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Characterization and Comparison of Oxidative Potential of Real-World Biodiesel and Petroleum Diesel Particulate Matter emitted from a Nonroad Heavy Duty Diesel Engine

Nathan R. Martin¹, Patrick Kelley^{1,2}, Rachel Klaski^{1,3}, Andrew Bosco^{1,3}, Brian Moore^{1,2,3}, Nora Traviss^{1,*}

¹Department of Environmental Studies, Keene State College, Keene NH, 03431

²Department of Chemistry, Keene State College, Keene NH, 03431

³Department of Biology, Keene State College, Keene NH, 03431

Abstract

Little is known regarding the oxidative potential of biodiesel particulate matter (PM) relative to diesel PM emitted from heavy duty diesel (HDD) nonroad engines generated in real-world occupational settings. The composition of biodiesel and diesel PM can include transition metals, polar, and nonpolar organic species which can increase oxidative potential via production of reactive oxygen species (ROS). Elevated ROS can lead to oxidative stress and induce antioxidant defense, inflammation, and toxicity. This study characterized the chemical composition of PM (water soluble organic carbon and elemental metals) collected in a real-world occupational setting. ROS production in a human epithelial cell line (BEAS-2B) treated with biodiesel and diesel PM extracts was compared to oxidative potential measured by an acellular dithiothreitol (DTT) assay. The oxidative potential (DTT consumption rate) of diesel PM was 21% greater than biodiesel PM at the highest treatment concentration (60 µg/mL), yet the ROS generated in vitro were similar between fuel types. Average concentrations of Cu, Cr and Zn were higher in diesel PM compared to biodiesel PM. Additionally, there was a significant correlation between DTT consumption and Cu in diesel PM ($r=0.98$), but not B20 PM. There was a strong correlation between WSOC content in diesel PM and ROS generated in vitro ($r=0.83$), but no correlation between WSOC content in biodiesel PM and ROS. Taken together, the results indicate the influence of fuel type on the chemical composition and oxidative potential of PM generated by a nonroad HDD engine operated at a recycling center. While acknowledging the potential influence of other species of interest not measured (i.e., quinones), real-world petroleum diesel PM emissions had higher oxidative potential compared to biodiesel PM suggesting that biodiesel use may reduce risk to human health.

*Corresponding author. Department of Environmental Studies, Keene State College, Keene NH, 03435. ntraviss@keene.edu.

Results suggest PM emissions from B20 (nonroad engine) less harmful to human health

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Keywords

Biodiesel; Particulate Matter; Reactive Oxygen Species; Oxidative Potential; BEAS-2B

1. Introduction

Due to its manufacture from plant-based or waste grease feedstocks, many consider biodiesel fuel blends a more environmentally friendly alternative to petroleum diesel fuel. However, less is known regarding the toxicological responses of biodiesel PM relative to diesel PM, especially from PM generated in real-world combustion operations utilizing heavy duty diesel (HDD) nonroad engines. The composition of biodiesel and diesel PM can include transition metals, and polar and nonpolar organic species, many of which can induce production of reactive oxygen species (ROS) in vitro (Verma et al. 2010; Hemmingsen et al. 2011; Fukagawa et al. 2013). While ROS is integral to many cellular processes, increased exposure to ROS has been shown to cause damage to intracellular components like DNA, lipids and proteins (Droge 2002, Nathan & Cunningham-Bussel 2013). Sustained elevation of ROS levels could result in numerous systemic health effects, as ROS have been associated with diabetes, neurodegenerative disorders, and age-related diseases such as Alzheimer's disease, Parkinson's disease and cardiovascular diseases (Zhang et al. 2000; Qin et al. 2006; Nelin et al. 2012; Brieger et al. 2012).

Oxidative stress resulting from exposure to diesel PM produces a variety of responses within human cells including antioxidant defense, inflammation, and at more concentrated levels, toxicity (Li et al. 2008, Verma et al. 2010; Jalava et al. 2010; Totlandsdal et al. 2015). The production of ROS can create varying levels of oxidative stress, depending on the concentration, duration, and location of exposure within human tissues. Studies of the oxidative potential of biodiesel PM determined by both intracellular ROS assays and acellular methods have increased in recent years. The results have often been contradictory, with some studies indicating biodiesel PM has increased oxidative potential compared to diesel PM (Jalava et al. 2012; Fukagawa et al. 2013; Gali et al. 2017) whereas other studies have shown biodiesel PM has similar or reduced oxidative potential when compared to diesel PM (Cheung et al. 2010; Jalava et al. 2010; Gerlofs-Nijland et al. 2013). Interpretation of the potential health implications of biodiesel combustion is further complicated by the varying engine configurations (catalyst, no catalyst), driving cycles (urban, rural), and normalization of results (on a PM per unit mass or PM per kilometer driven basis). The above referenced studies collected PM emissions from passenger car engines in a lab-based setting. To the best of our knowledge, studies of occupational exposure to biodiesel and petroleum diesel PM collected from heavy duty diesel (HDD) nonroad engines operating at a transfer station are nearly absent from the literature, other than previous work by our group (Traviss et al., 2012; Traviss et al. 2014; Martin et al. 2017).

