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Effect of biodiesel fuel on “real-world”, nonroad heavy duty diesel engine particulate matter emissions, composition and cytotoxicity

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Abstract

Biodiesel is regarded by many as a “greener” alternative fuel to petroleum diesel with potentially lower health risk. However, recent studies examining biodiesel particulate matter (PM) characteristics and health effects are contradictory, and typically utilize PM generated by passenger car engines in laboratory settings. There is a critical need to analyze diesel and biodiesel PM generated in a “real-world” setting where heavy duty-diesel (HDD) engines and commercially purchased fuel are utilized. This study compares the mass concentrations, chemical composition and cytotoxicity of real-world PM from combustion of both petroleum diesel and a waste grease 20% biodiesel blend (B20) at a community recycling center operating HDD nonroad equipment. PM was analyzed for metals, elemental/organic carbon (EC/OC), polycyclic aromatic hydrocarbons (PAHs), and nitro-polycyclic aromatic hydrocarbons (N-PAHs). Cytotoxicity in a human lung epithelial cell line (BEAS-2B) following 24 h exposure to the real-world particles was also evaluated. On average, higher concentrations for both EC and OC were measured in diesel PM. B20 PM contained significantly higher levels of Cu and Mo whereas diesel PM contained significantly higher concentrations of Pb. Principal component analysis determined Mo, Cu, and Ni were the metals with the greatest loading factor, suggesting a unique pattern related to the B20 fuel source. Total PAH concentration during diesel fuel use was 1.9 times higher than during B20 operations; however, total N-PAH concentration was 3.3 times higher during B20 use. Diesel PM cytotoxicity was 8.5 times higher than B20 PM ($p < 0.05$) in a BEAS-2B cell line. This study contributes novel data on real-world, nonroad engine sources of metals, PAH and N-PAH species, comparing tailpipe PM vs. PM collected inside the equipment cabin. Results suggest PM generated from burning petroleum diesel in nonroad engines may be more harmful to human health, but the links between exposure, composition and toxicity are not straightforward.

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Graphical Abstract

Keywords

Biodiesel; Particulate matter; Metals; PAHs; Cytotoxicity; N-PAHs

1 Introduction

Diesel particulate matter (DPM) exposure is associated with an array of chronic and acute cardiopulmonary health risks, including lung and cardiovascular inflammation, asthma exacerbation, and lung cancer (USEPA 2002a; HEI 2002; Attfield et al., 2012). U.S. regulatory agencies such as NIOSH and the USEPA have concluded that petroleum diesel exhaust is a “potential occupational carcinogen” (NIOSH 1988) and “likely to be carcinogenic to humans by inhalation” (USEPA 2002a). According to Pope et al. (2009), even low-to-moderate levels of fine particulate matter (PM) exposure may result in a linear relationship between exposure to PM and negative health effects, suggesting that there may not be a safe exposure threshold for humans. Reductions in PM emitted by diesel engines may help reduce the impact of PM exposure on public health. One potential method to reduce PM emissions from diesel engines is switching from petroleum diesel to biodiesel fuels.

Biodiesel fuel is biodegradable, made from renewable feedstocks, has high engine lubricity characteristics (USDOE 2004), and its use results in an overall reduction in greenhouse gas emissions (Hill et al., 2006). Previous studies have demonstrated that replacing diesel fuel with biodiesel fuel can reduce emission of PM, carbon monoxide and total hydrocarbons in tailpipe exhaust (USEPA 2002b; Lapuerta et al., 2007; Di et al., 2009; McCormick et al., 2006; Robbins et al., 2009; Yanowitz et al., 2009; Argawal et al., 2015). Less is known about the impact of biodiesel on PM composition (such as polycyclic aromatic hydrocarbons) and associated health effects, but this is an area of active research (Karavalakis et al., 2009; Cheung et al., 2010; Hemmingsen et al., 2011; Fukagawa et al., 2013; Gerlofs-Nijland et al.,

2013). In the aforementioned studies and most other diesel emission studies, the predominant method for collecting source or tailpipe PM is to follow standardized testing procedures using passenger car diesel engines (i.e., the Federal Testing Procedure at 40 CFR Part 86). These emissions studies collect particles directly from the tailpipe exhaust under controlled laboratory conditions of air dilution, temperature, humidity, and engine operating mode. While laboratory studies mimic dilution conditions and capture emissions trends, in-use studies of nonroad heavy duty diesel (HDD) equipment operating in occupational or public community settings provide an important complement to engine dynamometer tests. Performing an exposure assessment at an active work site better approximates PM inhalation levels and incorporates real world variations in fuel consumption and engine activity patterns (i.e., equipment moving with a heavy load) that ultimately influence PM characteristics. According to the most recent EPA diesel engine nonroad population estimates, there are over 300,000 large front loaders, 300,000 tractors and 100,000 excavators in use across the U.S. (EPA, 2010). These type of nonroad vehicles are commonplace in commercial, agricultural and construction type settings. Yet, to the best of our knowledge, biodiesel exposure studies in these types of settings are nearly absent from the literature, other than our previous work (Traviss et al., 2010; Traviss et al., 2012; Traviss et al., 2014). Traviss et al. (2012) determined that PM_{2.5} mass concentration significantly decreased during B20 usage (compared to petroleum diesel). Such reductions in PM mass are often linked to biodiesel's increased oxygen content within the fuel (~ 11% w/w) (Agarwal et al., 2015).

