Published in final edited form as:

Air Qual Atmos Health. 2014 March ; 7(1): 59–70. doi:10.1007/s11869-013-0231-x.

Petrodiesel and Waste Grease Biodiesel (B20) Emission Particles at a Rural Recycling Center: Characterization and Effects on Lung Epithelial Cells and Macrophages

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Abstract

Diesel engine emissions are an important source of ultrafine particulate matter (PM) in both ambient air and many occupational settings. Biodiesel is a popular, ‘green’ alternative to petroleum diesel fuel, but little is known about the impact of ‘real world’ biodiesel combustion on workplace PM concentrations and particle characteristics including size, morphology, and composition; or on biological responses. The objectives of the present work were to characterize PM workplace concentrations and tailpipe emissions produced by the combustion of commercially purchased low sulfur petrodiesel and a waste grease B20 blend (20% biodiesel/80% petrodiesel by volume) in heavy duty diesel (HDD) nonroad equipment operating in a ‘real world’ rural recycling center. Furthermore, we assessed the *in vitro* responses of cell lines representing human lung epithelial cells (BEAS-2B) and macrophages (THP-1) after 24 h of exposure to these real-world particles. Compared to petroleum diesel, use of B20 in HDD equipment resulted in lower mass concentrations of PM_{2.5}, PM_{<0.25} (particle diameter less than 2.5 and 0.25 micrometer, respectively), and elemental carbon. Transmission electron analysis of PM showed that primary particle size and morphology were similar between fuel types. Metals composition analysis revealed differences between fuels, with higher Fe, Al, V, and Se measured during B20 use, and higher As, Cd, Cu, Mn, Ni and Pb concentrations measured during petrodiesel use. *In vitro* responses varied between fuels but data supported that waste grease B20 particles elicited inflammatory responses in human macrophages and lung epithelial cells comparable to petrodiesel particles. However, the effects were more pronounced with B20 than petrodiesel at the same mass concentration. Since the primary particle size and morphology were similar between fuels, it is likely that the differential results seen in the *in vitro* assays points to differences in the composition of the PM. Future research should focus on the organic carbon and metals speciation and potential impact of real world particles on reactive oxygen species generation and mechanisms for differences in the cellular inflammatory responses.

Keywords

Biodiesel; Diesel; Particulate Matter (PM); Elemental Carbon; Organic Carbon; Metals; *In vitro* assays; Transmission Electron Microscope

Introduction

Occupational exposure to diesel engine emissions that contain fine and ultrafine particulate matter (PM) are typically much higher than environmental or ambient levels (Wheatley and Sadhra 2004; Smith et al. 2006; Pronke et al. 2009). Since 2007, on-road diesel engines have been engineered to yield significantly lower emissions, but nonroad engine and vehicle upgrades are not required until after model year 2014 (U.S. EPA 2013a; U.S. EPA 2013b). The Environmental Protection Agency (EPA) has estimated that older nonroad engines have contributed up to 47% of fine PM and 25% of total NO_x (nitrogen oxide) emissions from mobile sources (U.S. EPA 2004). Despite new regulatory requirements, some older diesel engines will continue to be in use for many years because of the long life expectancy of most on-road and nonroad heavy-duty diesel (HDD) engines.

Biodiesel is a renewable fuel that can be blended into existing petroleum diesel fuel supplies, and B20 blends (20% biodiesel/80% petrodiesel, by volume) may be substituted immediately in most diesel engines. Considered more environmentally friendly, biodiesel fuel is biodegradable, reduces greenhouse gas emissions, and has lubricity characteristics that may help extend engine life (NREL 2009). Pure biodiesel (B100) has a higher cloud point than petrodiesel, posing challenges for use during winter weather operations. However, winterized B20 blends have been developed (by the addition of kerosene or other cold flow additives) and used successfully across the U.S. in the coldest climates (NREL 2009). Biodiesel production from a variety of feedstocks, including soy, rapeseed and waste grease, is touted as an important strategy for energy independence and reduced environmental impact from the transportation sector. Despite the common perception that biodiesel fuels are better for the environment and human health, there is limited information about the biological and health effects of 'real world' biodiesel PM collected in occupational settings. Numerous laboratory studies have shown that use of biodiesel instead of petrodiesel fuel reduces emissions of PM, carbon monoxide, and total hydrocarbons in HDD tailpipe exhaust (Bagley et al. 1998; Graboski and McCormick 1998; Durbin et al. 2000; McCormick et al. 2001; Chen and Wu 2002; U.S. EPA 2002b; McCormick et al. 2006; Lapuerta et al. 2007; Robbins et al. 2009; Yanowitz and McCormick 2009). Yet, exposure assessment data from the use of biodiesel blends in real-world operating scenarios is quite limited. Traviss et al. (2012) recently reported a significant (56 to 76%) reduction in the 8 hour average PM_{2.5} (fine PM $\leq 2.5 \mu\text{m}$ in diameter) exposure concentrations after a fuel switch to a B20 soy blend from pure petrodiesel in HDD equipment. These findings suggest that using B20 in non-road equipment may lead to reductions in workplace exposures to PM_{2.5}. However, adverse health effects have been reported at unexpectedly low mass concentrations of fine PM, leading scientists and public health officials to conclude that long-term exposure to vehicle-generated particulate air pollution is a significant environmental risk factor for heart and lung diseases (Grahame and Schlesinger 2009; HEI 2010).

Recent studies evaluating the *in vitro* toxicological responses to PM generated from combustion of petrodiesel and biodiesel fuels in the laboratory have yielded mixed results. Cheung et al. (2010) reported that both soy-based biodiesel and petrodiesel PM elevated oxidative potential measured with the dithiothreitol (DTT) consumption assay. Swanson et al. (2009) reported that the soluble organic fraction (SOF) in soy-based biodiesel PM may be a more potent pro-inflammatory stimulant in human airway epithelial cells than the SOF in petrodiesel PM. In contrast, Hemmingsen et al. (2011) suggested that canola-based biodiesel PM was less toxic to a macrophage cell line (THP-1) than conventional petrodiesel PM when treated with equal mass concentrations. The PM samples in the above referenced studies were collected from engines operating under laboratory conditions or were standard reference samples. Comparing and contrasting responses to ‘real world’ biodiesel exposure scenarios and defining associated health effects and biological responses have been identified as critical research needs (Swanson et al. 2007; Swanson et al. 2009; Madden et al. 2010).

