Science of the Total Environment 584–585 (2017) 1230–1238

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Impact of biodiesel on regulated and unregulated emissions, and redox and proinflammatory properties of PM emitted from heavy-duty vehicles

Georgios Karavalakis [a,b,*], Nicholas Gysel [a,b], Debra A. Schmitz [c,d], Arthur K. Cho [c,d], Constantinos Sioutas [e], James J. Schauer [f], David R. Cocker [a,b], Thomas D. Durbin [a,b]

[a] University of California, Bourns College of Engineering, Center for Environmental Research and Technology (CE-CERT), 1084 Columbia Avenue, Riverside, CA 92507, USA
[b] Department of Chemical and Environmental Engineering, Bourns College of Engineering, University of California, Riverside, CA 92521, USA
[c] Department of Molecular and Medical Pharmacology, UCLA Center for Health Sciences, Los Angeles, CA 90095, USA
[d] Department of Environmental Health Sciences, UCLA Center for Health Sciences, Los Angeles, CA 90095, USA
[e] University of Southern California, Department of Civil and Environmental Engineering, 3620 South Vermont Avenue, Los Angeles, CA 90089, USA
[f] University of Wisconsin-Madison, Environmental Chemistry and Technology Program, 660 North Park Street, Madison, WI, USA

HIGHLIGHTS

- Most harmful emissions decreased with biodiesel, except NOx.
- Unsaturated biodiesel produced higher NOx emissions.
- The aftertreatment controls effectively reduced particle and PAH emissions.
- The oxidative potential (DTT) decreased with the biodiesel blends.
- Diesel exhaust PM were more reactive and showed the highest cellular responses.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form 25 January 2017
Accepted 26 January 2017
Available online 30 January 2017

Editor: D. Barcelo

Keywords:
Biodiesel
Heavy-duty diesel trucks
Emissions
Particles
Toxicity
Oxidative stress

ABSTRACT

The emissions and the potential health effects of particulate matter (PM) were assessed from two heavy-duty trucks with and without emission control aftertreatment systems when operating on CARB ultra-low sulfur diesel (ULSD) and three different biodiesel blends. The CARB ULSD was blended with soy-based biodiesel, animal fat biodiesel, and waste cooking oil biodiesel at 50 vol%. Testing was conducted over the EPA Urban Dynamometer Driving Schedule (UDDS) in triplicate for both trucks. The aftertreatment controls effectively decreased PM mass and number emissions, as well as the polycyclic aromatic hydrocarbons (PAHs) compared to the uncontrolled truck. Emissions of nitrogen oxides (NO_x) exhibited increases with the biodiesel blends, showing some feedstock dependency for the controlled truck. The oxidative potential of the emitted PM, measured by means of the dithiothreitol (DTT) assay, showed reductions with the use of biodiesel blends relative to CARB ULSD for the uncontrolled truck. Overall, the cellular responses to the particles from each fuel were reflective of the chemical content, i.e., particles from CARB ULSD were the most reactive and exhibited the highest cellular responses.

* Corresponding author at: University of California, Bourns College of Engineering, Center for Environmental Research and Technology (CE-CERT), 1084 Columbia Avenue, Riverside, CA 92507, USA.
E-mail address: gkaraval@cert.ucr.edu (G. Karavalakis).

http://dx.doi.org/10.1016/j.scitotenv.2017.01.187
0048-9697/

1. Introduction

There has been an increase in the number of heavy-duty vehicles (HDVs) worldwide as a result of economic growth. In the United States (US), HDVs accounted for 12.1% of total petroleum consumption in 2012 and transported 70% of freight by tonnage (Askin et al., 2015). The emissions from these trucks can have adverse consequences on the environment and human health. Several studies have demonstrated an association between diesel particulate matter (PM) and increased lung cancer, airway inflammation, and exacerbation of pulmonary diseases, such as asthma (Castranova et al., 2001; Mills et al., 2005; Nel et al., 1998). In addition, nitrogen oxides (NO_x), carbon monoxide (CO), and volatile organic compounds (VOCs) have direct health implications and can act as precursors for the formation of photochemical ozone and secondary aerosols (Kampa and Castanas, 2008; Wofsy et al., 1990). In response to health and environmental concerns, the US Environmental Protection Agency (USEPA) implemented significant reductions in 2007 in diesel PM mass emission standards for on-highway HDVs or engines. Manufacturers with the use of diesel particulate filters (DPFs) met these reductions. For model year 2010, USEPA promulgated strict control policies for reducing NO_x emissions from on-highway HDVs (Johnson, 2016). Currently, urea-based selective catalytic reduction (SCR) aftertreatment has emerged as the most feasible strategy in reducing NO_x emissions and meeting 2010 USEPA standards (Johnson, 2016).

