD exposure sessions. During one session of a diesel exposure day a wind speed measurement of approximately 2.0 m/s was observed. During one session of a B75 exposure day a wind speed measurement of approximately 1.0 m/s was recorded at the decline rib. It was noted that, in each case, it was particularly windy above ground. There were no other recordable wind speeds measured.

Compared to regular D fuel, our pilot study demonstrated reduction in rDPM with use of B75 and an even greater decrease with GD. Given that the MSHA standard is based on total carbon rDPM exposures, use of both alternative fuel types would likely increase compliance with this federal standard (30 CFR 57.5060). However, other components of vehicle exhaust contribute to adverse health effects, and the increases in certain analytes associated with B75 use should therefore be considered. GD fuel use resulted in decreased exposures for every analyte measured in our study, with the exception of CO, suggesting that from the perspective of the analytes evaluated, its use would likely reduce adverse health effects as compared with use of diesel and B75 fuels.

Previous studies have shown mixed results comparing particulate exposures and DPM across diesel and biodiesel blends, with some finding no difference[12,17], others showing significant reductions[13,14,16,71-74], and one finding a small increase[37] . Our research revealed increasing tOC and decreasing tEC concentrations associated with B75 use, consistent with previously published studies[15,17,37,72,75]. These studies used a variety of biodiesel blends, pollution control devices (or lack thereof), loading procedures, and engine configurations, all of which can influence vehicle emissions.

Our study failed to demonstrate a statistically significant difference in CO and NOx exposure concentrations between D and B75, consistent with reported results from several prior studies[12,17,18,37]. However, other studies have shown an increase in NO_2 or reductions in CO B75 emission concentrations compared to D fuel[15,37]. Our findings are also inconsistent with prior studies showing increased aldehyde concentrations with biodiesel use[12,18]. However, neither of the engines used in these studies used a DOC, as was done in our study. Further, we found decreasing naphthalene concentrations with use of B75, consistent with other studies[12,73,74].

Compared to D, use of natural gas has been shown to reduce particulate emissions[83-85]. Although there are no other published peer-reviewed studies comparing its use to D, reduction in exposures with a GD fuel would seem reasonable given the cleaner burning qualities of natural gas. Our results are also consistent with those described by the manufacturer of GD[24,25]. However, the Nevada Division of Environmental Protection reported that use of GD may cause small increases in CO and total hydrocarbon emissions[24].

Previous studies have shown that diesel emissions are influenced by a variety of factors including type of equipment, load, and pollution control equipment, including catalytic converters and particle traps[15,17,18,37,74,81]. The LHD vehicle in our study was chosen because it is commonly used in underground mine settings, and is often associated with high exposures to diesel exhaust[17,81]. Pollution control devices present on the LHD included a DOC, but no DPF. Other studies used neither DOC nor DPF[12,18,74], only a DOC[15], or combination of pollution control configurations[37,81]. Evidence suggests that DOCs can reduce biodiesel's CO and hydrocarbon emissions, but have little effect on NOx exposures[82]. Although our LHD does not represent the newest or most advanced LHD available, it is representative of the types of equipment frequently found in current use.

Limitations: In addition to the limitations cited for the D vs B75 Exposure Study, the potential for biased introduced by different operators, for shorter sampling sessions, and during a more condensed time period may have influenced the GD observations.

Although the current study was limited to the underground mining setting, our results suggest that use of GD could potentially decrease exposures to harmful emissions from the use of diesel engine-powered vehicles. Both occupational and general population exposures could be impacted.

GD Extension Pilot Study: There were a total of 20 D and 16 GD samples collected, with a few variations due to equipment malfunction. The median temperatures during D and GD sessions were 88.8 and 90.1˚F, respectively, and the median dew point during D and GD sessions was 29.5 and 44.9 ˚F, respectively. The median relative humidity for D and GD sessions was 11.4 and 18.9%, respectively, while the median wind speeds were 9.5 and 8.8 MPH, respectively. This study demonstrated little statistical difference between D and GD exhaust exposures in a JCI LHD with Purimuffler at the SX mine. While median rOC, tDPM, tOC, and NO_2 TWA_8 exposure concentrations were reduced with use of GD, only NO_2 was significantly so (p=0.023). Use of GD resulted in increased median concentrations of rDPM, rEC, tEC, and NO, though only tEC was significantly greater (p=0.012). Median formaldehyde concentrations were identical.