In summary, little is known about the impact of ‘real world’ biodiesel combustion on important particle characteristics (size, morphology, and composition) or about the relationship between human health and exposure to ‘real world’ biodiesel PM. In addition to evaluating the physical and chemical characteristics of PM, the performance of *in vitro* assays is an initial step in the evaluation of the impact of particles on biological responses in a model cell system. One approach is to determine whether biodiesel particles induce inflammatory mediators similar to published data on petrodiesel particles. Increased chemokine/cytokine levels reflect activation of pro-inflammatory processes that may contribute to disease pathogenesis. Previous work studying *in vitro* responses of laboratory-generated petrodiesel particles on cell lines representing human lung epithelial cells (BEAS-2B) and/or macrophages (THP-1) generally indicate increased inflammatory cytokine expression in cells treated with petrodiesel particles (Li et al. 2003; Totland et al. 2010; Gerlofs-Nijland et al. 2013).

The objectives of the present work were to characterize PM workplace concentrations and tailpipe emissions produced by the combustion of commercially purchased low sulfur petrodiesel and a waste grease B20 blend in HDD nonroad equipment operating in a ‘real world’ rural recycling center. Furthermore, we assessed the *in vitro* responses of BEAS-2B and THP-1 cells after 24 h of exposure to these real-world particles collected from HDD equipment. In contrast to earlier work using soy-based B20 (Traviss et al. 2012), the biodiesel fraction in this study was made from waste yellow grease, or used cooking oil, representing a unique feedstock with potential for sustainability. This approach provided an additional novel opportunity to explore if waste grease biodiesel feedstock influenced particle characteristics and *in vitro* responses compared to the more widely available soy-based biodiesel.

Materials and Methods

Field Site Description & Study Design

PM sampling was conducted during the summer of 2009 at the Keene Recycling Center (KRC), a rural municipal materials recovery facility in southwestern New Hampshire. Like

many public works facilities in rural areas, the KRC uses HDD nonroad construction equipment to move materials (trash and recyclables) throughout the site. The site characteristics and study design have been previously described in detail (Traviss et al. 2010 and Traviss et al. 2012). Briefly, the air sampling strategy regarded the KRC nonroad vehicles as pollutant sources for diesel-related PM, with in-cabin measurements over the 8 hour workshift approximating employee exposures. The KRC is a smoke-free worksite eliminating employee smoking as a confounding factor. Although PM was collected from multiple work area locations at the site, the focus of this study is on the PM collected from inside the cabin (near the employee breathing zone – referred to as “in-cabin” concentration) and near the tailpipe exhaust of a model year 2005 John Deere 624J (180hp @ 1800 rpm) front loader (designated as P5B). The same employee operated this equipment for each of the 22 sampling days, and kept both the cabin door and window open throughout the study. The particles used in the cell culture treatments were collected from the tailpipe exhaust of this front loader as described below.

Diesel exposure monitoring was performed while the KRC equipment operated on nonroad quality diesel fuel meeting ASTM D975 standards (sulfur content less than 500 ppm). Yellow grease-based biodiesel (B100) meeting ASTM D6751 quality standards was blended into the site’s main fuel tank to create a B20 (20% biodiesel by volume) blend. Fuel and lube oil composition analyses were not performed. B20-associated PM data collection began two weeks after fuel blending to allow time for the new fuel to transition into use with the equipment.

Exposure monitoring was conducted during 10 days of petrodiesel fuel use and 12 days of B20 fuel use between June and August, 2009. Temperature, relative humidity, wind speed and wind direction data were collected daily to evaluate if there were notable differences between fuel campaigns. Activity patterns for P5B were assessed using multiple approaches. Equipment activity generally consisted of similar repetitive tasks, such as the P5B front loader moving, pushing and lifting garbage off the tipping floor and into a pile. As the front loader did not have real time engine data logging capability, we assessed equipment activity by daily fuel consumption, daily hours of operation, and visual task observations.

Particulate Matter Collection: Sampling and Analysis

Size-segregated PM data ranging from >2.5 μm diameter to <0.25 μm diameter were collected inside the cabin of P5B using Sioutas personal cascade impactors (SKC Inc., Eighty Four, PA) and SKC Leland Legacy pumps (SKC Inc., Eighty Four, PA), calibrated to a flow rate of 9 L/min with a Bios Defender 510 primary calibrator. Each impactor contained (4) 25 mm and (1) 37 mm Teflon (PTFE) filters (SKC # 225-2708 and # 225-1709, respectively) which captured particulate matter across five size ranges. Prior to impactor assembly, filters were equilibrated at a constant temperature and humidity for 24 hours and pre-weighed in a temperature and humidity controlled environment using a Mettler Toledo (Columbus, OH) XP-6 microbalance. Post weighing of the filters was completed in the same manner. Fine particulate matter ($\text{PM}_{2.5}$) was calculated as the sum of the B plate through the afterfilter (AF). $\text{PM}_{>2.5}$ (A plate) was considered “coarse” particulate matter and $\text{PM}_{<0.25}$

(AF) was given the designation “quasi-ultrafine” particulate matter, similar to the categorization approach used by Arhami et al. (2009).

Elemental carbon and organic carbon (EC and OC) samples were collected inside the cabin of P5B using preassembled SKC Diesel Particulate Matter (DPM) quartz filter cassettes (SKC#225-317) and SKC PCXR8 pumps, calibrated to a flow rate of 2 L/min with a Bios Defender 510 primary calibrator. The DPM cassettes used in this study were designed to exclude respirable dust particles > 1.0 μm in diameter. Each cassette contained two quartz filters; all results were corrected for organic adsorbed artifact using the bottom filter. At the end of each sampling day, DPM cassettes were sealed and catalogued for shipment to an external laboratory (NIOSH, Pittsburgh, PA or Wisconsin Occupational Health Laboratory, Madison, WI) for thermo-optical analysis per the NIOSH 5040 method (Birch and Cary 1996).

A subset of Teflon filters from Sioutas impactors located inside the cabin of P5B were sent to the Trace Elements Analysis Core Facility at Dartmouth College for metals analysis by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for the following metals: Al, Fe, V, Cr, Cd, Mn, Ni, Cu, Zn, As, Cd and Pb. These metals were chosen because of their known toxic health effects and/or their association with vehicular emissions (Becker et al. 2005; Chen and Lippmann 2009; Cheung et al. 2010). Filters from two petrodiesel and two B20 days (20 filters total) were selected for metals analysis. Upwind samples (located 50 yards upwind of P5B) collected with Sioutas impactors for the same 2 days were also analyzed to characterize ambient background metals concentrations. Concentrations of metals on the B through AF plates ($\text{PM}_{2.5}$) are reported.

Primary particle size and morphology of the petrodiesel and B20 particles collected from the P5B tailpipe exhaust were compared using a Tecnai F20ST Transmission Electron Microscope (accelerating voltage 200kV). Particles were collected by attaching a 300 mesh copper grid with formvar support film (Electron Microscopy Sciences FF#300-Cu) to the D plate ($d_{50} = 0.25 \mu\text{m}$) of a Sioutas impactor using double backed tape. Samples were collected at a flow rate of 9 L/min for less than 2 minutes within 3 feet of the tailpipe exhaust while the front loader was stationary and idling.