Emissions reductions can also be achieved with the use of biofuels, which have attracted attention for their potential to reduce net carbon dioxide (CO_2) emissions in the transportation sector (Coronado et al., 2009). Currently, fatty acid methyl esters (FAMEs), commonly known as biodiesel, appear to be the biofuel of choice in both the U.S. and Europe for petroleum diesel fuel displacement. In the US, biodiesel has been promoted by several legislative measures, including the Energy Independence and Security Act (EISA, 2007) and the Renewable Fuel Standard (RFS), which was initiated in 2005 and expanded in 2007 (EISA, 2007). With the recently released proposed rule by the US EPA for the 2014 to 2017 RFS volume requirements, there is a significant growth for biodiesel usage ranging from 1.63 to 1.90 billion gallons per year (US EPA, 2015). There are numerous studies available in the open literature reporting the effects of pure and blended biodiesel fuels on the gaseous and PM emissions from light- and heavy-duty engines/vehicles (Hajbabaei et al., 2014; Kousoulidou et al., 2010; Na et al., 2015). Most studies have found reductions in total hydrocarbon (THC), CO, and PM emissions with biodiesel application, while a NO_x increase is usually acknowledged (Di et al., 2009; Eckerle et al., 2008; Karavalakis et al., 2016; Mueller et al., 2009). For example, Hajbabaei et al. (2012) reported systematic increases in NO_x emissions when they tested soy-based and waste cooking oil-based biodiesel blends at 20 vol% and 5 vol% with CARB ultra-low sulfur diesel (ULSD) in a heavy-duty diesel engine. They also reported NO_x neutrality with low level blends of more saturated animal fat-based biodiesel blends compared to CARB ULSD. Fewer studies have addressed the impact of biodiesel application on particle number emissions and particle size distributions (Fontaras et al., 2010; Westphal et al., 2013). In these studies, increased particle number emissions and smaller particle sizes with biodiesel were generally, but not always, observed, and the reasons for such inconsistencies are not properly understood. Fontaras et al. (2009) found that total particle number increased with B50 and B100 fuels, whereas solid particle number progressively decreased. The authors of that study also found increases in semi-volatile PM that they attributed to lower volatility of biodiesel components.

Several other studies have provided detailed emission profiles of VOCs, carbonyl compounds, and polycyclic aromatic hydrocarbons (PAHs), with the majority of these studies not reaching a consensus on the impact of biodiesel due to conflicting trends (George et al., 2014; Prokopowicz et al., 2015; Rouce et al., 2012). Magara-Gomez et al. (2012) found decreases in toluene, ethylbenzene, *m*/*p*-xylenes, *o*-xylene, benzene, formaldehyde, and acetaldehyde emissions with pure and blended soy-based and beef tallow-based biodiesel. Cahill and Okamoto (2012) showed higher emission rates for unsaturated aldehydes, such as acrolein, and benzaldehyde when they tested 50% and pure soy-based and animal fat-based biodiesel in a HDV. Ratcliff et al. (2010) showed reductions in formaldehyde and acetaldehyde emissions, as well as PM associated PAH emissions when they tested a heavy-duty diesel engine with a DPF on soy-based biodiesel, whereas Karavalakis et al. (2010) showed an increase in low molecular-weight PAH compounds and carbonyl emissions with biodiesel blends from a light-duty diesel vehicle.

Information on health effects in relation to biodiesel fuel usage in modern engines is also limited (Mutlu et al., 2015; Andre et al., 2015; Zhang and Balasubramanian, 2014). The mechanisms affecting adverse PM health effects involve oxidative stress, initiated by the formation of reactive oxygen species (ROS) within cells (Shafer and Buettner, 2001). High levels of ROS in the cells can trigger a cascade of events associated with inflammation and, at higher concentrations, apoptosis (Nel et al., 1998). A recent study utilizing a 50% rapeseed oil methyl ester in Euro 4 light-duty vehicles equipped with or without DPFs showed increased cytotoxicity and IL-6 release by bronchial epithelial cells (BEAS-2B) compared to diesel fuel (Gerlofs-Nijland et al., 2013). Another study reported that the application of 20% soy-based biodiesel in an uncontrolled light-duty diesel engine resulted in 20–30% higher concentrations of inflammatory mediators (IL-6, IP-10, G-CSF) in treated BEAS-2B cells and in bronchoalveolar lavage fluid in mice than diesel fuel (Fukagawa et al., 2013). Bhavaraju et al. (2014) suggested that particle composition is important for driving cellular inflammatory signals and demonstrated that alveolar macrophage exposure to 20% biodiesel resulted in increased production of prostaglandin E_2 (PGE_2) relative to diesel. Grigoratos et al. (2014) showed higher oxidative activity with increasing biodiesel blending when testing two light-duty vehicles with and without a DPF over the NEDC and Artemis cycles. Other studies have also demonstrated increases in oxidative activity with biodiesel blends (Cheung et al., 2009; Godoi et al., 2016; Guarieiro et al., 2014).