A previous study by Traviss et al. (2012) demonstrated significant reductions in biodiesel PM mass concentrations (measured inside the equipment cabin) relative to diesel PM when HDD equipment operated on a B20 (20% biodiesel/80% diesel) blend compared to 100% petroleum diesel fuel. The observed reduction in PM emissions at a real-world site is a

benefit of biodiesel, yet other considerations are important to public health outcomes, such as changes in chemical composition and biological responses. Additionally, for public health consideration, PM collected near the breathing zone can differ in chemical composition from PM collected from the tailpipe due to dilution and other processes as demonstrated in our previous work noting the differences in PAH and n-PAH composition comparing in-cabin vs. tailpipe PM (Martin et al. 2017). While the present study does not examine secondary aerosol formation effects, other studies have shown that aged particles, i.e. PM exposed to atmospheric oxidants, ozone, and ultra-violet light, results in PM with increased volatility and oxidative potential (Li et al. 2009; Pourkhesalian et al. 2015). In short, a number of chemical and physical changes to diesel and biodiesel PM emissions can occur in the ambient environment before human exposure, thus affecting PM toxicity. Understanding the composition of this PM is imperative to understanding the health implications related to exposure. Studies have demonstrated that varying feedstocks and concentrations of biodiesel can result in differing oxidative potentials and pro-inflammatory responses in vitro (Steiner et al. 2012; Fukugawa et al. 2013; Traviss et al. 2014; Pourkhesalian et al. 2015). Reactive metals and various organic species may influence the oxidative potential of PM (Cheung et al. 2010; Charrier and Anastasio 2012; Fukagawa et al. 2013; Fang et al. 2015; Totlandsdal et al. 2015; Godoi et al. 2016); however, understanding the relationship between PM composition and health effects is a continued challenge.

This study characterized the chemical composition of PM collected in an occupational setting utilizing HDD nonroad engines and assessed ROS production in a human epithelial cell line (BEAS-2B) exposed to real-world biodiesel and diesel PM extracts. This was compared to the oxidative potential of the PM measured by an acellular dithiothreitol (DTT) assay. Water soluble organic carbon (WSOC) and metal concentrations of the PM were measured to investigate associations between composition and oxidative potential. Understanding PM composition and the link to toxicological endpoints is important in understanding the health implications of various fuels used in real-world conditions.

2. Materials and Methods

2.1 Overall Approach and PM Collection

Details of the overall study design, location and approach have been previously described (Traviss et al. 2012; Traviss et al. 2014) and are briefly summarized here. During an 8-week period in the summer of 2014, daily PM samples were collected from inside the equipment cabin (near the operator breathing zone, over the work shift) of a heavy duty front loader (2010 John Deere 624K, six cylinder diesel engine [Tier III], 198 horsepower at 1800 rpm). As with most Tier III nonroad engines, the John Deere 624K in this study did not have aftertreatment emissions control technology installed. EPA regulations have required stricter emissions controls in new nonroad engines sold since 2014, but many older nonroad HDD engines are still in use due to the durability of diesel engines in general. There are about 300,000 large front loaders/tractors/backhoes (from 75 to 300 HP), as well as 100,000 excavators (75 to 300 HP) with Tier III nonroad engines in use per the most recent official EPA population estimate (EPA 2010).

This front loader operated at the Keene Recycling Center on a daily basis, moving trash on the tipping floor of the waste transfer station. The equipment was operated by the same employee during the study period and this employee left the cabin door open. The fuels were purchased in bulk by the City of Keene and available commercially from a regional supplier. The front loader operated on two commercially purchased nonroad fuel blends: a 20:80 biodiesel/diesel waste grease based blend (B20, ASTM D6571) and a nonroad petroleum diesel fuel (ASTM D975 <500 ppm sulfur). PM was collected inside the cabin for 5 days per fuel type (n = 5 days B20, n = 5 days diesel). There was a 3-week interval in between the B20 and petroleum diesel operations to allow time for the equipment fuel tank to be emptied and conditioned to the new fuel.

The PM was collected on pre-weighed Teflon filters (Pall, Cat. No. 7221, 25 mm and 37 mm) placed inside a Sioutas™ impactor attached to a Leland Legacy vacuum pump pulling approximately 9 LPM over an 8-hour work day. The Sioutas™ impactor size segregates PM from greater than 2.5 to < 0.25 micrometer (μm) in diameter. 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). Filters were removed at the end of each field day from the impactors, placed into labeled Omega Filter Keepers (SKC, Cat. No. 225–8303), and then allowed to equilibrate again (24 hours) prior to post-weighing. Filters were kept cooled (4°C) awaiting gravimetric analysis and covered in aluminum foil. PM_{2.5} mass concentration was calculated from the mass of the B - D plates and the after-filter (AF) of the impactor. PM_{2.5} used in ROS/DTT experiments (see next sections) was the PM extracted and combined from the B -D and AF Teflon filters. TOC and metals characterization data is reported as PM_{2.5} (combined B-D, AF filters) as well. Additionally, ROS/DTT experiments were run only for individual days (per fuel type) with sufficient PM_{2.5} mass to develop the 60 μg/ml stock concentration necessary for direct comparison between fuel types per work shift.