In vitro cell culture experiments: Particles for Cell Culture Treatments

Particles collected directly from the tailpipe exhaust of P5B onto filters during in-field combustion of petrodiesel and waste grease (WG) B20 were used for the biological experiments. Briefly, a Leland Legacy pump pulled 15 L/min of raw exhaust mixed with HEPA-filtered air (at an approximate 1:1 dilution ratio) onto a 47 mm Pallflex Emfab filter contained in a stainless steel filter holder. The high mass loading on the filters enabled particles to be gently scraped off the filter surface into ethanol to a final concentration of 1 mg/ml. The suspension was sonicated for 10 minutes and triturated through a 22-gauge needle to obtain a homogenous suspension prior to use in treatments. THP-1 and BEAS-2B cells were exposed to particles at mass concentrations of 10 and 20 $\mu\text{g/ml}$ (<10% ethanol in the final medium suspension). These treatments are designated as Petro-10, Petro-20, WG-10, and WG-20, indicating the fuel used and dose of particles administered. Controls were exposed to the ethanol/medium suspension without particles. After 24 h of exposure to

the particles, culture medium and total protein from cell lysates were collected for subsequent *in vitro* assays including levels of cytokines/chemokines and Western Blots to assess cell signaling pathways. Details of the procedures for cell culture, cytokine/chemokine assays, and Western Blot analysis may be found in the Electronic Supplementary Material online.

Data Analysis Methods

The PM mass concentration, EC and OC data were not normally distributed so non-parametric statistics (Mann-Whitney U test) were used to compare concentrations between fuels (PASW Statistics 18 Version 18.0, SPSS Inc., Chicago, IL). Results from the *in vitro* experiments were analyzed using ANOVA (Tukey multiple comparison test) with fuel as the grouping variable. Statistical significance was determined at $p = 0.05$.

For the TEM analysis, images were collected on particles selected randomly within the grid at 15,000 $\times$ and 71,000 $\times$ magnifications. Approximately 100 agglomerates per fuel type from the Sioutas D plate ($d_{50} = 0.25$ micrometer) were analyzed for primary particle diameter size and fractal dimension calculations. Over 300 primary particle diameters per fuel type were measured to determine each primary particle size distribution. Public domain Image J software was used to measure the maximum length, $L_{\max}$ of the PM agglomerate, the area of the primary (spherical) particles (A_p) and the projected area of the PM agglomerate (A_c), as illustrated in Figure 1.

Results

Weather & Activity

Overall the weather conditions (temperature, humidity, wind speed) were similar for both fuel campaigns. Wind speed and direction were consistent with the average wind speed on any given field day not exceeding 2 mph. The calm conditions are likely due to the distinct bowl like topography at the site. The only notable difference was in temperature (average temperature of 64° F [petrodiesel] compared to 76° F [B20 fuel], $p < 0.05$). However, we do not believe temperature influenced PM concentrations during the study. Average daily fuel consumption was slightly greater during B20 operation (14.4 (+/- 1.5 SE) vs. 13.8 (+/- 1.5 SE) gallons per day). The 9% lower energy content of biodiesel (on a BTU/lb basis) may account for the higher fuel consumption (NREL 2009). The daily average operating time was 6.3 (+/- 0.3 SE) hours (B20) vs. 4.5 (+/- 0.2 SE) hours (petrodiesel) for P5B and likely also contributed to the difference in fuel consumption. It is unknown why the recycling center operation was busier during the B20 fueling campaign.

Particulate Matter: In-cabin concentrations and tailpipe characterization

In-cabin concentrations—Table 1 summarizes the data obtained from P5B in-cabin monitoring ($n=10$ days for diesel; $n=12$ days for B20). The in-cabin results are not different at 95% confidence. $PM_{2.5}$ mass in-cabin concentrations ranged from 25 to 221 $\mu\text{g}/\text{m}^3$ during petrodiesel operation compared to a range of 31 to 164 $\mu\text{g}/\text{m}^3$ during B20 fueling. After switching to B20, the average $PM_{2.5}$ concentration was 5% lower in the cabin. $PM_{<0.25}$ or “quasi-ultrafine” concentrations during petrodiesel fuel use ranged from 14 to 177 $\mu\text{g}/\text{m}^3$

compared to a range of 9 to 66 $\mu\text{g}/\text{m}^3$ measured during B20 use. After switching to B20, the average $\text{PM}_{<0.25}$ concentration inside the cabin was 43% lower on average compared to petrodiesel fueling days. The level of equipment activity was higher during the B20 sampling period when evaluating activity based on observed hours of engine operation and gallons of fuel consumed over the 8 hour workshift; however, the mean $\text{PM}_{2.5}$ exposure mass concentration was lower during B20 operation. To account for the difference in source activity based on fuel use and hours of equipment operation, we computed PM emissions factors on a per gallon and per hour basis. The average $\text{PM}_{2.5}$ mass measured in the cabin was 24.9 $\mu\text{g}/\text{gal}$ (± 7.0 SE, $n=10$) for petrodiesel compared to 19.0 $\mu\text{g}/\text{gal}$ (± 4.2 SE, $n=12$) during B20 operation. On a time-operation basis, the average $\text{PM}_{2.5}$ mass measured during diesel fueling was 74.0 $\mu\text{g}/\text{hr}$ (± 20.0 SE, $n=10$) compared to 40.3 $\mu\text{g}/\text{hr}$ (± 8.7 SE, $n=12$) during B20 use. These emissions factors do not account for differences in the equipment operating load conditions during either fuel campaign. To evaluate operating intensity, one would have to log engine data, which was not available for this study.

After the switch from diesel to B20, the average elemental carbon exposure concentration in the cabin was 10% lower, but the average organic carbon level was 46% higher. The ratio of EC/OC was 1.04 during petrodiesel operation compared to 0.64 during B20 use, suggesting that PM composition is different between fuels.

TEM analysis—PM size and morphology per fuel type were determined using the TEM images. Circles were drawn around primary particles with clear boundaries within an agglomerate and the average primary particle diameter, d_p , was calculated using the average projected area, A_p , per Equation 1 below. Results for d_p are presented in Figures 2a and 2b. The primary particle diameters ranged from 4.4 to 23.0 nm for petrodiesel fueling compared to 4.7 to 23.7 nm for B20 fueling. There was little difference in primary particle diameter between fuel types.

$$d_p = \sqrt{\frac{4A_p}{\pi}} \quad \text{Equation 1}$$

The number of primary particles per agglomerate, N , was calculated using Equation 2, where K_a represents an empirical constant and α represents an empirical coefficient (Oh and Sorensen 1997; Mustafi and Raine 2009).

$$N = K_a \left(\frac{A_c}{A_p} \right)^\alpha \quad \text{Equation 2}$$

For this study, values of 1.35 and 1.1 were selected for K_a and α , respectively, based on literature values for diesel engine operation at conditions other than high fueling/heavy load and considering the overlap of the primary particle spheres in the typical agglomerate (Oh and Sorensen 1997; Mustafi and Raine 2009). After N was determined per Equation 2, the fractal dimension, D_{FL} , was calculated by solving for the slope of the $\ln(N)$ versus $\ln(L_{\max}/d_p)$ plot (Mustafi and Raine 2009).