The objective of this study was to assess the potential emissions and health implications of the widespread use of high concentration biodiesel blends in HDVs with and without aftertreatment systems. The impacts of different biodiesel feedstocks were compared to those of a typical CARB ULSD. Emission measurements were conducted on two HDVs over the EPA Urban Dynamometer Driving Schedule (UDDS). The results are discussed in the context of changing fuel type, engine technology, and exhaust aftertreatment.

2. Experimental

2.1. Test fuels, vehicles, and measurement protocol

Four fuels were tested for this study, including a typical on-road CARB ULSD, which served as the baseline fuel. Three fatty acid methyl esters that span the degree of unsaturation levels common in the marketplace were used as biodiesel blendstocks with the CARB ULSD. The biodiesels were blended at a 50% proportion by volume. A methyl ester produced from soybean oil (SME), a waste cooking oil methyl ester (WCO), and a methyl ester produced from animal fat (AFME) were used. The basic physicochemical properties of the CARB ULSD and the three methyl esters are given in the Supplementary Material, Table SM1.

Two trucks were used in this study, including a vehicle with a 2010 model year Cummins ISX-15 engine with cooled exhaust gas recirculation (EGR) and a turbocharger, at a rated horsepower of 344 kW at 1700 rpm, and a vehicle with a 2002 model year Cummins ISX-450 engine with EGR at a rated horsepower of 336 kW at 1700 rpm. The vehicle with the Cummins ISX-450 engine was not equipped with aftertreatment control devices. The vehicle with the Cummins ISX-15 engine was equipped with SCR for NO_x removal, and a diesel oxidation

catalyst (DOC)/DPF for PM, CO, and THC removal. Uncontrolled trucks are heavily used in many regions of the world, including the US. While California has imposed strict regulations on heavy-duty diesel trucks, vehicles without emission controls from other states operate in the greater Los Angeles basin by transporting goods from the Ports of Los Angeles and Long Beach to the rest of the country.

Both vehicles on each fuel were tested over the EPA Urban Dynamometer Driving Schedule (UDDS) in triplicate. A double UDDS cycle was conducted for each of the three tests for the Cummins ISX-15 truck to obtain sufficient PM mass for gravimetric analysis. The vehicles were preconditioned at the start of each test day by performing a power map to bring the vehicle up to its operational temperature. A preconditioning UDDS was then performed on each fuel prior to beginning testing on each fuel. The preconditioning cycle warmed up both the vehicle and dynamometer to the conditions of the test configuration, thus reducing the emissions variability between tests. Between tests, there was a 'hot soak', where the engine was turned off for about 20 min. All tests were conducted as 'hot running' tests and the UDDS cycle was run on triplicate for each fuel.

It should be noted that newer Cummins engines have been approved for 20 vol% of biodiesel, while other manufacturers approved their engines for up to 5 vol%. For this study, higher concentration biodiesel blends were chosen in order (a) to better investigate the effect of biodiesel on emissions, especially for a heavily controlled vehicle, and (b) to provide a potential suggestion in terms of environmental performance as to whether higher than 20 vol% biodiesel concentrations could be used in niche markets, such as the heavy-duty vehicle transportation sector.

2.2. Emissions testing and analysis

All tests were conducted at CE-CERT's Heavy-Duty Chassis Dynamometer facility. Emissions measurements were obtained using the CE-CERT Mobile Emissions Laboratory (MEL). The facility and sampling setup have been described in detail previously, and are only discussed briefly here (Cocker et al., 2004). For all tests, emissions of THC, CO, NO_x, CO_2, and PM, were measured using standard instruments, as shown in the Supplementary Material. Measurements of ammonia (NH_3) were also obtained on a real-time basis using a Unisearch Associates Inc. LasIR S Series tunable diode laser (TDL) near infrared absorption spectrometer. Carbonyl compounds and 1,3-butadiene, benzene, toluene, ethylbenzene, and xylene (BTEX) compounds, PM mass, total particle number emissions, and particle size distributions were also measured. Detailed information on the methods used to collect and analyze these compounds, as well as the experimental setup for particle measurements is provided in the Supplementary Material. Measurements were also conducted for PAHs, hopanes and steranes, and metals and trace elements. A detailed description of the sampling procedures and analysis are also given in the Supplementary Material.