A better understanding of the potential human health impacts related to “real world” biodiesel and diesel PM exposure requires evaluation of the PM mass concentration, chemical composition, and cytotoxicity. Biodiesel feedstocks vary and may include soybean oil, rapeseed oil, waste grease, and animal fats (with varying degrees of saturation/unsaturation) which can impact PM composition and result in differences in metal, polycyclic aromatic hydrocarbon (PAH), nitro-polycyclic aromatic hydrocarbon (N-PAH), and elemental carbon/organic carbon (EC/OC) content between diesel and biodiesel fuels (Karavalakis et al., 2009; Cheung et al., 2010; Ballesteros et al., 2010; Karavalakis et al., 2011). Traviss et al. (2012) reported a significant reduction (up to 76%) in PM_{2.5} mass concentration during B20 use (soy feedstock), despite an associated increase in OC concentration (467% greater than diesel OC concentration). However, during the previous study the OC fraction was not chemically characterized, and toxicological responses were not examined. Polycyclic aromatic hydrocarbons are most likely a component of the overall OC fraction and currently, the EPA lists 16 PAHs as potential carcinogens (US EPA, 2014), each with varying chemical structure and toxicological potential (Ravindra et al., 2008). Agarwal et al. (2013) showed that diesel PM had slightly higher toxic potential than a 20% biodiesel blend due to PAH composition. However, in a study comparing emissions from various biodiesel feedstocks, Karavalakis et al. (2011) found that use of waste grease based biodiesel increased total PAH emissions by 27% (urban drive cycle) compared to the reference diesel fuel. The authors suggest this may be due to the chemical composition of used frying oil, possibly containing already oxidized compounds; burning used frying oil also resulted in higher levels of N-PAH's compared to other biodiesel blends, but reference diesel had the highest overall N-PAH emissions (Karavalakis et al., 2011). Nitration of PAHs, i.e. the formation of N-PAHs, can result from combustion processes as well as

exposure of PAHs to the ambient environment (Finlayson-Pitts and Pitts, 1997). Previous studies demonstrate that ambient concentrations of N-PAHs are associated with increased mutagenic activity relative to PAHs (Umbuzeiro, et al., 2008), possibly because N-PAH's do not require metabolism to become reactive, genotoxic compounds (Fu and Herreno-Saenz, 1999). Finally, metals composition could also contribute to the negative health impacts associated with diesel and biodiesel PM. Previous studies show that metals, particularly transition metals such as Cu, are a major component of diesel and biodiesel PM (Betha and Balasubramanian, 2011; Traviss et al., 2014; Godoi et al., 2016). The presence of transition metals, and polar and nonpolar organic species in biodiesel and diesel PM can induce production of Reactive Oxygen Species (ROS) in vitro (Verma et al., 2010; Hemmingsen et al., 2011; Fukagawa et al., 2013; Godoi et al., 2016). While ROS is integral to many cellular processes, increased exposure to ROS causes damage to intracellular components like DNA, lipids and proteins (Dröge, 2002). Excessive ROS levels can result in cytotoxic effects.

The goals of this study were to compare the mass concentration, chemical composition, and cytotoxicity of diesel vs. B20 PM collected in a “real world” occupational setting. We performed an exposure assessment at a community recycling facility, where HDD nonroad vehicles were first fueled with commercially purchased nonroad diesel fuel before switching to a B20 biodiesel blend (20% waste grease biodiesel/80% nonroad petrodiesel). Work area and equipment-cabin concentrations of size fractionated PM mass (2.5 μm and $<0.25 \mu\text{m}$), EC/OC, metals, PAH, and N-PAH were quantified and compared. Bulk PM was collected directly from inside the exhaust tailpipe for each fuel and used to perform in vitro cytotoxicity assays on a human epithelial lung cell line (BEAS-2B).

2 Methods

2.1 Site Description and Study Design

During the summer of 2011, particulate matter (PM) samples were collected at the Keene Recycling Center (KRC), a small, rural materials recovery facility for Keene, New Hampshire and surrounding towns. As described in previous studies (Traviss et al., 2010; Traviss et al., 2012; Traviss et al., 2014), KRC is an ideal field site due to its relative isolation from other PM sources, its consistency in operations on a weekly basis, and its usage of nonroad HDD equipment. Figure 1 is a schematic of the KRC building layout, showing the separation of the trash and recycling operations, as well as the sampling locations. Our sampling strategy regarded the KRC nonroad vehicles as pollutant sources for diesel PM, with in-cabin measurements representing the highest employee exposures. The KRC is a smoke-free worksite, eliminating employee smoking as a confounding factor. The primary tasks of the KRC's HDD nonroad equipment include moving trash and recyclable materials throughout the site. In this study, we measured PM concentrations inside the cabins of three pieces of equipment: a small front loader (2004 JCB Model 409B, four cylinder diesel engine [Tier II], 75 hp @ 2,500 rpm - designated “P4”), a large front loader (2010 John Deere Model 624K, six cylinder diesel engine [Tier III], 198 horsepower @ 1800 rpm - designated “P5B”), and a track excavator (2010 Hitachi 2X160LC, four cylinder diesel engine [Tier III], 110 hp @ 2,150 rpm - designated “P5A”). P5A and P5B operated at the trash tipping floor side of the KRC, and P4 operated inside the recycling materials side

(Figure 1). None of the vehicles were equipped with emissions control aftertreatment devices. We did not interfere with day-to-day operations, which included time periods of equipment idle, moving, lifting with load, shut down and restart. Equipment cabin windows were open for all pieces of equipment except P5A. We collected PM from inside the recycling materials side of the building (designated “P2”) to represent the general “work area” concentration for employees. PM was collected at all the in-cabin and work area locations (P2, P4, P5A, P5B) from 7 am to 3 pm over an approximately 8 hour work shift. Equipment ran first on commercially purchased nonroad diesel fuel (ASTM 975, <500 ppm sulfur) and then on an 80/20 diesel/biodiesel blend (ASTM D6751, White Mountain Biodiesel, North Haverhill, NH). Waste grease was the biodiesel feedstock source. Additional fuel data are located in Table S1, but of note is that the diesel fuel contained over 300 ppm S. Diesel sampling (16 days) occurred between June 7, 2011 and July 1, 2011. Following the diesel sample collection cycle, sample collection was suspended for 3 weeks while the fuel was switched to B20. During this 3 week sampling hiatus, the bulk fuel storage tank on-site and the fuel tank of each piece of equipment were emptied twice, allowing the equipment to condition to the B20 fuel. B20 sampling (12 days) was performed between July 21, 2011 and August 8, 2011.