Plots of $\ln(N)$ versus $\ln(L_{\max}/d_p)$ for the agglomerates are presented in Figures 3a and 3b. Fractal dimension values were quite similar between fuel types, with values of 1.63 and 1.61 determined for petrodiesel and B20 fueling, respectively. Next, the average agglomerate diameter of gyration, d_g , and the fractal prefactor, k_f , were determined per the steps outlined in Mustafi (2008). The fractal prefactor is considered another characteristic of particle morphology, whereby a smaller k_f value suggests more elongated particulates (Neer and Koylu 2006).

The average agglomerate diameter of gyration, d_g was smaller during B20 fueling, 99 nm compared to 109 nm during diesel use. The values for the fractal prefactor, k_f , were likewise similar between fuels. The average primary particle diameters and the agglomerate gyration diameters per fuel type are summarized in Table 2.

Metals—Figures 4, 5 and 6 display metals concentrations in $PM_{2.5}$ (in ng/mg PM) for Fe, Al, Cr, Mn, Cu, Ni, V, As, Cd, Se and Pb measured in the cabin during both B20 and petrodiesel days of operation. It should be noted that Cr levels were consistently found to be higher in the upwind samples for both fuel types, suggesting another potential local source for this metal. The concentrations of all other metals in upwind samples were either lower than the in-cabin levels or below detection limits, indicating the HDD equipment as the major source of metals in $PM_{2.5}$. Higher concentrations for Fe, Al, V, and Se were measured during B20 use, and higher As, Cd, Cu, Mn, Ni and Pb concentrations were noted during petrodiesel use. The highest metal concentration on a ng/mg PM basis was observed for Fe, averaging 13893 ng/mg during petrodiesel fueling and 15951 ng/mg during B20 use. Concentration of Cu was 2310 ng/mg PM for petrodiesel compared to 2012 ng/mg PM for B20 use; Ni was 165 ng/mg for diesel vs. 128 ng/mg for B20. Higher levels of V and Se were observed during B20 use compared to diesel (21 vs. 16 ng/mg and 2 vs. 1 ng/mg, respectively) which was surprising as V and Se are associated with petroleum sources not biodiesel. However, the petroleum diesel fuel and B100 were hand blended at the tank to create the B20 mixture, and this process was repeated for new petroleum shipments during the summer sampling period using the same B100 source. It's possible the composition of the commercial petroleum diesel base fuel was slightly different between shipments and between petrodiesel vs. B20 sampling campaigns.

Comparing the metals composition of $PM_{2.5}$ in exposure units of concentration, ng/m^3 , gives slightly different results. This is likely because the average total $PM_{2.5}$ mass concentration (for the two day subset of filters per fuel type that was analyzed for metals) was higher during B20 operation: 131 $\mu g/m^3$ for B20 compared to 97 $\mu g/m^3$ for petrodiesel. Correspondingly, most concentrations of metals measured in ng/m^3 were higher during B20 use. Of note, Fe concentration values were highest at 2180 ng/m^3 for B20 vs 954 ng/m^3 for petrodiesel followed by Cu values of 229 ng/m^3 (B20) vs. 202 ng/m^3 (petrodiesel). Ni was measured at 16 ng/m^3 (B20) compared to 12 ng/m^3 (petrodiesel). The total mass of metals analyzed in this study contributed about 3 to 4% of total $PM_{2.5}$ mass for both fuel types; much of this mass contribution was from Al and Fe, likely from fine resuspended dust, though Fe is also associated with engine wear (Cheung et al. 2009).

In Vitro Cell Responses to Exposure to PM

Pro-inflammatory Cytokines/Chemokines—A total of 27 cytokines/chemokines (see kit information for details) were screened to determine responsiveness of the cells to petrodiesel and B20 particles. Many of the readings were not detectable so only those with significant changes or with clear trends are shown in the results.

THP-1 cells: These cells represent the macrophage that is often considered the sentinel cell in the immune response because they are typically the first to encounter pathogens or inhaled particulates. Cytokines/chemokines were measured to determine the pro-inflammatory responses elicited by PM exposure. As shown in Figure 7, Tumor-necrosis Factor alpha (TNF- α) increased at both WG PM concentrations although only the WG-20 treatment was significantly greater than the control. Petrodiesel PM at both concentrations had minimal effects on TNF- α release. IL-8 significantly increased after Petro-10, Petro-20 and WG-10 treatments, but not after the WG-20 treatment. Macrophage inflammatory protein-1 α (MIP-1 α) significantly increased after all particle treatments. As determined by post-hoc analysis, the WG-10 treatment had a greater MIP-1 α response than the Petro-10 treatment and the WG-20 treatment also had a greater response than the Petro-20. No statistically significant differences were found in the levels of IL-1 β compared to the control.

BEAS-2B cells (lung epithelial cells): As shown in Figure 7, BEAS-2B cells responded to particle treatments by differentially secreting IL-6 and IL-8 into the culture medium. Particles from combustion of both fuels induced IL-6. The WG-10 effect on IL-6 was greater than the Petro-10 effect ($p < 0.05$). There was no statistically significant difference between the WG-20 and Petro-20 treatments on IL-6. IL-8 was secreted primarily in response to Petro-10 and Petro-20 particles. The IL-8 response to WG-10 and WG-20 particles was not significantly different than the control. The Petro-20 effect on IL-8 was significantly greater than the WG-20 effect ($p < 0.05$).

Western blot Analyses

THP-1 cells: Involvement of Toll-like Receptor 2 (TLR2) and TLR4 in inflammatory immune responses induced by PM and DEP has been reported in alveolar macrophages (Shoenfelt et al. 2009). Expression of TLR2 appeared to be slightly higher in WG-treated THP-1 cells compared with untreated control (e.g. Petro-20 increased 1.6 ± 0.26 fold over control) but differences were not statistically significant (Figure 8). No TLR4 response could be detected.

BEAS-2B cells: PM or DEP have been suggested to upregulate the expression of the epidermal growth factor receptor (EGFR) and extracellular signal-regulated kinase (ERK) pathways and sustain the proinflammatory response in human bronchial epithelial cells (Blanchet et al. 2004). We examined activation of EGFR and ERKs in WG PM treated BEAS-2B cells compared with petrodiesel, and found that phosphorylated ERK1 and 2 was increased in all particle-treated cells, especially WG10 ($p < 0.05$); phosphorylation of EGFR at T1068 was not significant, as seen in Figure 8.