Potential health effects of the PM emissions were examined for both vehicles using cell-based and cell-free assays, and stress-based inflammatory and adaptive responses by a mouse macrophage cell line (Raw 264.7) assays. Detailed information about the chemical and cellular assays utilized for this study is also provided in the Supplementary Material.

3. Results and discussion

The results for both vehicles as a function of different fuel types with the UDDS cycle are presented below. The error bars denote the standard deviation of the average for each fuel. Statistical comparisons between fuels for a given vehicle were made using a 2-tailed, 2-sample, equal variance *t*-test. For the purpose of this discussion, results are considered to be statistically significant for $p \le 0.05$ and marginally statistically significant for $0.05 < p \le 0.1$.

3.1. Gaseous emissions

The CO and THC emissions for both vehicles are presented in Table 1, with the uncontrolled vehicle showing clear trends towards lower CO and THC emissions with the biodiesel blends. For the 2010 vehicle, there were no distinguishable fuel trends with the exception of AFME-50, which showed CO emissions reductions relative to CARB ULSD at a statistically significant level. THC emissions were below the detection limit of the instruments, and thus are not reported in Table 1. CO emissions for the 2010 vehicle were approximately one order of magnitude lower than those for the 2002 vehicle. The presence of a DOC played a major role in reducing CO emissions. The lower CO and THC emissions from the 2002 vehicle with biodiesel can be attributed to the higher oxygen content in the methyl ester molecules, which allows for leaner and more complete combustion (Giakoumis et al., 2012). In addition, biodiesel is a straight-chain hydrocarbon compared with the more complex molecular structure of petroleum diesel, including aromatics and many different carbon chain lengths. As a result, biodiesel would be expected to have a thinner turbulent flame front and therefore less mixing of post flame gasses with cooler regions of unburnt gasses, thus leading to lower THC emissions (Giakoumis et al., 2012; Lapuerta et al., 2008).

NO_x emissions as a function of fuel type are shown in Fig. 1 for both vehicles. It is evident from the results that the newer vehicle produced significantly lower amounts of NO_x compared to the uncontrolled vehicle. The 2010 SCR-equipped vehicle emitted 91%, 85.3%, 88.5%, and 84.2%, respectively, for CARB ULSD, SME-50, AFME-50, and WCO-50 less NO_x emissions than the uncontrolled vehicle. For the SCR-equipped vehicle, NO_x emissions showed statistically significant increases of 74% and 101%, respectively, for SME-50 and WCO-50 and a marginally statistically significant increase of 47% for AFME-50 compared to CARB ULSD. For the uncontrolled vehicle, NO_x emissions showed statistically significant increases of 8.4% and 7.9%, respectively, for AFME-50 and WCO-50 compared to CARB ULSD. Our results of higher NO_x emissions with the biodiesel blends are consistent with previous studies (Borillo et al., 2015; Eckerle et al., 2008; Fontaras et al., 2009; Hajbabaei et al., 2014; Karavalakis et al., 2016; Karavalakis et al., 2010; Kousoulidou et al., 2010; Mueller et al., 2009; Na et al., 2015). Mueller et al. (2009) showed that an increase in oxygen for biodiesel brings the charge air mixture closer to stoichiometric conditions at ignition and in the standing premixed auto-ignition zone near the flame lift-off length, which can lead to NO_x increases. It has been shown that more unsaturated methyl esters have lower cetane numbers and higher densities than saturated methyl esters (Giakoumis et al., 2012; McCormick et al., 2001; Lapuerta et al., 2008). Double-bond structuring of methyl esters tends to decrease cetane number and lengthen ignition delay. The longer ignition delay can lead to greater premixed combustion, which can lead to greater NO_x emissions (Sun et al., 2010). Under the present test conditions, the effect of biodiesel source material was not as clear since the unsaturated SME blend showed higher NO_x emissions compared to the saturated AFME blend for one vehicle but not for the other. The results reported here also showed a rather strong fuel effect with biodiesel blends compared to CARB ULSD for the SCR-equipped vehicle. This is in contrast with previous studies showing that the effect of SCR negates the effect of biodiesel blends and pure biodiesel on NO_x emissions (Lammert et al., 2012), but in agreement with other studies reporting increases in NO_x emissions with biodiesel from vehicles equipped with SCR catalysts (Borillo et al., 2015; Kawano et al., 2010).

Further analyses showed that for all test fuels, a relatively large portion of the NO_x emissions for the SCR-equipped vehicle was produced at the start of the UDDS when the SCR system temperature was <250 °C, as illustrated in Fig. 2. The lowest NO_x emissions for all fuels were found when the SCR system was at a temperature above 250 °C. This phenomenon can be explained by the fact that with the SCR system NO_x is converted into N_2 by reaction with NH_3 over a catalyst. When operating temperatures are >250 °C, an aqueous solution of urea is injected into the exhaust upstream of the SCR catalyst. The heat converts the urea

Table 1
Gaseous and PM mass and number emissions for the 2002 Cummins ISX-450 and 2010 Cummins ISX-15 vehicles over the UDDS cycle.