Fuel usage and hours of operation per day were recorded by undergraduate researchers for the HDD equipment at the P5B location. This information was used to normalize PM_{2.5} concentrations per liter of fuel consumed and per hour of operation. Undergraduate researchers also collected data on temperature, relative humidity, wind speed, and wind direction using a Kestrel 4500 portable weather station.

2.2 Particulate Matter Sample Collection and Mass Concentration Determination

PM collection strategy and methods are described more fully in Traviss et al. (2012) and Traviss et al. (2014) and are briefly summarized here. A Sioutas cascade impactor (SKC Inc., Eighty Four, PA) was placed inside the equipment cabin, near the employees breathing zone, at P4, P5A, P5B, and in the stairwell at P2. Each impactor contained four 25 mm (0.5 µm pore size, Pall Corporation P5PQ025) filters and one 37mm (2.0 µm pore size, SKC #225–1709) polytetrafluoroethylene (PTFE) filter in order to collect size-segregated PM ranging from >2.5 µm diameter to <0.25 µm diameter. The impactors were connected to a SKC Leland Legacy Pump (SKC Inc., Eighty Four, PA) operating at a flow rate of 9 L/min. The pumps were calibrated at the beginning and end of every field day using a Bios Defender 510 primary calibrator. Prior to placement in the impactors, the Teflon filters were allowed to equilibrate in a temperature- and humidity-controlled environment for 24 hours and weighed using a Mettler Toledo XP-6 microbalance (1 µg readability). At the end of each sample collection day the filters were removed from the impactors, placed into Omega Filter Keepers (SKC #225–8303), and placed in the temperature and humidity controlled environment for 24 hours prior to gravimetric analysis. As described in our previous study (Traviss et al. 2014), diesel and B20 PM were also collected directly from the exhaust pipe of P5B onto a 47 mm Tissuquartz Pallflex filter held in a stainless steel filter holder. At the end of the sampling day, the filter was archived, wrapped in foil, and stored in a –20 °C freezer. These tailpipe exhaust samples were analyzed for metals, PAH and N-PAH concentrations and provided the bulk PM used to assess cytotoxicity. The tailpipe chemical

analyses data can only be interpreted qualitatively for the presence or absence of species. This is because after sample collection, the perimeter edges of the tailpipe filters stuck to the filter holder so that an accurate total PM mass could not be determined.

Samples for EC/OC analyses were collected on quartz filter cassettes (SKC #225–317) attached to a GS-1 cyclone (SKC #225–105), placed next to the Sioutas impactors, and connected to SKC Universal Pumps (SKC PCXR8) operating at a flow rate of 2 L/min. These cassettes, collecting PM_{1.0}, were stored in a –20 °C freezer prior to analyses. All pumps were pre and post calibrated at the beginning and end of every sample collection day using a Bios Defender 510 primary calibrator.

Detailed organics speciation was performed on PM_{1.0} samples (16 days diesel, 10 days B20) collected inside the cabin of P5B using a PM_{1.0} cyclone separator (BGI Instruments GK2.055H) packed with a 37 mm Tissuquartz filter (Pallflex 7201) connected to a SKC Universal Pump (SKC PCXR8) operating at a flow rate of 4.0 L/min. Filters were wrapped in foil and stored at –20 °C prior to analysis. All pumps were pre and post calibrated at the beginning and end of every field day using a Bios Defender 510 primary calibrator.

2.3 Metals and EC/OC Analyses of Particulate Matter

As described previously (Traviss et al. 2014), following gravimetric analysis, PM collected on PTFE filters (at P5B) was sent to the Dartmouth College Trace Elements Laboratory (Hanover, NH) for metals analysis performed via inductively coupled plasma-mass spectrometer (ICP- MS) for the following metals: Na, Al, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Mo, Cd, Ba, Hg, Pb. These metals were selected based on their previously described toxicides (Becker et al., 2005; Chen and Lippmann, 2009; Gerlofs-Nijland et al., 2013) and for their association with vehicle sources or fuels (HEI 2010). The concentrations of the above metals in the PM_{<0.25} size cut are reported. Elemental carbon (EC) and organic carbon (OC) analyses were performed by WOHL (Wisconsin Occupational Health Laboratory) following the NIOSH 5040 method.

2.4 PAH and N-PAH Analyses of Particulate Matter

The PM_{1.0} samples collected inside the cabin near the employee breathing zone in P5B and bulk tailpipe PM were analyzed for PAHs and N-PAHs. Analytes were extracted from filters following methods described in Havey et al., 2006 and Ratcliff et al., 2010. Briefly, filters were spiked with deuterated internal standards consisting of 250 pg of nitrobenzene-d₅, 1-nitronaphthalene-d₇, 1,5-dinitronaphthalene-d₆, 9-nitroanthracene-d₉, and 1-nitropyrene-d₉ for N-PAH analysis and 2.5 ng of naphthalene-d₈, phenanthrene-d₉, and chrysene-d₁₂ for PAH analysis (CDN Isotopes, Pointe-Claire, Quebec). Filters were then Soxhlet-extracted into 200 mL of dichloromethane (HPLC grade, Avantor, Center Valley, PA) for at least 200 cycles. Extracts were chilled and concentrated under flowing nitrogen into 200 µL of toluene (HPLC grade, Sigma-Aldrich, St. Louis, MO).