These biological data support the notion that particles from WG combustion have a pro-inflammatory effect on exposed cells comparable to petrodiesel and with possibly greater effects at the same mass concentration.

Discussion

In this work, PM generated by use of petrodiesel fuel and B20 (waste grease feedstock) in a non road heavy duty engine operating under real world conditions was evaluated for workplace concentrations and biological responses in two cell lines. We characterized the PM collected inside the cabin near the operator breathing zone by measuring size segregated gravimetric mass ($PM_{2.5}$ and $PM_{<0.25}$), EC, OC, and metals concentrations. We also conducted morphological analysis of PM collected at the tailpipe from the same piece of equipment. We assumed that tailpipe emissions were the major contributor to the PM measured in-cabin, a reasonable assumption due to the type of work the front loader performed, essentially pushing or lifting loads, but consistently moving back and forth through its own exhaust plume. Other biodiesel emissions studies have concluded metals emitted in the exhaust are mainly from a fuel source (Dwivedi et al. 2006). However, we note that vehicle related PM and associated metals such as Fe, Pb, and Cu may also be impacted by lubrication oil, tire and brake emissions (HEI 2011). In addition, due to changes in PM from dilution and aging as PM moves from the tailpipe to the equipment cabin, future work should examine potential differences in composition and morphology between tailpipe and in-cabin PM, as well as analyze the source fuel for metals.

Tailpipe PM primary particle size and morphology were similar between fuels. The primary particle diameter (~10 nm size range) for B20 and diesel was smaller compared to the primary particle diameters (in the 20 to 35 nm size range) reported for diesel soot by other researchers (Park et al 2004; Chung et al. 2008; Mustafi and Raine 2009). The smaller primary particle diameter could be a function of the larger nonroad type engine and different fuel configurations used in this study compared to the above referenced work. The average PM agglomerate size (represented by the gyration diameter, d_g) for both fuels was similar to Mustafi (2008), though the latter reports a wider range (120 to 270 nm) likely due to the examination of multiple engine loads and fuels. We note that this study only examined particles collected during idle mode, and that operating under load would likely change particle characteristics. For example, Mustafi (2008) found the average d_g for diesel PM was highest during high load conditions.

The fractal dimension values in this study were similar between diesel and B20 and were comparable to other literature reports of diesel soot fractal dimension values ranging from 1.61 to 1.88 (Park et al. (2004); Chung et al. 2008; Mustafi and Raine (2009)). Smaller fractal dimension values are associated with more chain-like, irregular agglomerates (Mustafi and Raine 2009), and our results were in the smaller range of values reported in the literature, indicating an increase in chain-like agglomerates. This type of particle would likely have increased surface area for adsorption of chemical components.

While the tailpipe PM size and morphology were similar between fuel types, the in-cabin PM mass concentrations were lower during B20 fueling, even when normalizing for the

higher fuel consumption and hours of operation during B20 operations. We note the reduction in $PM_{2.5}$ mass concentration from B20 use was not statistically significant in this study compared to previous work (Traviss et al. 2012). The average EC concentration was higher during petrodiesel fueling, and the average OC concentration was higher during B20 use; the EC/OC ratio was 1.04 during petrodiesel use and 0.64 during B20 use. Our prior study at this same field site determined an average EC/OC ratio across multiple sampling locations of 1.29 [petrodiesel] compared to 0.21 [B20] (Traviss et al. 2012). However, EC/OC concentrations were not measured at P5B during the earlier study as that vehicle was not part of the City of Keene fleet at the time. Comparing our EC/OC results to the literature is difficult due to the use of different engine types, fuel combinations, operating modes and our measurement of workplace concentrations vs. tailpipe emissions. Generally, biodiesel operation results in a lower EC/OC ratio compared to petroleum diesel fueling, which is consistent with our results. Use of biodiesel and ULSD blends in a 56 hp nonroad engine operated in a simulated underground environment found that the OC increased with increasing fraction of biodiesel in the fuel, with EC/OC ratios of 1.56 (lower load) during ULSD fueling compared to 0.69 (lower load) during B50 fueling (Bugarski et al. 2010). Chung et al. (2008) also determined that biodiesel use decreased the EC/OC ratio in particulate emissions from a small nonroad diesel engine, with the EC/OC ratio ranging from 0.5 (diesel, low load) and 0.09 (biodiesel, low load) to 3.8 (diesel, maximum load) and 3.0 (biodiesel, maximum load). Finally, Magara-Gomez et al. (2012) determined the highest OC emissions from B100 use in a tractor engine, which was attributed to unburned fatty acid methyl esters.

Differences were noted in $PM_{2.5}$ metals concentrations between fuel types. Higher in-cabin concentrations for Fe, Al, V, and Se (on a ng/mg PM basis) were measured during B20 use, and higher As, Cd, Cu, Mn, Ni and Pb concentrations were noted during petrodiesel use. Comparing our results to the literature, Betha and Balasubramanian (2011) found higher levels of Fe, Cu, Ba and Ni in B100 exhaust (waste grease feedstock), and higher levels of Al, Mn, As, Cd, and Pb in ultra low sulfur diesel exhaust. Conversely, Gangwar et al. (2011) found higher levels of Mn and Pb in B20 exhaust from karanja oil, and values of Fe were not above detection limits for either fuel.

From a human exposure perspective, emerging evidence exists that inhalation of some metals such as Ni, V and Pb at concentrations not much higher than ambient levels is associated with adverse health effects (Chen and Lippman 2009). Comparing the in-cabin $PM_{2.5}$ metals concentrations to ambient levels of $PM_{2.5}$ metals measured in urban areas by the EPA (Chen and Lippmann 2009), concentrations of metals were highly elevated at the KRC compared to the mean ambient $PM_{2.5}$ metals data in the northeastern cities of Burlington VT and Philadelphia, PA. For example, the mean workplace concentration of Fe during the B20 campaign was 26 times higher than the mean concentration of 84 ng/m³ reported for Philadelphia PA and 53 times higher than the mean concentration of 41 ng/m³ for Burlington, VT. Cu levels during both fuel campaigns were about 10 times higher in the workplace compared to levels reported in northeastern cities. Pb, Ni and Mn were elevated in the workplace compared to various urban areas, ranging from 2 to 6 times higher than ambient concentrations. With only two days of data per fuel type, it is difficult to draw conclusions about differences in metals concentrations between fuels. Our results emphasize

the need for ongoing exposure assessment research in microenvironments such as workplaces as these microenvironments may experience highly elevated metals concentrations compared to more frequently studied urban areas.