	CO (g/mile)	THC (g/mile)	NO_x (g/mile)	NH_3 (g/mile)	PM Mass (g/mile)	Particle number (particles/mile)
2002 Cummins ISX-450						
CARB ULSD	2.64 ± 0.07	0.79 ± 0.07	12.91 ± 0.49	24.6 ± 12.1	0.336 ± 0.043	1.90E+14 ± 3.25 + 12
SME-50	2.14 ± 0.06	0.37 ± 0.10	13.02 ± 0.31	22.5 ± 14.3	0.110 ± 0.027	1.94E+14 ± 4.96E+12
WCO-50	2.09 ± 0.00	0.54 ± 0.01	14.09 ± 0.25	29.7 ± 5.1	0.111 ± 0.029	1.99E+14 ± 6.51E+12
AFME-50	2.06 ± 0.06	0.62 ± 0.21	14.02 ± 0.03	18.3 ± 7.6	0.088 ± 0.001	1.89E+14 ± 1.82E+12
2010 Cummins ISX-15						
CARB ULSD	0.23 ± 0.02	ND	1.10 ± 0.22	134.2 ± 127.9	0.0032 ± 0.001	2.82E+11 ± 3.69E+11
SME-50	0.25 ± 0.05	ND	1.91 ± 0.12	362.5 ± 282.8	0.0031 ± 0.001	4.91E+11 ± 1.09E+11
WCO-50	0.15 ± 0.02	ND	1.61 ± 0.22	384.2 ± 138.8	0.0026 ± 0.000	8.83E+11 ± 1.51E+11
AFME-50	0.26 ± 0.02	ND	2.22 ± 0.36	317.3 ± 233.4	0.0028 ± 0.000	6.47E+11 ± 3.52E+11

into NH_3, which reacts to convert NO_x to nitrogen, and water. At temperatures <250 °C, urea is not injected so the full engine-out NO_x are emitted. Under the present test conditions, a significant portion of the UDDS temperature was <250 °C, precluding urea injection, and thus NO_x control for the newer vehicle. It is interesting to note that for the low-temperature period (<250 °C), the fuel effect was particularly strong, with WCO-50 blend showing higher NO_x emissions relative to CARB ULSD, followed by SME-50 and AFME-50 blends. The WCO-50 blend systematically caused higher NO_x emissions throughout the entire driving cycle and generally showed lower exhaust temperatures. In fact, all biodiesel blends (which had lower heating values) had lower exhaust temperatures, suggesting that the amount of urea solution injected may have decreased due to the decreased temperature. These phenomena could be due to the original engine calibration parameters that did not compensate for the lower exhaust temperatures with biodiesel blends higher than 20 vol%.

Emissions of NH_3 for both vehicles are shown in Table 1, with the SCR-equipped vehicle producing significantly higher NH_3 than the uncontrolled vehicle. The use of biodiesel blends produced increases in NH_3 emissions relative to CARB ULSD, which trended with the increases in NO_x emissions for the SCR-equipped vehicle. The differences in NH_3 emissions with the biodiesel blends were not statistically significant for the individual biodiesel blends, but when the biodiesel results are considered as a whole, a marginally statistically significant increase for the biodiesel blends was seen. It is reasonable to assume that the high NH_3 emissions (NH_3 slip) for the biodiesel blends could be due to a response of the dosing control system for the SCR to the higher engine-out NO_x emissions. Owing to biodiesel's lower heating value the engine control module (ECM) may dictate a higher fuel flow rate that under certain operating conditions will result in NO_x increases. It is, therefore, expected that the SCR system will inject more urea to be hydrolyzed into NH_3 to suppress NO_x emissions.