2.2 PM Extraction for ROS and Total Organic Carbon (TOC) Analysis

The in-cabin PM_{2.5} was extracted from the Teflon filters into 15 mL of 18.2 Ω Millipore water via sonication, with 7.5 mL stored at –20 °C until analysis was performed for water soluble organic carbon (WSOC) using a Shimadzu TOC analyzer (TOC-L CSH/CSN). The remaining 7.5 mL of water extract was filtered using 0.45 μm syringe filter (Pall, Cat. No. 4438T) attached to a 20 mL NORM-JECT® syringe with Luer Lock (Sigma-Aldrich, Cat. No. Z248037–1PAK). The filtered samples were frozen to –80°C, lyophilized to dryness, and then the PM was resuspended in 18.2 Ω Millipore water to a final concentration of 60 μg PM/mL.

2.3 Chemical Composition of PM

2.3.1 TOC Analysis—The Shimadzu Total Carbon Analyzer was used to analyze Total Organic Carbon (TOC) of the water extracted PM. PM extracts were injected into a 680 °C heated combustion tube, where all organic and inorganic carbon converted to CO₂. The combustion products (after cooling and removing water) were scrubbed to remove any

halogens. Total carbon (TC) was determined by measuring CO₂ using a Non-Dispersive Infrared Detector (NDIR). To determine total organic carbon, inorganic carbon was measured by acidifying the sample to convert any carbonates to CO₂, which was measured via NDIR. The inorganic carbon result was subtracted from TC to calculate TOC. Percentage WSOC content was calculated by dividing TOC by the measured PM_{2.5} mass.

2.3.2 Metals Analysis—Metals content for in-cabin PM_{2.5} was analyzed via ICP- MS by the Dartmouth College Trace Elements Laboratory (Hanover, NH) for the following metals: Na, Al, S, Cr, Mn, V, Fe, Ni, Cu, Zn, Mo, Cd, Ba, Pb. These metals were selected based on previous work performed in Traviss et. al (2014) and Martin et al. (2017) and for their association with vehicle sources or fuels (HEI 2010).

2.4 Cellular Culture

An immortalized BEAS-2B human bronchial epithelial lung cell-line (ATCC, Manassas, VA) was used for the ROS assay. Falcon 75cm² Blue Vented Cap Tissue Culture Flasks (Fisher Scientific, Cat. No. 13–680-65) were pre-coated with Vitrogen Plating Medium (VPM) and incubated (37°C, 5% CO₂) for 24 hours prior to cell seeding. The cells were cultured in Bronchial Epithelial Growth Medium (BEGM) supplemented with the following: 2 mL BPE, 0.5 mL insulin, 0.5 mL HC, 0.5 mL GA-1000, 0.5 mL retinoic acid, 0.5 mL transferrin, 0.5 mL triiodothyronine, 0.5 mL epinephrine, and 0.5 mL hEGF per 500 mL of medium. (Lonza, Cat. No. CC-3170). Cellular culture medium was replaced inside of the culture flasks every 2–3 days and cells were subcultured into freshly coated culture flasks (with VPM) every 5–7 days. For experimental assays, BEAS-2B cells were cultured onto 96-well plates at a seeding density of 30,000 cells per well.

2.5 Reactive Oxygen Species (ROS) Assay

The BEAS-2B cells (ATCC) were seeded on a black Costar 96-well plate (ImmunoChemistry Technologies, Cat. No. 266) for 24 hours prior to treatment with previously extracted PM at various concentrations (0, 15, 30, and 60 µg PM/mL). An ROS Assay was performed using the ROS Oxiselect™ Intracellular Assay Kit (Cell Biolabs, Cat. No. STA-342, <https://www.cellbiolabs.com/reactive-oxygen-species-ros-assay>) according to manufacturing protocols. Briefly, cells were exposed to cell permeable DCFH-DA, which was deacetylated into non-fluorescent DCFH upon entry into the cell. DCFH was oxidized to highly fluorescent DCF when in the presence of elevated ROS following PM exposure. Fluorescence was measured using a BioTek HT Synergy Plate Reader at 480nm and 530nm emissions every 15 minutes for 2 hours. The 2 hour time point results were used for all ensuing data analyses.

2.6 Dithiothreitol (DTT) Assay

The DTT assay was performed using 100 µL of extracted PM stock solution (60 µg/mL). The DTT assay was performed according to the well-defined method outlined in Charrier and Anastasio (2012). Briefly, the assay measured consumption of DTT in a potassium phosphate buffer at 37 °C. Blanks consisting of 3 mL of buffer plus 100 µM DTT were run alongside every PM extract. All experimental procedures were performed in a dark room due to the light sensitive nature of the chemicals. Results presented were blank corrected.