Particulate matter extracts were analyzed on a JEOL JMS-700T MStation magnetic sector mass spectrometer (MS) fitted with a tunable electron energy monochromator (TEEM™) ionization source and a Hewlett-Packard 6890 gas chromatograph (GC) inlet. Electron monochromator mass spectrometry has been shown to be a sensitive and selective method

for PAH and NPAH analysis (Havey et al., 2006; Ratcliff et al., 2010; Jensen and Voorhees, 2015; Jensen et al., 2014).

Injections were done directly onto a 0.25 mm i.d. 30 m RTX 5Sil-MS column (Restek Corp., Bellefonte, PA) with Integra-Guard®. The GC temperature profile was as follows: 100°C for 1 min, ramp 10°C/min to 190°C and hold for 2 min, ramp 5°C/min to 240°C and hold for 1 min, ramp 10°C/min to 310°C and hold for 3 min. Temperatures of 310°C and 280°C were used for the MS transfer line and ion source, respectively. For PAH detection, samples were analyzed in full-scan, positive-ion mode from m/z 10–600 at an electron energy of 25 eV. The lower ionization energy allows for a greater chance at seeing the molecular ion. Selected ion monitoring (SIM) in negative-ion mode at m/z 46 and 3.5 eV ionization energy was used to analyze N-PAH compounds. Here, 3.5 eV is used to selectively fragment the NO_2^- group from nitro compounds, and subsequent SIM monitoring will display an NO_2^- peak for each nitro compound as it exits the column.

Compounds were identified by comparison against standard retention times. Peaks that could not be identified based on retention time were analyzed with a JEOL JMS-700T MStation using standard electron ionization (EI) mode with an ionization current of 700 μA and a source temperature of 200°C. A NIST/EPA/NIH Standard Reference Library 1A search was conducted for initial identification.

2.5 Cytotoxicity Assay

2.5.1 Human BEAS-2B bronchial epithelial cell culture—BEAS-2B human bronchial epithelial lung cells (ATCC, Manassas, VA) were used in the cytotoxicity assay as a model cell line to compare results to other in vitro studies of diesel PM (Totlandsdal et al. 2010, Fukagawa et al. 2013). Cellular culture flasks (75 cm^2) were pre-coated with Vitrogen Plating Medium and incubated (37° C, 5% CO_2) for 24 hours prior to cell seeding. The cells were cultured in Bronchial Epithelial Growth Medium (BEGM) (Lonza CC-3170).

2.5.2 PM extraction & Cytotoxicity Assay—The 47 mm Tissuquartz Pallflex filters containing tailpipe PM were cut in half and soaked in ethanol overnight. Next, the filters were sonicated for 60 min in ethanol, followed by syringe-filtering the extracts and allowing them to evaporate. A PM stock solution of approximately 1 mg/mL in cell culture media (with <0.1 % v/v ethanol) was prepared. The BEAS-2B cells were treated with 100 μl aliquots of PM at the concentrations of 300, 150, and 50 $\mu\text{g/mL}$. Blank Tissuquartz filters with no PM were also extracted with ethanol as a method blank. Cell culture media alone was a negative control (0 $\mu\text{g/mL}$). The cells were incubated (5% CO_2 , 37°C) for 24 hours following treatment prior to the start of the cytotoxicity assessment. Cytotoxicity was measured by the lactose dehydrogenase (LDH) assay using a Promega CytoTox 96 kit and following the manufacturer's instructions.

2.6 Data Analyses

Nonparametric (i.e., Mann-Whitney U test) tests were run to compare PM mass concentrations, EC and OC data, metals data, PAH, and N-PAH data between fuel types (PASW Statistics Version 18.0, SPSS Inc., Chicago, IL). Cytotoxicity results were analyzed

with ANOVA (Tukey multiple comparison test) using the fuel source as the grouping variable. Statistical significance was determined at $p < 0.05$. PM mass concentrations were separated into two groups: fine ($PM_{2.5}$) and “quasi”-ultrafine ($PM_{<0.25}$) in order to assess how PM size emissions may differ between the two fuel varieties.

Principal component and factor analyses for metals concentrations in $PM_{<0.25}$ were calculated with JMP 9 software. Principal components were determined on the correlations and varimax factor loadings were determined from factor analysis on the principal components. Principal components with eigenvalues greater than one were used in the factor loading calculations.

3 Results and Discussion

3.1 Weather and Activity

The average temperature and humidity were similar during both fuel campaigns. The average temperature during diesel operation/sampling was 24.4°C versus 27.1°C during biodiesel operation. The average relative humidity was 55% (diesel) versus 59% (biodiesel). None of the weather parameters were significantly different. The average daily fuel consumption was very similar between diesel and biodiesel operation (11.9 versus 12.0 gal/day, respectively). The average daily operating hours were 6.9 hours for diesel and 7.0 hours for biodiesel. In summary, weather patterns and activity characteristics were not significantly different between the different fuel types and sampling periods.