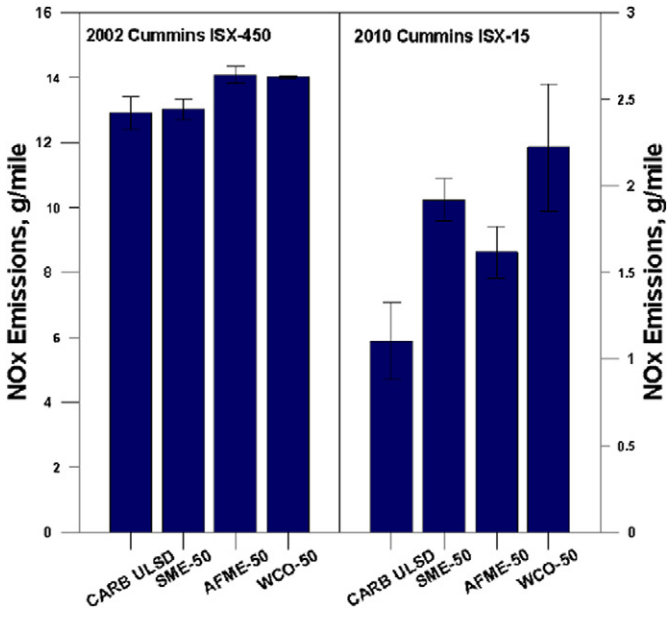

Fig. 1. NO_x emissions of the CARB ULSD and the biodiesel blends for the 2002 Cummins ISX-450 and 2010 Cummins ISX-15 vehicles over the UDDS cycle.

3.2. PM mass, particle number emissions, and particle size distributions

PM mass emissions are presented in Table 1. For the uncontrolled vehicle, the use of biodiesel blends, independent their source material resulted in dramatic PM mass reductions ranging from 67% to 74%, that were statistically significant. These reductions could be attributed to the increased oxygen concentration in the biodiesel blend, which reduces locally fuel-rich regions and limits soot nucleation in the formation process (Giakoumis et al., 2012; Lapuerta et al., 2008). In addition to the oxygen content, the absence of aromatics, which act as soot precursors, could also contribute to the PM mass decrease with biodiesel (Giakoumis et al., 2012). For the DPF-equipped vehicle, PM mass emissions were substantially lower than those of the uncontrolled vehicle and very low on an absolute basis due to the presence of the DPF. Fuel effects on PM mass emissions were indistinguishable for the DPF-equipped vehicle, suggesting the biodiesel did not provide significant added benefits in PM reduction beyond those that were already being achieved with the DPF. Similar to PM mass, total particle number emissions for the 2010 vehicle were found to be significantly lower than those for the 2002 vehicle, as shown in Table 1. It should be stressed that particle number emissions for the controlled vehicle were below the background levels of the CVS. For the 2002 vehicle, particle number emissions were about the same with all the test fuels.

Average particle size distributions are shown in Fig. 3 (A–B) for the Cummins ISX-15 and Cummins ISX-450 vehicles, respectively. For the 2010 vehicle, particle size distributions were dominated by nucleation mode particles peaking at around 11 nm, with a relatively minor soot mode at about 40 to 45 nm. The CARB ULSD exhibited higher nucleation mode particle concentrations compared to the biodiesel blends, regardless their feedstock. The reductions in nucleation mode particle concentrations with biodiesel could be due to the higher molecular oxygen content in biodiesel, which favored soot oxidation, or the lack of aromatics (Young et al., 2012). The particle formation mechanism from the DPF and SCR-equipped vehicle could be due to sulfur oxidation over the catalyst configuration, consisting mainly of sulfuric acid-based, ammonium sulfate-based, and ammonium nitrate-based particles.

For the 2002 vehicle, particle size distributions exhibited a characteristic bimodal distribution for all fuels. Particle concentrations were found to be substantially higher than for the controlled vehicle, suggesting that the aftertreatment system was effective in trapping larger and smaller size particles. All fuels showed nucleation mode particles with peaks at around 10 nm in diameter and accumulation mode particles with peaks ranging from 45 to 50 nm in diameter. Fuel type had varying effects on particle size distributions, with CARB ULSD producing more

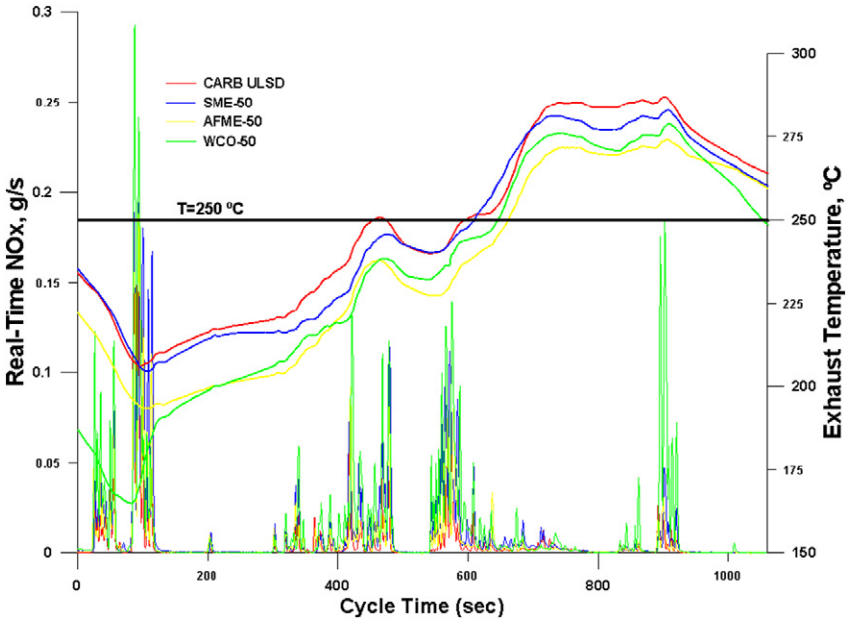

Fig. 2. Real-time NO_x emissions as a function of SCR temperature.