2.7 Data Analyses

Differences in ROS production by BEAS-2B cells were analyzed with ANOVA (Tukey multiple comparison test) and differences in DTT consumption were analyzed with the MannWhitney U test (non-parametric analysis) using SPSS Statistical Analysis Software (PASW Statistics Version 23.0, SPSS Inc., Chicago, IL). The fuel source (B20 vs. diesel) was used as the grouping variable for the ANOVA analyses. Statistical significance was determined at $p = 0.05$. Correlations between oxidative potential (ROS production or DTT consumption) and WSOC were determined by Pearson Correlation Coefficient using Microsoft Excel Version Windows 2010.

3. Results and Discussion

3.1 ROS and DTT Assays

BEAS-2B cells exposed to B20 and diesel extracts resulted in an initial decrease in ROS at lower doses (15, 30 $\mu\text{g/mL}$) when compared to control treatments (0 $\mu\text{g/mL}$) (Fig. 1). Average ROS production at the 60 $\mu\text{g/mL}$ treatment was significantly greater ($p = 0.05$) than control for both fuel sources (Fig. 1). Diesel PM exposure resulted in slightly higher ROS levels (11% greater) than B20 at the 60 $\mu\text{g/mL}$ dose; however, this difference was not statistically significant (Fig. 1). There was no variation in ROS production between fuel sources at the 30 $\mu\text{g/mL}$ (2%) and 15 $\mu\text{g/mL}$ (1%) doses. While Fukagawa et al. (2013) did not observe a ROS response in BEAS-2B cells, this may be due to their lower treatment concentrations (20 $\mu\text{g/mL}$) and different PM sources (passenger car vs. HDD equipment). Previous works have demonstrated that fuel related PM induces dose-dependent increases in ROS production in other human cell lines (Baulig et al. 2003; Hemmingsen et al. 2013); however, ROS production was consistently decreased at 15 and 30 $\mu\text{g/mL}$ treatment concentrations (compared to controls), suggesting a hormetic response (Fig. S1A & S1B). Low doses of PM induce an upregulation of antioxidants (Li et al. 2004), which may be sufficient to reduce ROS production below untreated cells.

The acellular DTT assay also demonstrated that diesel PM has a higher oxidative potential than B20 PM (Fig. 2). The oxidative potential of diesel PM was 21% greater than B20 PM at 60 $\mu\text{g/mL}$ concentration; however, while quantitatively higher, this difference was not statistically significant.

3.2 WSOC and DTT/ROS Correlation Results

When all PM WSOC results were pooled together for both fuel types, there was a positive correlation between increasing WSOC (calculated as the % WSOC of PM mass) and ROS production ($n=9$) and DTT consumption ($n=10$).

There was a modest correlation ($r = 0.44$) observed between cellular ROS production and WSOC content for the pooled data set (Fig. 3); however, this correlation was not significant.

Correlations were then evaluated by fuel-type (B20 [$n=5$] vs. diesel [$n=4$] each fuel). Although the sample size per fuel type is small, a noticeable difference was observed between the two PM sources. ROS generated by petroleum diesel PM was highly correlated

with WSOC content ($r = 0.83$) (Fig. 4A), though the correlation was not significant. Interestingly, ROS activity associated with B20 PM was negatively correlated (Fig. 4B) with WSOC content ($r = -0.14$). Ultimately, exposure to both diesel and B20 PM resulted in similar ROS fold-increases at 60 $\mu\text{g/ml}$ (Fig. 1), despite the average WSOC content being 1.3 times greater in diesel than in B20 PM (16.0 vs. 11.5) (Figure S3).

There was a significant correlation ($p = 0.05$) observed between oxidative potential, measured using the acellular DTT assay, and WSOC for the pooled data set ($r = 0.64$) (Fig. 5). When analyzed by fuel type (B20 [$n=5$] vs. diesel [$n=5$] each fuel), DTT consumption by diesel and B20 PM were positively correlated with WSOC content, though neither correlation was significant. DTT consumption and ROS production were only slightly correlated ($r = 0.21$). (Fig. S2). Higher WSOC content in diesel PM may partially explain the increased DTT consumption rate. Lower DTT consumption by PM generated by combustion of biodiesel blends has been noted by other researchers for passenger car engines (Cheung et al. 2010; Gerlofs-Nijland et al. 2013). Petrodiesel PM was also found to generate higher levels of ROS compared to biodiesel PM (Cheung et al. 2010; Hemmingsen et al. 2011), although these researchers used different engine configurations, ROS assays and cell lines.