3.2 Particulate Matter In-Cabin Concentrations

The results for $PM_{2.5}$ concentrations were similar (Fig. 2) at P2, P4 and P5B (ranging from 35–110 $\mu\text{g}/\text{m}^3$), but lower at P5A (ranging from 9–54 $\mu\text{g}/\text{m}^3$). $PM_{<0.25}$ concentrations were generally lower than $PM_{2.5}$ concentrations (Fig. 3). There is a notable difference between PM concentrations measured at P5A compared to other sampling locations. This is likely due to differences in the equipment cabin windows during operation, since at P4 and P5B the equipment windows were open during operation, but at P5A the windows were closed and the air conditioning system (containing a HEPA-filter) was used during operation. Mean mass concentrations between diesel and biodiesel fuel use were not significantly different at any monitoring location for either $PM_{2.5}$ or $PM_{<0.25}$ size cuts. These results vary from previous results reported in Traviss et al. (2012), where average $PM_{2.5}$ mass concentrations measured during diesel use were significantly higher than during B20 use. However, in the present study, newer and different HDD equipment was in use at the recycling center than the equipment in our previous study. Additionally, the feedstock in this study was waste grease and not soy based as in the 2012 study. The current $PM_{2.5}$ results are more consistent with Traviss et al. (2014), which used a waste grease feedstock. However, $PM_{<0.25}$ results at both P4 and P5B in the present study differed from Traviss et al. (2014); $PM_{<0.25}$ mass concentration inside the cabin of P5B was 32% higher during B20 use, whereas Traviss et al. (2014) found $PM_{<0.25}$ mass concentration to be 43% higher during diesel use.

$PM_{2.5}$ mass and $PM_{<0.25}$ mass concentrations collected at P5B were evaluated on a per hour and per liter basis for each fuel type in an attempt to normalize for potential activity

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differences in equipment operation between the two fuel campaigns (Fig. 4). In contrast to previous work (Traviss et al. 2014), higher $PM_{<0.25}$ mass concentrations were observed during B20 operation compared to diesel whether normalized by hours of operation or fuel consumption. When evaluated on a per hour of operation basis, B20 $PM_{<0.25}$ levels were significantly higher compared to diesel ($p < 0.05$). Most biodiesel emissions studies have determined that burning biodiesel reduces PM mass concentration; our previous exposure studies (Traviss et al., 2012; Traviss et al., 2014) also found that PM mass concentrations were lower during B20 fuel usage. However, some researchers have found increased PM emissions from biodiesel and suggest this is related to the lower volatility of biodiesel fuel, which contributes to increased formation of a soluble organic fraction (SOF) (Karavalakis et al., 2009; Bakeas et al., 2011). Unexpected increases in PM emissions were found during combustion of oxidized biodiesel blends (such as frying oil blends), which were attributed to a possible combination of fuel type and driving conditions, specifically the incomplete combustion of the oxidized biodiesel fuels in the catalyst and the cold start conditions of the driving cycle (Bakeas et al., 2011). The above referenced studies on common rail injection system engines utilized robust emissions controls, whereas the equipment in this study did not have aftertreatment controls. However, we speculate that the slight increases observed in B20 $PM_{<0.25}$ concentrations may be related to the waste grease feedstock (more oxidized) and/or the frequent “stop and go” mode of operation of the HDD equipment.

In summary, the $PM_{2.5}$ mass concentrations measured within the equipment cabin and in the work area during operation with both fuels were similar. This may also be due to the benefits of cleaner engine technology as the equipment model for P5B in Traviss et al. (2014) was a 2005 John Deere 624J (Tier II Engine) and the equipment model for P5B in this study was a 2010 John Deere 624K (Tier III Engine). The benefits from using B20 to reduce $PM_{2.5}$ mass concentration may diminish over time with the implementation of new technology such as cleaner engines and diesel particulate filter (DPF) technology. Yet, while $PM_{2.5}$ mass concentration remains a benchmark for public health standards, future research is needed to examine the potential of various fuel and engine combinations to generate smaller, ultrafine particles.

3.3 EC/OC Concentrations

Elemental carbon concentrations were consistently elevated during diesel operations compared to B20 operations across all sampling locations (Fig. 5), similar to previous observations (Traviss et al. 2014). EC concentrations ranged from a low of 1.17 to a high of 3.35 $\mu\text{g}/\text{m}^3$. EC concentrations at P2 and P4 were 2.0–2.5 fold higher during diesel operation compared to B20 use. EC concentrations inside the cabins of the HDD equipment (P5A and P5B) were generally lower for both fuel types compared to P2 and P4. The lower EC concentrations at P5A and P5B may be related to the consistent movement of these vehicles in and out of the KRC facility, providing some in-cabin ventilation, while P2 and P4 were in a more enclosed building allowing for EC to build up over time.

Organic carbon concentrations were also elevated during diesel operations (Fig. 6) at most locations (except P2). OC concentrations ranged from a low of 2.51 to a high of 7.19 $\mu\text{g}/\text{m}^3$. The higher levels of OC measured during diesel fueling contrast with our previous work

(Traviss et al., 2012; Traviss et al., 2014) and the work of other researchers (Bugarski et al., 2010; Magara-Gomez et al., 2012). P5A and P5B OC concentrations were 1.2–1.8 fold higher during diesel use compared to B20. OC concentrations were slightly higher than EC concentrations. OC concentrations were lower than expected at each sampling location based upon previous work (Traviss et al., 2012; Traviss et al., 2014). While the reduced OC levels can be explained in part by the lower total PM mass in this study, we note the thermal optical method may not capture important contributions to PM mass from other organic, oxidized species such as organic acids and esters (Magara Gomez et al., 2012). Other researchers who have performed more detailed speciation of biodiesel and diesel PM have determined organic functional groups such as acids can contribute substantially to PM composition (Chueng et al., 2010, Magara Gomez et al., 2012). The variability in OC content between different field campaign years could also be due to updated equipment standards. A number of studies have demonstrated that exhaust aftertreatment devices and updated engines can have significant impacts on the elemental and organic components of emitted particles (Pakbin et al., 2009; Lui et al., 2009; Agarwal et al., 2013). In this study, averaging the relative ratios of EC to OC for each fuel type resulted in a similar ratio at every location. EC/OC ratio was approximately 0.26 for biodiesel and 0.29 for diesel. While there were relative differences between elemental and organic carbon concentrations per fuel type, these differences were not statistically significant at any of the sampling locations. Table S3 contains the EC/OC data at each sampling location.