A unique aspect of this work is the determination of *in vitro* cell responses to particles collected from in use HDD engines, in addition to the use of waste grease as the feedstock for the B20 fuel. The 4 pro-inflammatory cytokines (IL-6, IL-8, TNF- α and MIP-1 α) measured in this study have been reported in PM- and diesel exhaust particles (DEP)-exposed cells or animals (Arimoto et al. 2007; Sawyer et al. 2010; Levesque et al. 2011; McConnell et al. 2013). The present work is the first to show that WG-derived B20 particles also induced these responses. Dose response effects were noted for some of the cytokines, such as in TNF- α (THP-1) and IL-6 (BEAS-2B), but not seen in others, like IL-8 (THP-1). This is not unusual in PM-treated cells because mass concentration is only one way to induce responses (Steenhof et al. 2013). Surface properties may play a more important role than mass concentration in ultrafine particle toxicity. Unfortunately, treating cells with the same mass concentration in PM suspensions may not necessarily assure uniformity in the particle size and surface area distribution.

The data demonstrate that exhaust particles collected from in-field combustion of waste grease B20 elicits different patterns of inflammatory responses in human macrophages and lung epithelial cells when compared to PM generated by petrodiesel combustion. Variable responses were also seen in the activation of pro-inflammatory signaling pathways. Since the primary particle size and morphology are similar between fuels, it is likely the differential results seen in the *in vitro* assays points to differences in the composition of the PM, as suggested by others (Hemmingsen et al. 2011).

Additionally, both B20 and petrodiesel particles collected in the cabin contained elevated levels of transition metals Fe, Cu and Ni. These transition metals are associated with generation of reactive oxygen species (ROS) and/or cellular inflammatory effects (Chen and Lippmann 2009; Cheung et al. 2010; Jalava et al. 2010 and Schwarze et al. 2010). Further analysis of ROS generation, as well as speciation of the organic composition of the PM, is desirable to better understand the results of this study. In summary, different cell signaling pathways and subsequent inflammatory responses appear to be activated by the two fuel types, suggesting that the type and pathogenesis of disease will be dependent on fuel source, PM composition, as well as the cell types exposed.

Conclusion

The national interest in biodiesel from various feedstocks emphasizes the need to study health effects associated with exposure to real world particles. This study provides important novel data on real-world PM workplace concentrations, particle morphology and *in vitro* responses to particles collected from nonroad heavy duty diesel engines fueled by petrodiesel and waste grease biodiesel. Understanding PM composition and toxicological effects resulting from biodiesel blend combustion is an important area for future research as biodiesel feedstocks and engine configurations evolve and there is sustained national interest in biofuel production as a means to achieve independence from fossil fuels. The present

work suggests that PM from B20 combustion in a workplace setting elicits differential inflammatory responses than petrodiesel PM and additional data are needed to further examine these differences, such as the impact of biodiesel/petrodiesel PM on ROS. Finally, future work should consider the real-world combustion of biodiesel in higher blend percentages (>20%), to determine if the increased level of biodiesel in is an important factor in PM composition and associated biological response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Steve Russell, Duncan Watson, the City of Keene, New Hampshire, the staff of the Keene Recycling Center, and the Keene State College Safety Studies and Environmental Studies students and staff who directly supported this work. The following Keene State students assisted with field data collection: Kyle Barnett, Alissa Couturier, Greg Goupil, Ryan Hall, Daniel O'Brien and Ben Lazich. We also thank Dr. Joseph Helble, Thayer School of Engineering, Dartmouth College, for advice regarding the TEM analysis and Dr. Charles Daghljan, Dartmouth College, for assistance in using the TEM. Research reported in this publication was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20-GM103413 as well as National Institute of Environmental Health Sciences grant number 1R21ES021417.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, National Institute of General Medical Sciences or the National Institute of Environmental Health Sciences.

References

- Arhami M, Sillanpaa M, Hu S, Olson M, Schauer JJ, Sioutas C. Size-segregated inorganic and organic components of PM in the communities of the Los Angeles harbor. *Aerosol Science and Technology*. 2009; 43(2):145–160.
- Arimoto T, Takano H, Inoue K, Yanagisawa R, Yoshino S, Yamaki K, Yoshilawa T. Pulmonary exposure to diesel exhaust particle components enhances circulatory chemokines during lung inflammation. *Int J Immunopathol Pharmacol*. 2007; 20(1):197–201. [PubMed: 17346445]
- Bagley ST, Gratz LD, Johnson JH, McDonald J. Effects of oxidation catalytic converter and a biodiesel fuel on the chemical, mutagenic, and particle size characteristics of emissions from a diesel engine. *Environ Sci Technol*. 1998; 32(9):1183–1191.
- Becker S, Dailey LA, Soukup JM, Grambow SC, Devlin RB, Huang YC. Seasonal variations in air pollution particle induced inflammatory mediator release and oxidative stress. *Environmental Health Perspectives*. 2005; 113(8):1032–1038. [PubMed: 16079075]
- Betha R, Balasubramanian R. Emissions of particulate-bound elements from stationary diesel engine: characterization and risk assessment. *Atmospheric Environment*. 2011; 45:5273–5281.
- Betha R, Balasubramanian R. Particulate emissions from a stationary engine fueled with ultra-low-sulfur diesel and waste-cooking-oil-derived biodiesel. *Air & Waste Management Association*. 2011; 61:1063–1069.
- Birch M, Cary R. Elemental carbon-based method for monitoring occupational exposures to particulate diesel exhaust. *Aerosol Science and Technology*. 1996; 25:221–241.
- Blanchet S, Ramgolam K, Baulig A, Marano F, Baeza-Squiban A. Fine particulate matter induces amphiregulin secretion by bronchial epithelial cells. *Am J Respir Cell Mol Biol*. 2004; 30(4):421–427. [PubMed: 14701705]
- Bugarski AD, Cauda EG, Janisko SJ, Hummer JA, Patts LD. Aerosols emitted in underground mine air by diesel engine fueled with biodiesel. *J Air Waste Manag Assoc*. 2010; 60:237–244. [PubMed: 20222537]