3.3 Metals Results

There were notable differences observed in average metals concentrations in the $\text{PM}_{2.5}$ size cut when comparing diesel vs. B20 PM (Fig. 7). On a ng/mg basis, the concentrations of metals in PM were generally similar for Ba, Mo, Ni, Fe, Cd, Al and V during equipment operation for both fuel types (Table S1). There were notable quantitative differences in Cr (1301 ng/mg [petrodiesel] vs. 455 ng/mg [B20]), Zn (16081 ng/mg [petrodiesel] vs 11666 ng/mg [B20]), Mn (352 ng/mg [petrodiesel] vs 622 ng/mg [B20]), Cu (30730 ng/mg [petrodiesel] vs. 16031 ng/mg [B20]); however, none of these differences were statistically significant. These results are in contrast to previous results where we observed significantly higher levels of Cu and Mo in B20 PM (from a waste grease feedstock) and Ni was not detected (Martin et al. 2017). In the current study, $\text{PM}_{2.5}$ was extracted to obtain sufficient mass for cellular experiments. To compare PM composition to toxicological endpoints, $\text{PM}_{2.5}$ was also used for TOC and metals analyses. The previous study (Martin et al. 2017) reported metals concentrations on the $\text{PM}_{<0.25}$ size fraction. With this caveat, it is still interesting to draw comparisons to our previous work. In both studies, Zn concentrations were higher in petrodiesel PM, while Fe content was similar (Martin et al. 2017). In addition to the differences in size cuts, the variability between these studies is likely due to variability in waste grease feedstock, compared to biodiesel made from pure rapeseed oil or soybean oil. Other researchers have also attributed fuel source as an important contributing factor to the concentrations of specific metals like Cr, Cu, Mn in PM from biodiesel and diesel exhaust (Gangwar et al. 2012; Godoi et al. 2016). Gangwar et al. (2012) found that B20 PM (biodiesel from karanja oil) contained higher concentrations of Fe, Mn, and Cr, whereas the Cu concentration in diesel PM was higher. Betha and Balasubramanian (2011) determined that Zn, Cr, Cu, Fe, and Ni concentrations were higher in B100 PM (waste grease derived biodiesel). The current study suggests the importance of fuel sources and the biodiesel feedstock to metals (versus contributions from engine wear).

Metals concentrations were examined as an explanatory factor for the DTT and ROS results. Cheung et al. (2010) found that Ba and Zn were significantly correlated with DTT consumption, while other studies demonstrated that Fe, Mn, and Cr were significantly correlated with cellular ROS activity (Verma et al. 2010). Godoi et al (2016) showed that Cu was strongly correlated with acellular oxidative potential of biodiesel PM (soy based) emitted from an HDD engine. In a detailed study on the influence of metals on the DTT assay, Charrier and Anastasio (2012) determined Cu and Mn to be the most reactive contributor to DTT consumption.

DTT consumption by diesel PM was significantly correlated with Cu ($r=0.98$, $p=0.05$) but there was no correlation between DTT and Cu for B20 PM. There was a strong but not significant correlation of ROS with Cu in petroleum diesel PM ($r=0.82$). Again, this was not the case for B20 PM. With respect to metals in general, except for the DTT/Cu relationship in diesel PM, there were no significant correlations between metals content and oxidative potential (both DTT and ROS); in fact, most correlations of metals species with ROS/DTT were negative (Table 1). Similar to this study, Karavalakis et al. (2017) found that ultralow sulfur diesel emissions from an HDD engine produced higher DTT activity compared to biodiesel. Fe and Cu in HDD engine diesel emissions were significantly correlated with DTT consumption, but this was for pooled PM data, all fuel types. Interestingly, when all metals were removed by chelation, the DTT consumption was reduced dramatically for diesel samples and almost eliminated in biodiesel samples (Karavalakis et al 2017).

Both DTT consumption and ROS production were strongly correlated with Cu only for diesel PM (not B20 PM). The DTT/Cu correlation was significant for diesel PM. This suggests the diesel fuel source as a factor in oxidative potential, as the Cu content was 48% higher in diesel PM (Table S1). Diesel emissions from an HDD engine have been shown to have higher redox potential (increased DTT consumption) as well generate higher ROS (as determined by increased expression of HO-1) compared to biodiesel fuels (Karavalakis et al. 2017). DTT consumption was positively but not significantly correlated with WSOC when analyzing per fuel type (diesel PM vs B20 PM), suggesting Cu in diesel PM may be the more important factor in that DTT result.

ROS generation was positively correlated with WSOC for diesel PM but negatively associated with B20 PM, suggesting the WSOC composition differed between fuel types. To explore the relationship between Cu and WSOC, we ran additional an correlational analysis. There was a negative correlation between Cu and WSOC for B20 PM, but a moderate (not significant) positive correlation ($r=0.74$) between Cu and WSOC for diesel PM. While this does not eliminate possible confounding between Cu/WSOC in our diesel PM DTT/ROS results, WSOC has been found to be more significant in ROS generation (cellular assays) but not DTT consumption (Karavalakis et al.2017) potentially due to the different mechanisms in each assay and redox pathways (Cheung et al. 2010, Ghio et al 2012). Additionally, other species not measured in this study, such as PAHs and quinones, have also been suggested as confounders influencing redox activity (Cheung et al 2010, Totlandsdal et al. 2015, Ghio et al. 2012, Sheng and Lu 2017). The difference in the diesel vs. B20 PM DTT/ROS assay results suggest not only the role of Cu, but that organic composition in general (which may include other species such as quinones and PAHs) may be quite different in diesel PM

compared to B20 PM. Additional chemical analysis of diesel and B20 PM for these species of interest would be an area for future research.