3.5 Metals Concentrations

Metals concentrations in the $PM_{<0.25}$ size cut differed between fuel types (Fig. 7 and Table S4). Cu and Mo levels were significantly higher during B20 use, and Pb levels were significantly higher during diesel use ($p < 0.05$). Mn and Zn concentrations were higher in diesel PM and Ni was higher in B20 PM. Fe made up the largest metal fraction of PM and levels of Fe in diesel were on average 44% higher than in B20 PM. These results differed from previous metals characterization of PM completed by Traviss et al. (2014), in which Cu levels were relatively similar between fuels, Fe was high in B20 PM, and Ni was higher in diesel PM. This highlights the importance of evaluating combinations of feedstock sources and/or combustion technologies which can influence PM chemical composition, even within similar equipment at the same sampling location. While both John Deere 624 series front loaders (in this study and Traviss et al. 2014), followed the same maintenance schedules, and did not have aftertreatment technology, this study's model was a 2010 624K engine [Tier III] compared to the previous 2005 624J engine [Tier II].

Differences in metals concentrations between biodiesel and diesel PM have also been reported in the literature. For instance, in several studies Pb was measured at higher concentrations in diesel PM than in biodiesel PM (Betha and Balasubramanian 2011; Agarwal et al., 2010); however, a study by Gangwar et al. (2012) showed elevated levels of Pb in biodiesel PM in comparison to diesel PM. Metals composition may impact biological responses evaluated in vitro and establishes the importance of a complete characterization of particulate matter in order to better understand the health implications of exposure.

Table 1 shows the correlations among the metals species in $PM_{<0.25}$, within each fuel type. Significant correlations are highlighted in red. The data in Table 1 suggest patterns in metals concentrations, which may be related to the fuel sources. For example, correlations between Cu and Ba in B20 PM contrast with patterns from diesel PM. Further analysis by principle component analysis (PCA) indicated that three factors account for 85.8% of the variance in the metals concentrations. Individual analyte factor loadings for these three factors were determined via varimax factor rotation (Table S7). The metals with the greatest loading to factor one are Mo, Cu, and Ni. This further suggests a unique metals pattern related to the B20 fuel source as concentrations of Mo, Cu, and Ni were higher in B20 PM relative to diesel PM. The second factor is largely comprised of Pb, Na, and Ba. This component is indicative of general emissions associated with vehicle traffic (Pb and Ba) (HEI 2010). The third factor consists primarily of Mn, Al, and to a lesser extent Fe. Sources of these metals could include engine wear. All of the identified PCA factors are associated with vehicle emissions sources, such as fuel, engine lubrication oils, or brake and engine wear (HEI 2010). In B20 PM, sodium is highly correlated with chromium, manganese, nickel, copper, molybdenum, and barium. This was likely due to the use of sodium in the biodiesel manufacturing process. The lack of correlation of Na, Ni and Cu with each other in diesel PM seems to suggest these metals may be related to the B20 fuel source.

3.7 PAH and N-PAH Concentrations

PAHs and N-PAHs in $PM_{1.0}$ inside the cabin of P5B were measured for 16 days during diesel fuel usage and 8 days during B20 fuel usage. Only individual PAHs and N-PAHs with levels measured above the limit of detection for at least 3 sampling days per fuel type (at P5B) are presented below. Total PAH (Fig. 8; Table S5) and total N-PAH (Fig. 9; Table S6) include only results that meet this 3 day threshold. Almost all daily results of in-cabin PM for PAHs and N-PAHs were below the detection limit for both fuel types. Elevated levels of PAHs and N-PAHs were measured in tailpipe PM (Table S9).

In-cabin total PAH concentrations were 1.92 times greater during diesel operations than during B20 operations (Fig. 8). Our results indicated that lower molecular weight PAH's were typically found in the gas phase, not particle phase. We note that the use of quartz filters introduces the potential for adsorbed organic vapors, and positive artifacts (May et al 2015; Margara-Gomez et al. 2012; Lipsky and Robinson 2006). Additionally, gas-particle partitioning dynamics of organic species are complex and affected by dilution, temperature and adsorption onto black carbon at ambient conditions (Lipsky and Robinson 2006; Liu et al. 2015; May et al. 2015). Finally, we observed higher concentrations of gas and particle-associated PAH's in the tailpipe PM sample, with heavier molecular weight PAH species (benzo[a]pyrene) measured in tailpipe diesel PM (Table S9) but not in the in-cabin PM (Table S8). Thus, it is difficult to tease apart gas vs. particle associated PAH's in the in-cabin (P5B) samples.

In reference to the higher PAH values we observed during diesel operations, other researchers who have analyzed dynamometer PAH emissions have found higher levels of total PAHs in diesel PM compared to biodiesel PM (Karavalakis et al. 2009; Cheung et al., 2010; Ratcliff et al. 2010; Magara-Gomez et al. 2012), with the exception of used frying oil

based biodiesel PAH emissions, which were reported to be 27% higher in a B20 blend (Karavalakis et al 2011). Conversely, in this study total in-cabin N-PAH concentrations were significantly higher (3.3 times) during B20 operations than during diesel fuel use (Fig. 9). Tailpipe PM N-PAH levels are reported in Table S9. In dynamometer studies, biodiesel fuel was found to reduce overall N-PAH emissions (Karavalakis et al. 2009; Ratcliff et al. 2010), although Karavalakis et al. (2011) determined the used frying oil blend had the highest N-PAH levels of the bioblends. Although the concentrations of N-PAHs are an order of magnitude lower than the PAH concentrations, N-PAHs are highly mutagenic and carcinogenic (WHO 2003). The in-cabin N-PAH data are novel because the PM was collected in the worker breathing zone, in a “real world” community setting utilizing typical HDD equipment and a waste grease based B20 fuel.