accumulation mode particles with larger mobility diameters compared to the biodiesel blends. The lower accumulation particle concentrations with biodiesel could be a consequence of the reduced aromatics content in the fuel blends (Young et al., 2012). The general trend showing decreased geometric mean diameters (GMD) with the biodiesel blends, could also be due to a decrease in the percentage of carbon and corresponding increase of oxygen, favoring the reduction of elemental carbon cores and decreasing the accumulation and agglomeration phenomena and hence the GMD, as well as the lower PM mass for the biodiesel blends (Tsolakis, 2006). Another interesting feature is that the unsaturated SME-50 blend showed higher nucleation mode particle concentrations followed by CARB ULSD, WCO-50, and AFME-50. Soy-based biodiesel, in particular, contains unsaturated fatty esters, which have been shown to increase nucleation mode particles, possibly because of the emission of organic species with high boiling points, which tend to be more condensable than hydrocarbon components (Schönborn et al., 2009).

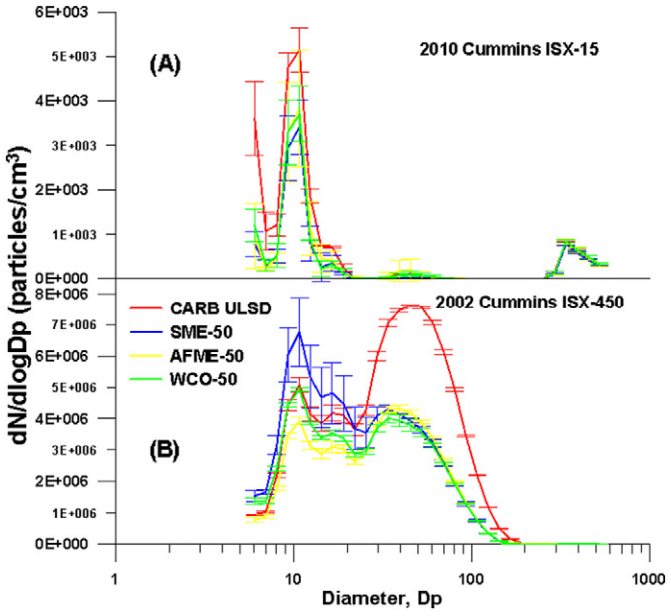

Fig. 3. (A–B) Average particle size distributions for the 2010 Cummins ISX-15 vehicle (A) and the 2002 Cummins ISX-450 vehicle.

3.3. Volatile organic compound emissions

Benzene, toluene, ethylbenzene, *m,p*-xylenes, and *o*-xylene, collectively known as BTEX compounds, and 1,3-butadiene emissions are given in Table 2. Overall, the uncontrolled vehicles did not show any strong fuel trends in 1,3-butadiene emissions, while the DPF-equipped vehicle produced significantly lower 1,3-butadiene and BTEX emissions than the uncontrolled vehicle. This observation suggests that these compounds were effectively oxidized in the DOC/DPF system. Benzene emissions showed some statistically significant reductions for the biodiesel blends for the uncontrolled vehicle, but not for the controlled vehicle, where the presence of aftertreatment negates the fuel effect with respect to benzene emissions. Reasons contributing to benzene reductions include the lower aromatics in the fuel blend as well as the oxygen enrichment with biodiesel, which may promote the oxidation of benzene, leading to a decrease of benzene emissions. Both increases and decreases were seen for toluene, *m,p*-xylenes and *o*-xylene emissions with biodiesel fuels depending on the vehicle.