BEAS-2B cells treated with petroleum diesel extracts produced slightly higher ROS levels at every dose; however, none of the observed differences were statistically significant. The similarity in ROS produced in vitro (petroleum diesel vs. B20 PM) may be due to an initial antioxidant response from the cells, as it was consistently observed that ROS levels decreased at low doses but increased with increasing dose. The highest achievable concentration from the in-cabin PM collection method was 60 µg/ml. It is possible that differences in ROS production exist between fuel types, but higher PM treatment concentrations (100 to 150 µg/ml), such as those used in other studies (Cheung et al. 2010; Gerlofs-Nijaland 2013), are required to assess this endpoint. Nevertheless, this study demonstrates that biodiesel PM is less redox active, which is in line with several studies of diesel/biodiesel PM from passenger cars (Cheung et al. 2010; Gerlofs-Nijaland 2013). Cheung et al. (2010) determined a modest correlation between organic acid content and DTT consumption ($r=0.77$) but a significant correlation between PAH content and DTT consumption ($r=0.94$). PAH content (or oxygenated PAHs) in diesel PM may have been a factor in the present study as well. In total, these results suggest the influence of fuel type on the oxidative potential of PM generated by a HDD nonroad engine. In our occupational setting, utilizing a HDD nonroad engine, B20 PM was less redox active (lower oxidative potential) suggesting biodiesel may be less harmful to human health.

Conclusion

Examining the influence of biodiesel on PM composition and oxidative potential is important to understanding of the impact of various biodiesel blends used in real-world operating nonroad HDD engines. PM generated from combustion of both petroleum diesel and B20 in an HDD engine resulted in a dose-dependent increase in ROS production in vitro (BEAS-2B cells). Petroleum diesel PM had a 21% higher DTT consumption rate compared to B20 PM. The ROS produced by BEAS-2B cells were not significantly different per fuel type, although generally, ROS levels were slightly higher post-treatment with petroleum diesel PM. There was a significant correlation between WSOC content in the PM (pooled diesel and B20 data) and DTT consumption ($r=0.64$), and a strong but not significant correlation between the WSOC content (petroleum diesel PM) and ROS generation ($r=0.83$). Cu also was a factor in the oxidative potential of diesel PM only, as DTT consumption was significantly correlated with Cu ($r=0.98$) and ROS production was strongly correlated with Cu ($r=0.82$). Higher WSOC and Cu content in diesel PM may partially explain the increased DTT consumption rate, but other compounds such as quinones may be a factor. This study suggests the organic carbon and Cu content of the PM are key drivers for oxidative potential in real-world diesel PM emitted from an HDD nonroad engine. The results indicate that petroleum diesel PM has higher oxidative potential compared to B20 PM. Overall, B20 PM is less redox active and therefore may reduce health risk compared to burning petroleum diesel. Future studies should examine the influence of biodiesel feedstock, as well as PAHs, FAMES and quinone content in PM, as contributors to oxidative potential in PM emitted from HDD nonroad engines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Agarwal AK, Gupta T, & Kothari A (2010). Toxic potential evaluation of particulate matter emitted from a constant speed compression ignition engine: a comparison between straight vegetable oil and mineral diesel. *Aerosol Science and Technology*, 44(9), 724–733.
- Agarwal AK, Gupta T, Dixit N, & Shukla PC (2013). Assessment of toxic potential of primary and secondary particulates/aerosols from biodiesel vis-a-vis mineral diesel fueled engine. *Inhalation toxicology*, 25(6), 325–332. [PubMed: 23631768]
- Baulig A, Garlatti M, Bonvallot V, Marchand A, Barouki R, Marano F, & BaezaSquiban A (2003). Involvement of reactive oxygen species in the metabolic pathways triggered by diesel exhaust particles in human airway epithelial cells. *American Journal of Physiology Lung Cellular and Molecular Physiology*, 285(3), L671–L679.
- Betha R, & Balasubramanian R (2011). Particulate emissions from a stationary engine fueled with ultra-low-sulfur diesel and waste-cooking-oil-derived biodiesel. *Journal of the Air & Waste Management Association*, 61(10), 1063–1069. [PubMed: 22070039]
- Brieger K, Schiavone S, Miller FJ Jr, & Krause KH (2012). Reactive oxygen species: from health to disease. *Swiss medical weekly*, 142, w13659.
- Charrier JG, & Anastasio C (2012). On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: evidence for the importance of soluble transition metals. *Atmospheric chemistry and physics (Print)*, 12(5), 11317.
- Cheung KL, Ntziachristos L, Tzankiozis T, Schauer JJ, Samaras Z, Moore KF, & Sioutas C (2010). 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*, 44(7), 500–513.
- Dröge W (2002). Free radicals in the physiological control of cell function. *Physiological reviews*, 82(1), 47–95. [PubMed: 11773609]
- Delfino RJ, Staimer N, & Vaziri ND (2011). Air pollution and circulating biomarkers of oxidative stress. *Air Quality, Atmosphere & Health*, 4(1), 37–52.
- Fang T, Verma V, Bates JT, Abrams J, Klein M, Strickland MJ, ... & Russell AG. (2015). Oxidative potential of ambient water-soluble PM 2.5 measured by Dithiothreitol (DTT) and Ascorbic Acid (AA) assays in the southeastern United States: contrasts in sources and health associations. *Atmospheric Chemistry and Physics Discussions*, 15(21), 30609–30644.
- Fukagawa NK, Li M, Poynter ME, Palmer BC, Parker E, Kasumba J, & Holmén BA (2013). Soy biodiesel and petrodiesel emissions differ in size, chemical composition and stimulation of inflammatory responses in cells and animals. *Environmental science & technology*, 47(21), 12496–12504. [PubMed: 24053625]
- Gali NK, Yang F, Cheung CS, & Ning Z (2017). A comparative analysis of chemical components and cell toxicity properties of solid and semi-volatile PM from diesel and biodiesel blend. *Journal of Aerosol Science*, 111, 51–64.