In other studies, PAH and N-PAH emissions are measured via dynamometer studies with different operating protocols and often passenger car engines. Ratcliff et al. (2010) quantified engine out PAH and N-PAH emissions from an HDD engine operated under an 8 mode test cycle to replicate a wide range of operating conditions. B20 combustion reduced PM associated PAH and N-PAH emissions compared to ultralow sulfur diesel fuel (ULSD), most notably reducing the emission rates of phenanthrene, anthracene and 1-nitropyrene (Ratcliff et al. 2010). While it is challenging to compare across studies using different experimental approaches and equipment configurations, we noted some individual PAH and N-PAH species measured in Karavalakis (2009) and Ratcliff et al. (2010) (such as 1-nitropyrene) were also detected in our tailpipe PM but other species identified in our in-cabin PM (such as 3 and 4-nitrotoluene) differed markedly. A closer examination of our in-cabin PM characterization results versus our tailpipe PM characterization results may help explain why. PAHs such as pyrene and benzo [a] pyrene were quantified in tailpipe PM during diesel operation but were not quantified in the corresponding in-cabin PM. Additionally, pyrene, benzo [a] pyrene, and fluoranthene were quantified only in diesel tailpipe PM and not in B20 tailpipe PM (Table S9). Individual N-PAH species in diesel and biodiesel tailpipe PM were similar and included 1-nitropyrene; however, 1-nitropyrene was not detected in the in-cabin PM filters during either diesel or B20 operation. These results suggest important differences in chemical composition between tailpipe PM and in-cabin PM collected near the breathing zone, likely due to dilution effects.

3.6 Cytotoxicity

Cells were treated with a range of concentrations (0–300 µg/mL) of both B20 and diesel tailpipe PM. There was a dose-dependent increase in cell death when cells were exposed to diesel PM at increasing concentrations, but in B20-treated cells there was essentially no change in cytotoxicity. At lower concentrations, B20 and diesel PM elicited cytotoxic results similar to control (0 µg/mL treatment). At higher concentrations (300 µg/mL), significantly increased cytotoxicity was observed in cells exposed to diesel PM compared to B20 PM ($p < 0.05$). These results suggest exposure to diesel fuel PM may have increased cytotoxic effects compared to B20 PM.

It is important to note that the particles used in the in vitro assay were collected directly from the tailpipe to obtain sufficient mass for the biological assessments. As described in the

previous section, several PAHs and N-PAHs measured in the diesel tailpipe PM (pyrene, fluoranthene, benzo [a] pyrene, 2-nitrophenol) were not present in B20 tailpipe PM (Table S9). There were no metals found in diesel tailpipe PM that were not in B20 tailpipe PM, except for trace amounts of As and Hg. N-PAH species such as 1-nitropyrene were measured in both diesel and biodiesel tailpipe PM; however, diesel tailpipe PM had consistently higher levels of N-PAH species in comparison to biodiesel (Table S9). Pulling together the multiple observations suggests that differences in PAH and N-PAH composition as factors in the diesel PM cytotoxicity results. Toxicological studies have demonstrated the genotoxic and carcinogenic effects of these analytes in a variety of model systems, especially for 1-nitropyrene which is considered a “marker” of diesel exhaust (WHO 2003). An important result from this study is the comparison of tailpipe PM to in-cabin PM species for PAHs and N-PAHs. For example, N-PAHs that were detected in the tailpipe PM for both diesel and B20 operation (such as 1-nitropyrene and 6-nitrobenzo[a]pyrene) were not measured in the in-cabin or “breathing zone” PM, indicating that atmospheric dilution and/or other reaction processes may influence the ultimate composition of PM, which will ultimately influence health effects. Other biodiesel cytotoxicity studies have yielded conflicting results; Steiner et al. (2013) determined the highest cytotoxic effect from B100 PM yet Swanson et al. (2009) reported no cytotoxicity in BEAS-2B cells treated with biodiesel PM.

4.1 Conclusion

Biodiesel and diesel PM collected at a work site utilizing nonroad HDD equipment [Tier III and Tier II] did not differ significantly in PM_{2.5} mass concentrations but did differ in chemical composition and cytotoxicity. In contrast to our previous work (Traviss et al. 2012; Traviss et al. 2014), PM_{2.5} mass concentrations between fuels were similar. B20 PM_{<0.25} mass concentration was significantly higher compared to diesel PM_{<0.25} when PM was normalized on a per hour basis. The chemical composition of the in-cabin PM (collected in the equipment cabin/employee breathing zone) differed between fuels with respect to PAH's, N-PAH's, and metals. Total PAH were higher in diesel PM, and total N-PAH were higher in B20 PM. Cu and Mo levels were significantly higher in B20 PM, and Pb was significantly higher in diesel PM. Differences in PM metals composition between diesel and biodiesel have been noted by other researchers, and elevated Cu may be related to waste grease feedstock (Cheung et al. 2010; Betha and Balasubramanian, 2011). There were marked differences in the PAH and N-PAH species identified when comparing diesel vs. B20 tailpipe PM, notably the absence of pyrene, fluoranthene, benzo [a] pyrene, and 2-nitrophenol in B20 tailpipe PM that may help explain the significantly higher cytotoxicity of diesel tailpipe PM. We note that we were unable to quantify the tailpipe PAH and N-PAH levels on a ng/mg basis due to the loss of filter fragments during handling. Pulling together the multiple observations suggests that differences in PAH and N-PAH composition factor into the diesel PM cytotoxicity results. Thus, PM generated from burning petroleum diesel in nonroad engines may be more harmful to human health than burning biodiesel, but we note the links between exposure, composition and toxicity are not straightforward. Polar organic species composition such as esters and organic acids were not measured in this study, but could contribute to cytotoxicity or other inflammatory effects in BEAS-2B cells (Fukagawa et al. 2013). Additional chemical analysis of polar organic components of PM between fuel types would be recommended for future work. Evaluation of other important