Table 1. TWA_8 exposure concentrations by analyte and fuel type

Analyte	rDPM		rEC		rOC	
Fuel	D	GD	D	GD	D	GD
n	20	15	20	15	20	15
Mean ($\mu g/m^3$)	275	305	116	138	159	168
S.D. ($\mu g/m^3$)	112	84	36	33	85	101
Median ($\mu g/m^3$)	250	283	113	141	151	138
IQR ($\mu g/m^3$)	133	74	54	27	94	41

Analyte	tDPM		tEC		tOC	
Fuel	**D**	**GD**	**D**	**GD**	**D**	**GD**
n	20	16	20	16	20	16
Mean ($\mu g/m^3$)	552	526	136	162	413	361
S.D. ($\mu g/m^3$)	142	75	34	20	121	53
Median ($\mu g/m^3$)	551	500	134	156*	406	344
IQR ($\mu g/m^3$)	150	90	40	23	142	66

Analyte	NO		NO_2		Formaldehyde	
Fuel	**D**	**GD**	**D**	**GD**	**D**	**GD**
n	20	16	20	16	19	16
Mean (ppm)	1.95	2.98	0.345	0.159	0.035	0.033
S.D. (ppm)	0.78	2.32	0.589	0.098	0.013	0.010
Median (ppm)	1.73	1.87	0.186	0.120*	0.030	0.030
IQR (ppm)	1.13	1.68	0.115	0.060	0.012	0.013

*Significantly different from D at the p<0.05 level.

This pilot study demonstrated mixed results regarding the effect of GD use in a JCI LHD with a Purimuffler for basic operation at the SX mine. These findings are inconsistent with those observed while using the Wagner LHD, where all contaminant concentrations except CO were significantly reduced. The results of this pilot study are consistent with previous work that demonstrated variability in exposure reductions based on the use of different equipment and pollution control configurations.[86] Specifically, the pollution control device difference (diesel oxidative catalyst v. Purimuffler) used on a diesel engine appears to have a marked effect on the ability of alternative fuels to reduce emissions.

Conclusion
In this project we evaluated multiple health parameters following acute exposure to diesel and B75 emissions. Most health endpoints, with the exception of changes in lung function, were similar when comparing the two fuel-types, despite the 20% reduction in rDPM achieved by the use of B75. Additional studies are needed to evaluate the potential differential health effects from emissions of these fuels at both lower concentrations and for more chronic exposure periods, as well as for a larger selection of biodiesel fuel sources, blend concentrations, and pollution control devices. The present study highlights the need to further evaluate the health effects associated with alternative fuels, and not assume that reduction in rDPM alone will lead to reduced health effects.

We also observed significant reduction in rDPM exposure concentrations with the use of B75. Although the current study was limited to the underground mining setting, our results suggest that use of alternative fuels, and GD in particular, could potentially decrease exposures to

harmful emissions from the use of diesel engine-powered vehicles, depending on the type of pollution control device used. Both occupational and general population exposures could be impacted. Despite this, our study was limited to two LHDs with limited pollution controls, and further investigation with additional vehicles and more pollution controls is needed.

Publications

1.   Lutz EA, Reed RJ, Lee VST, Burgess JL. Comparison of Personal Diesel and Biodiesel Exhaust Exposures in an Underground Mine. Journal of Occupational and Environmental Hygiene (accepted pending minor revisions).

2.   Mehus AA, Reed RJ, Lee VST, Littau SR, Hu C, Lutz EA, Burgess JL. Comparison of Acute Health Effects From Exposures to Diesel and Biodiesel Fuel Emissions. Journal of Occupational and Environmental Medicine. 2015;57(7):705-12. doi: 10.1097/jom.0000000000000473. PubMed PMID: WOS:000357944000001.

3.   Lutz EA, Reed RJ, Lee VST, Burgess JL. Occupational Exposures to Emissions from Combustion of Diesel and Alternative Fuels in Underground Mining-A Simulated Pilot Study. Journal of Occupational and Environmental Hygiene. 2015;12(3):D18-D25. doi: 10.1080/15459624.2014.987384. PubMed PMID: WOS:000349104900002.

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