- Gangwar JN, Gupta T, & Agarwal AK (2012). Composition and comparative toxicity of particulate matter emitted from a diesel and biodiesel fuelled CRDI engine. *Atmospheric environment*, 46, 472–481.
- Gerlofs-Nijland ME, Totlandsdal AI, Tzamkiozis T, Leseman DLC, Samaras Z, Låg M, ... & Cassee FR (2013). Cell toxicity and oxidative potential of engine exhaust particles: impact of using particulate filter or biodiesel fuel blend. *Environmental science & technology*, 47(11), 5931–5938. [PubMed: 23597117]
- Ghio AJ, Carraway MS, & Madden MC (2012). Composition of air pollution particles and oxidative stress in cells, tissues, and living systems. *Journal of Toxicology and Environmental Health, Part B*, 15(1), 1–21.
- Godoi RH, Polezer G, Borillo GC, Brown A, Valebona FB, Silva TO, ... & Neto RAP (2016). Influence on the oxidative potential of a heavy-duty engine particle emission due to selective catalytic reduction system and biodiesel blend. *Science of the Total Environment*, 560, 179–185. [PubMed: 27101453]
- HEI, 2010 Traffic-related air pollution: a critical review of the literature on emissions, exposure, and health effects; Special Report 17.
- Hemmingsen JG, Møller P, Nøjgaard JK, Roursgaard M, & Loft S (2011). 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 & technology*, 45(19), 8545–8551. [PubMed: 21842833]
- Jalava PI, Aakko-Saksa P, Murtonen T, Happonen MS, Markkanen A, Yli-Pirila P, ... & Jokiniemi J. (2012). Toxicological properties of emission particles from heavy duty engines powered by conventional and bio-based diesel fuels and compressed natural gas. *Particle and fibre toxicology*, 9(1), 37. [PubMed: 23021308]
- Karavalakis G, Gysel N, Schmitz DA, Cho AK, Sioutas C, Schauer JJ, ... & Durbin TD (2017). Impact of biodiesel on regulated and unregulated emissions, and redox and proinflammatory properties of PM emitted from heavy-duty vehicles. *Science of the Total Environment*, 584, 1230–1238. [PubMed: 28148458]
- Li N, Alam J, Venkatesan MI, Eiguren-Fernandez A, Schmitz D, Di Stefano E, ... & Wang M (2004). Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals. *The Journal of Immunology*, 173(5), 3467–3481. [PubMed: 15322212]
- Li N, Xia T, & Nel AE (2008). The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles. *Free Radical Biology and Medicine*, 44(9), 1689–1699. [PubMed: 18313407]
- Li Q, Wyatt A, & Kamens RM (2009). Oxidant generation and toxicity enhancement of aged-diesel exhaust. *Atmospheric Environment*, 43(5), 1037–1042.
- Martin N, Lombard M, Jensen KR, Kelley P, Pratt T, & Traviss N (2017). Effect of biodiesel fuel on “real-world”, nonroad heavy duty diesel engine particulate matter emissions, composition and cytotoxicity. *Science of The Total Environment*, 586, 409–418. [PubMed: 28236480]
- Nathan C, & Cunningham-Bussell A (2013). Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nature Reviews Immunology*, 13(5), 349–361.
- Nelin TD, Joseph AM, Gorr MW, & Wold LE (2012). Direct and indirect effects of particulate matter on the cardiovascular system. *Toxicology letters*, 208(3), 293–299. [PubMed: 22119171]
- Pourkhesalian AM, Stevanovic S, Rahman MM, Faghihi EM, Bottle SE, Masri AR, ... & Ristovski ZD (2015). Effect of atmospheric aging on volatility and reactive oxygen species of biodiesel exhaust nano-particles. *Atmospheric Chemistry and Physics*, 15(16), 9099–9108.
- Qin B, Cartier L, Dubois-Dauphin M, Li B, Serrander L, & Krause KH (2006). A key role for the microglial NADPH oxidase in APP-dependent killing of neurons. *Neurobiology of aging*, 27(11), 1577–1587. [PubMed: 16260066]
- Ristow M (2014). Unraveling the truth about antioxidants: mitohormesis explains ROS-induced health benefits. *Nature medicine*, 20(7), 709–711.