- Chen LC, Morton L. Effects of metals within ambient air particulate matter (PM) on human health. *Inhalation Toxicology*. 2009; 21:1–31.
- Chen YC, Wu CH. Emissions of submicron particles from a direct injection diesel engine by using biodiesel. *J Environ Sci Health*. 2002; 37(5):829–43.
- Cheung KL, Ntziachristos L, Tzamkiozis T, Schauer JJ, Samaras Z, Moore KF, Sioutas C. Emissions of particulate trace elements, metals and organic species from gasoline diesel and biodiesel passenger vehicles and their relation to oxidative potential. *Aerosol Science and Technology*. 2010; 44(7):500–513.
- Chung A, Lall AA, Paulson SE. Particulate emissions by a small non-road diesel engine: Biodiesel and diesel characterization and mass measurements using the extended idealized aggregates theory. *Atmospheric Environment*. 2008; 42:2129–2140.
- Durbin TD, Collins JR, Norbeck JM, Smith MR. Effects of biodiesel, biodiesel blends, and a synthetic diesel on emissions from light heavy-duty diesel vehicles. *Environ Sci Technol*. 2000; 34(3):349–355.
- Dwivedi D, Agarwal AK, Sharma M. Particulate emission characterization of biodiesel vs diesel-fueled compression ignition transport engine: a comparative study. *Atmospheric Environment*. 2006; 40:5586–5595.
- Gangwar J, Gupta T, Gupta S, Agarwal AK. Emissions from diesel versus biodiesel fuel used in a CRDI SUV engine: PM mass and chemical composition. *Inhalation Toxicology*. 2011; 23:449–458. [PubMed: 21689006]
- Gerlofs-Nijland ME, Totlandsdal AI, Tzamkiozis T, Leseman DL, Samaras Z, Lag M, Schwarze P, Ntziachristos L, Cassee FR. Cell toxicity and oxidative potential of engine exhaust particles: impact of using particulate filter or biodiesel fuel blend. *Environ Sci Technol*. 2013; 47(11):5931–5938. [PubMed: 23597117]
- Graboski MS, McCormick RL. Combustion of fat and vegetable oil derived fuels in diesel engines. *Prog Energy Combust Sci*. 1998; 24:125–164.
- Graham TJ, Schlesinger RB. Cardiovascular health and particulate vehicular emissions: a critical evaluation of the evidence. *Air Qual Atmos Health*. 2010; 3(1):3–27. [PubMed: 20376169]
- HEI. Traffic-related air pollution: A critical review of the literature on emissions, exposure, and health effects. Special Report. 2010; 17
- HEI. The future of vehicle fuels and technologies: anticipating health benefits and challenges. Communication. 2011; 16
- Hemmingsen JG, Møller P, Nøjgaard JK, Roursgaard M, Loft S. Oxidative stress, genotoxicity, and vascular cell adhesion molecule expression in cells exposed to particulate matter from combustion of conventional diesel and methyl ester biodiesel blends. *Environmental Science and Technology*. 2011; 45:8545–8551. [PubMed: 21842833]
- Jalava PI, Tapanainen M, Kuusalo K, Markkanen A, Hakulinen P, Happonen MS, Pennanen AS, Ihalainen M, Yil-Pirilä P, Makkonen U, Teinilä K, Mäki-Paakkanen J, Salonen RO, Jokiniemi J, Hirvonen MR. Toxicological effects of emission particles from fossil- and biodiesel-fueled diesel engine with and without DOC/POC catalytic converter. *Inhalation Toxicology*. 2010; 22(S2):48–58. [PubMed: 21029031]
- Lapuerta M, Armas O, Rodríguez-Fernández J. Effect of biodiesel fuels on diesel engine emissions. *Progress Energy Combust Sci*. 2007; 34(2):198–223.
- Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M, Oberley T, Froines J, Nel A. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environmental Health Perspectives*. 2003; 111:455–460. [PubMed: 12676598]
- Levesque S, Taetzsch T, Lull ME, Kodavanti U, Stadler K, Wagner A, Johnson JA, Duke L, Kodavanti P, Surace MJ, Block ML. Diesel exhaust activates and primes microglia: air pollution, neuroinflammation, and regulation of dopaminergic neurotoxicity. *Environ Health Perspect*. 2011; 119(8):1149–1155. [PubMed: 21561831]
- Madden, MC., Bhavaraju, L., Kodavanti, UP. Toxicology of Biodiesel Combustion Products. Biodiesel- Quality, Emissions and By-products. In: Montero, G., editor. Intech; Available from: <http://www.intechopen.com/books/biodiesel-quality-emissions-and-by-products>

- Magara-Gomez A, et al. Sensitivity of Diesel Particulate Material Emissions and Composition to Blends of Petroleum Diesel and Biodiesel Fuel. *Aerosol Science and Technology*. 2012; 46(10): 1109–1118.
- Martinon F, Burns K, Tschopp J. The Inflammasome: A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL- β . *Molecular Cell*. 2002; 10(2):417–426. [PubMed: 12191486]
- McConnell R, Wu W, Berthane K, Liu L, Verma G, Peden D, Diaz-Sanchez D, Fruin S. Inflammatory cytokine response to ambient particles varies due to field collection procedures. *Am J Respir Cell Mol Biol*. 2013; 48(4):497–502. [PubMed: 23306836]
- McCormick RL, Graboski MS, Alleman TL, Herring AM, Tyson KS. Impact of biodiesel source material and chemical structure on emissions of criteria pollutants from a heavy-duty engine. *Environ Sci Technol*. 2001; 35(9):1742–1747. [PubMed: 11355187]
- McCormick, RL., Williams, A., Ireland, J., Brimhall, M., Hayes, RR. Effects of biodiesel blends on vehicle emissions, NREL/MP-540-40554. National Renewable Energy Laboratory; Golden, CO: 2006.
- Mustafi, NN. Particle emissions of a dual fuel engine. Auckland: University of Auckland; 2008. Department of Mechanical Engineering PhD: 272
- Mustafi NN, Raine RR. Electron microscopy investigation of particulate matter from a dual fuel engine. *Aerosol Science and Technology*. 2009; 43:951–960.
- National Renewable Energy Laboratory (NREL). Biodiesel Handling and Use Guide (4th). 2009NREL/TP-540-43672
- Neer A, Koylu UO. Effect of operating conditions on the size, morphology, and concentration of submicrometer particulates emitted from a diesel engine. *Combustion and Flame*. 2006; 146:142–154.
- Park K, Kittleson DB, McMurry PH. Structural properties of diesel exhaust particles measured by transmission electron microscopy (TEM): Relationships to particle mass and mobility. *Aerosol Science and Technology*. 2004; 38:881–889.
- Oh C, Sorensen CM. The effect of overlap between monomers on the determination of fractal cluster morphology. *Journal of Colloid and Interface Science*. 1997; 193:17–25. [PubMed: 9299084]
- Pronk A, Coble J, Stewart PA. Occupational exposure to diesel engine exhaust: A literature review. *Journal of Exposure Science and Environmental Epidemiology*. 2009; 19:443–457. [PubMed: 19277070]
- Robbins C, Hoekman SK, Gertier A, Broch A. Biodistillate transportation fuels 2. – emissions impacts. 2009SAE paper 2009-01-2724
- Sawyer K, Mundandhara S, Ghio AJ, Madden MC. The effects of ambient particulate matter on human alveolar macrophage oxidative and inflammatory responses. *J Toxicol Environ Health A*. 2010; 73(1):41–57. [PubMed: 19953419]
- Schwarze PE, Totlandsdal AI, et al. Importance of sources and components of particulate air pollution for cardio-pulmonary inflammatory responses. *Air Pollution*. 2010:47–74.
- Shoenfelt J, Mitkus RJ, Zeisler R, Spatz RO, Powell J, Fenton MJ, Squibb KA, Mevedev AE. Involvement of TLR2 and TLR4 in inflammatory immune responses induced by fine and coarse ambient air particulate matter. *J Leukoc Biol*. 2009; 86(2):303–312. [PubMed: 19406832]
- Smith TJ, Davis ME, Reaser P, Natkin J, Hart JE, Laden F, Heff A, Garshick E. Overview of particulate exposures in the US trucking industry. *J Environ Monit*. 2006; 8(7):711–720. [PubMed: 16826284]
- Steenhof M, Mudway IS, Gosens I, Hoek G, Godri KJ, Kelly FJ, Harrison RM, Pieters RH, Cassee FR, Lebret E, Brunekreef BA, Strak M, Janssen NA. Acute nasal pro-inflammatory response to air pollution depends on characteristics other than particle mass concentration or oxidative potential: the RAPTES project. *Occup Environ Med*. 2013; 70(5):341–348. [PubMed: 23428835]
- Swanson KJ, Madden MC, Ghio AJ. Biodiesel exhaust: the need for health effects research. *Environ Health Perspectives*. 2007; 115(4):496–499.
- Swanson KJ, Kado NY, Funk WE, Pleil JD, Madden MC, Ghio AJ. Release of the pro-inflammatory markers by BEAS-2B cells following in vitro exposure to biodiesel extracts. *Open Toxicol J*. 2009; 3:8–15.