biological responses such as the impact of biodiesel PM on the generation of cellular reactive oxygen species (ROS) in the BEAS-2B cell line is also recommended. While ROS plays a key role in a myriad of cellular processes, increased exposure can result in oxidative stress, thus causing damage to DNA, lipids, and proteins (Dröge et al. 2002). Finally, an important result from this study is the difference in PAH and N-PAH composition observed when comparing fuel sources but also when comparing tailpipe PM to in-cabin PM. Future research should consider the relationships between PM origin, PM composition and other biological endpoints, ideally comparing tailpipe PM and “real world” PM collected in occupational or urban settings. While dynamometer studies offer a key advantage of controlled experimental conditions, occupational and community exposure assessments offer a key advantage of the collection of PM that reflects actual ambient conditions and PM composition relevant to understanding public health impacts. Both dynamometer studies and exposure studies have critical roles to play in better understanding the potential impact of biodiesel on air quality and human health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Compared diesel vs. biodiesel PM from "real world" setting utilizing nonroad engines
- Higher total PAHs, Pb, and *in vitro* cytotoxicity associated with diesel PM
- Higher total N-PAHs, Cu, and Mo associated with waste grease B20 PM
- Notable differences in PAHs and N-PAHs comparing tailpipe vs. "equipment-cabin" PM
- PM from burning diesel in non-road engines may be more harmful to human health

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Figure 2:
Mean PM_{2.5} concentrations. Error bars represent standard error from the mean.

Figure 3:
Mean PM_{0.25} concentrations. Error bars represent standard error from the mean.

Figure 4:

Diesel vs. biodiesel (at P5B) for $PM_{2.5}$ and $PM_{<0.25}$ reported on per hour and per liter bases. A) Differences in $PM_{2.5}$ mass emitted per hour. B) Differences in $PM_{2.5}$ mass emitted per liter of fuel. C) Differences in $PM_{<0.25}$ mass emitted per hour. D) Differences in $PM_{<0.25}$ mass emitted per liter of fuel. Asterisk (*) indicates significant difference between fuels ($p < 0.05$).

Figure 5:
Mean EC concentrations in PM_{1.0}. Error bars represent standard error from the mean.

Figure 6:
Mean OC concentrations in $\text{PM}_{1.0}$. Error bars represent standard error from the mean.

Figure 7: Concentrations of metals of interest in PM_{0.25} at P5B: Cu, Zn, Na, Mo, and Ni. Asterisk (*) indicates significant difference between fuels ($p < 0.05$). Error bars represent standard error from the mean.

Figure 8:
Concentrations of measured PAHs in PM_{1.0} at P5B. Error bars represent standard error from the mean.

Figure 9: Concentrations of N-PAHs in PM_{1.0} at P5B. Error bars represent standard error from the mean. Asterisk (*) indicates significant difference between fuels ($p < 0.05$).

Figure 10:

Cytotoxicity as represented by mean percentage of LDH released from BEAS-2B epithelial lung cells in response to increasing concentrations of petroleum diesel and B20 tailpipe PM. Asterisk (*) indicates significant difference from control ($p < 0.05$). Pound (#) indicates a significant difference between fuel types at the 300 µg/ml treatment concentration ($p < 0.05$). Error bars represent standard error from the mean.

Table 1:

Correlation coefficients (Spearman's rho), r , among metals species in PM ($<0.25 \mu\text{m}$) per fuel type. Significant correlations are highlighted in red ($p < 0.05$).

B20 AF	Na	Al	Mn	Fe	Ni	Cu	Zn	Mo	Ba	Pb
Na	1	0.6	0.7	-0.7	1	0.9	0.7	1	0.9	0.5
Al		1	0.9	-0.5	0.6	0.7	0.9	0.6	0.3	0.7
Mn			1	-0.3	0.7	0.6	1	0.7	0.4	0.9
Fe				1	-0.7	-0.9	-0.3	-0.7	-0.6	0.1
Ni					1	0.9	0.7	1	0.9	0.5
Cu						1	0.6	0.9	0.8	0.3
Zn							1	0.7	0.4	0.9
Mo								1	0.9	0.5
Ba									1	0.3
Pb										1

Diesel AF	Na	Al	Mn	Fe	Ni	Cu	Zn	Mo	Ba	Pb
Na	1	-0.8	-0.5	-0.1	-0.7	0.1	0.1	0	0.5	0.9
Al		1	0.7	0	0.7	0.4	0	0.3	-0.6	-0.9
Mn			1	0.4	0.3	0.1	-0.4	0	-0.5	-0.6
Fe				1	-0.6	-0.7	-1	-0.9	-0.7	-0.3
Ni					1	0.5	0.6	0.7	0.1	-0.5
Cu						1	0.7	0.9	0.2	0
Zn							1	0.9	0.7	0.3
Mo								1	0.5	0.1
Ba									1	0.8
Pb										1