- Sheng K, & Lu J (2017). Typical airborne quinones modulate oxidative stress and cytokine expression in lung epithelial A549 cells. *Journal of Environmental Science and Health, Part A*, 52(2), 127–134.
- Steiner S, Czerwinski J, Comte P, Popovicheva O, Kireeva E, Müller L, ... & RothenRutishauser B (2013). Comparison of the toxicity of diesel exhaust produced by bio-and fossil diesel combustion in human lung cells in vitro. *Atmospheric Environment*, 81, 380–388.
- Thompson C, & Nathanail P (Eds.). (2009). *Chemical analysis of contaminated land*. John Wiley & Sons.
- Totlandsdal AI, Låg M, Lilleaas E, Cassee F, & Schwarze P (2015). Differential proinflammatory responses induced by diesel exhaust particles with contrasting PAH and metal content. *Environmental toxicology*, 30(2), 188–196. [PubMed: 23900936]
- Traviss N, Li M, Lombard M, Thelen BA, Palmer BC, Poynter ME, ... & Fukagawa NK (2014). Petrodiesel and waste grease biodiesel (B20) emission particles at a rural recycling center: characterization and effects on lung epithelial cells and macrophages. *Air Quality, Atmosphere & Health*, 7(1), 59–70.
- Traviss N, Thelen BA, Ingalls JK, & Treadwell MD (2012). Evaluation of biodiesel's impact on real-world occupational and environmental particulate matter exposures at a municipal facility in Keene, NH. *Air Quality, Atmosphere & Health*, 5(1), 101–114.
- Verma V, Shafer MM, Schauer JJ, & Sioutas C (2010). Contribution of transition metals in the reactive oxygen species activity of PM emissions from retrofitted heavy-duty vehicles. *Atmospheric Environment*, 44(39), 5165–5173.
- Zhang Y, Dawson VL, & Dawson TM (2000). Oxidative stress and genetics in the pathogenesis of Parkinson's disease. *Neurobiology of disease*, 7(4), 240–250. [PubMed: 10964596]

Highlights

Oxidative potential of diesel PM was 21% higher than biodiesel PM (DTT assay).

No difference in ROS generated in vitro (diesel vs B20 PM)

Concentrations of Cu, Zn, Cr higher in diesel PM

Cu and WSOC associated with oxidative potential in diesel PM, not biodiesel PM.

Figure 1.

Average ROS production of BEAS-2B cells treated with B20 and diesel extracts at varying doses (0–60 µg/mL) from the Keene Recycling Center (n= 5 days per fuel type) as determined by ROS Oxiselect™ Intracellular Assay Kit. Asterisk (*) indicates significant difference from control (p=0.05). Error bars represent standard error from the mean.

Figure 2. Comparison of oxidative potential for biodiesel (n=5) and diesel (n=4) PM (60 $\mu\text{g/mL}$) from the Keene Recycling Center. Error bars represent standard error from the mean.

Figure 3.
Fold-increase in ROS production compared to percentage of WSOC in total PM mass

(pooled data, both fuel types, $n = 9$).

Figure 4.

Fold-increase in ROS production compared to percentage of WSOC in total PM mass by fuel. **(A)** Percentage of WSOC (diesel PM, $n=4$) versus ROS fold increase **(B)** Percentage of WSOC (B20 PM, $n=5$) versus ROS fold increase.

Figure 5.

DTT consumption compared to percentage of WSOC in total PM mass (pooled data, both fuel types, $n=10$). There was a significant correlation ($r = 0.64$) between the DTT consumption rate and WSOC percentage of PM mass as determined by Pearson Correlation Coefficient at $p=0.05$.

Figure 6. Percentage of WSOC in total PM mass by fuel versus DTT consumption. **(A)**Percentage of WSOC (diesel PM, n=5) versus DTT consumption. **(B)** Percentage of WSOC (B20 PM, n=5) versus DTT consumption.

Figure 7. Concentrations of metals of interest from diesel (n=6) and B20 (n=5) PM_{2.5}: Cu, Mn, Ni, Mo, Ba, Fe, Cu, and Zn. Error bars represent standard error from the mean.

Table 1.

Correlation coefficients, r , between metals species in $PM_{2.5}$ and DTT (top) or ROS (bottom) per fuel type. Significant correlations are highlighted in red ($p=0.05$).

<u>DTT</u>	Mn	Cu	Ba	Fe	Cr	Zn	S
Diesel	-0.46	0.98	-0.51	-0.49	-0.67	0.07	0.61
B20	-0.43	-0.06	-0.54	0.02	-0.28	-0.25	0.06
<u>ROS</u>	Mn	Cu	Ba	Fe	Cr	Zn	S
Diesel	-0.39	0.82	-0.08	-0.58	-0.93	-0.38	0.64
B20	-0.3	0.12	0.17	0.14	-0.1	-0.48	-0.04