- Totlandsdal AI, Cassee FR, Schwarze P, Refsnes M, Låg M. Diesel exhaust particles induce CYP1A1 and pro-inflammatory responses via differential pathways in human bronchial epithelial cells. *Particle and Fibre Toxicology*. 2010; 7:41. [PubMed: 21162728]
- Traviss N, Thelen BA, Ingalls JK, Treadwell MD. Biodiesel versus diesel: A pilot study comparing exhaust exposures for employees at a rural municipal facility. *J Air Waste Manag Assoc*. 2010; 60:1026–1033. [PubMed: 20863048]
- Traviss N, Thelen BA, Ingalls JK, Treadwell MD. Evaluation of biodiesel's impact on real-world occupational and environmental particulate matter exposures at a municipal facility in Keene, NH. *Air Qual Atmos Health*. 2012; 5:101–114.
- U.S. EPA. Control of Emissions from New and In-Use Highway Vehicles and Engines, 40 CFR Part 86. 2013a
- U.S. EPA. Control of Emissions from New and In-Use Nonroad Compression-Ignition Engines, 40 CFR Part 1039. 2013b
- U.S. EPA. A comprehensive analysis of biodiesel impacts on exhaust emissions, EPA420-P-02-001. 2002b. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/20021001_gen-323.pdf. Accessed 1 June 2010
- U.S. EPA. Clean air nonroad diesel rule, EPA420-F-04-032. 2004
- Wheatley AD, Sadhra S. Occupational exposure to diesel exhaust fumes. *Ann Occup Hyg*. 2004; 48(4):369–376. [PubMed: 15148050]
- Yanowitz J, McCormick R. Effect of biodiesel blends on North American heavy-duty diesel engine emissions. *Eur J Lipid Sci Technol*. 2009; 111(7):763–772.

Fig 1. TEM micrograph showing the parameters measured: the maximum length of the PM agglomerate (L_{max}), the projected area of the primary particle (A_p) and the projected area of the PM agglomerate (A_c).

Fig 2a. Distribution of primary particle diameters, d_p for tailpipe PM (petrodiesel fueling), shown with fitted normal distribution curve.

Fig 2b.
Distribution of primary particle diameters, d_p for tailpipe PM (biodiesel (B20) fueling), shown with fitted normal distribution curve.

Fig 3a.

Plot of $\ln(N)$ versus $\ln(L_{\text{max}}/d_p)$ for petrodiesel PM agglomerates, with fractal dimension represented by the slope. $N=1.4 (L_{\text{max}}/d_p)^{1.63}$

Fig 3b.
Plot of $\ln(N)$ versus $\ln(L_{\max}/d_p)$ for B20 PM agglomerates, with fractal dimension represented by the slope. $N=1.5 (L_{\max}/d_p)^{1.61}$

Fig 4. Metals concentration of Al, Fe, and Cu in PM_{2.5} (per fuel type) in ng/per mg PM (arithmetic mean $\pm$ SE).

Fig 5. Metals concentration of Cr, Mn, Ni and Pb in PM_{2.5} (per fuel type), in ng/per mg PM (arithmetic mean $\pm$ SE).

Fig 6. Metals concentration of V, As, Se and Cd in PM_{2.5} (per fuel type), in ng/mg PM (arithmetic mean $\pm$ SE).

Fig 7.
Observed cytokine responses to petrodiesel and WG (B20) particles in THP-1 (left) and BEAS-2B cells (right). * denotes significance at p=0.05 compared to control.

Fig 8.
Activation of pro-inflammatory pathways in response to petrodiesel and WG (B20) particles.

Table 1

Summary statistics for P5B (“in-cabin” concentrations), the large front loader used at the Keene Recycling Center. Fine particulate matter (PM_{2.5}), quasi ultrafine (PM_{<0.25}), elemental carbon, and organic carbon mass concentrations are measured in µg/m³. PM_{2.5} mass is also expressed as emission factors based on fuel usage (µg/gal) and time operation of equipment (µg/hr). There were no significant differences between fuel types.

Large Front Loader (“P5B”) 2005 John Deere 624J, 180 hp @1800 rpm	Petrodiesel (n=10)	B20 (n=12)
	<i>Exposure (“in cabin”)</i>	<i>Exposure (“in cabin”)</i>
PM _{2.5} (µg/m ³) (arithmetic mean ± SE)	77.2 ± 19.0	73.1 ± 11.2
PM _{<0.25} (µg/m ³) (arithmetic mean ± SE)	54.5 ± 21.1	31.1 ± 5.3
PM _{2.5} (µg/gal) (arithmetic mean ± SE)	24.9 ± 7.0	19.0 ± 4.2
PM _{2.5} (µg/hr) (arithmetic mean ± SE)	74.0 ± 20.0	40.3 ± 8.7
EC (µg/m ³) (arithmetic mean ± SE)	7.0 ± 0.9	6.3 ± 1.0
OC (µg/m ³) (arithmetic mean ± SE)	6.7 ± 1.9	9.8 ± 4.2
EC/OC ratio	1.04	0.64

Table 2

Summary of TEM analysis of particles collected at the tailpipe exhaust. The differences in d_p and d_g were not significant at 95% confidence.

Large Front Loader (“P5B”) 2005 John Deere 624J	<i>Petrodiesel</i>	<i>B20</i>
Primary particle diameter, d_p (nm) (arithmetic mean $\pm$ SE)	10.8 ± 3.8	10.7 ± 3.2
d_g , diameter of gyration (nm) (arithmetic mean $\pm$ SE)	109 ± 16.1	99 ± 9.7
Fractal dimension	1.63	1.61
Fractal prefactor, k_f	2.60	2.68