Carbonyl emissions for both vehicles are presented in Table 2. For both vehicles, low molecular-weight carbonyls, such as formaldehyde and acetaldehyde, were the dominant species in the exhaust. These results are comparable to previous studies, showing that formaldehyde and acetaldehyde are the most abundant carbonyls in biodiesel exhaust, followed by butyraldehyde and methacrolein (Fontaras et al., 2009; George et al., 2014; Karavalakis et al., 2016; Magara-Gomez et al., 2012; Ratcliff et al., 2010). Heavier carbonyls were also present in the tailpipe for the uncontrolled vehicle, but in lesser amounts. The uncontrolled vehicle produced substantially higher carbonyl emissions than the controlled vehicle. The use of biodiesel resulted in systematic decreases in formaldehyde emissions for the uncontrolled vehicle, with some differences being statistically significant, whereas there were no fuel effects on formaldehyde emissions for the controlled vehicle. Acetaldehyde emissions did not show any fuel effects for the uncontrolled vehicle, while for the controlled vehicle acetaldehyde emissions were well below the background levels. Previous studies have shown decreases in carbonyl emissions with the use of biodiesel (Cahill and Okamoto, 2012). Reductions in carbonyls with biodiesel could be attributed to the decomposition of esters via decarboxylation, which could

Table 2
Emissions of BTEX, 1,3-butadiene, formaldehyde, and acetaldehyde compounds, expressed in g/mile, for the 2002 Cummins ISX-450 and 2010 Cummins ISX-15 vehicles over the UDDS cycle.

	Benzene	Toluene	Ethylbenzene	*m,p*-Xylene	*o*-Xylene	1,3-butadiene	Formaldehyde	Acetaldehyde	Total Carbonyls
2002 Cummins ISX-450									
CARB ULSD	0.079 ± 0.017	0.035 ± 0.007	0.007 ± 0.002	0.013 ± 0.004	0.006 ± 0.002	0.021 ± 0.022	0.075 ± 0.003	0.011 ± 0.003	0.107 ± 0.014
SME-50	0.060 ± 0.00	0.024 ± 0.00	0.006 ± 0.00	0.011 ± 0.00	0.005 ± 0.00	0.025 ± 0.00	0.062 ± 0.003	0.008 ± 0.003	0.101 ± 0.029
AFME-50	0.048 ± 0.008	0.015 ± 0.001	0.003 ± 0.00	0.007 ± 0.001	0.002 ± 0.00	0.003 ± 0.00	0.067 ± 0.004	0.008 ± 0.003	0.099 ± 0.016
WCO-50	0.069 ± 0.002	0.018 ± 0.002	0.004 ± 0.00	0.007 ± 0.001	0.003 ± 0.00	0.018 ± 0.020	0.066 ± 0.003	0.008 ± 0.002	0.099 ± 0.013
2010 Cummins ISX-15									
CARB ULSD	0.002 ± 0.001	0.007 ± 0.00	0.001 ± 0.00	0.003 ± 0.00	0.001 ± 0.00	0.003 ± 0.001	0.014 ± 0.004	0.003 ± 0.00	0.021 ± 0.009
SME-50	0.006 ± 0.006	0.016 ± 0.008	0.003 ± 0.002	0.011 ± 0.005	0.004 ± 0.002	0.003 ± 0.00	0.007 ± 0.003	0.002 ± 0.00	0.015 ± 0.006
AFME-50	0.010 ± 0.012	0.023 ± 0.027	0.004 ± 0.004	0.012 ± 0.013	0.005 ± 0.005	0.004 ± 0.003	0.004 ± 0.004	0.001 ± 0.00	0.011 ± 0.017
WCO-50	0.002 ± 0.002	0.005 ± 0.001	0.001 ± 0.00	0.002 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.010 ± 0.003	0.003 ± 0.00	0.022 ± 0.010

decrease the probability of forming oxygenated combustion intermediates with respect to conventional diesel combustion (Lapuerta et al., 2008).

3.4. PAHs, and hopanes and steranes emissions

Overall, 26 particle-phase PAH compounds were identified and quantified in the exhaust for each vehicle/fuel combination over the UDDS, as shown in Supplementary Material, Table SM2. Fig. 4 shows the total PAH emissions for both test vehicles. For the controlled vehicle, low molecular-weight PAHs with three to four rings were the only PAH compounds present in the tailpipe. Medium and high molecular-weight PAHs were practically undetectable due to the presence of the DOC/DPF configuration, which retained these compounds and facilitated their oxidation. For the uncontrolled vehicle, the PAH profile was dramatically different than the DPF-equipped vehicle, showing significantly higher PAH emission levels and more PAH species detected in the exhaust. Lower and medium molecular-weight PAHs were the dominant compounds found in the exhaust, suggesting that these compounds were pyrolyzed from incomplete combustion of the fuel (Lea-Langton et al., 2008). Previous vehicle and engine studies have also reported the dominance of light PAHs in biodiesel exhaust (Fontaras et al., 2009; George et al., 2014; Karavalakis et al., 2010; Magara-Gomez et al., 2012; Ratcliff et al., 2010). Some heavier PAHs were also found in the exhaust, but in lesser amounts than those of light and medium molecular-weight PAHs. The formation of these species might be due to pyrosynthesis of lower molecular-weight aromatic compounds to larger PAHs and to the contribution of the lubricant oil (Lim McKenzie et al., 2007).

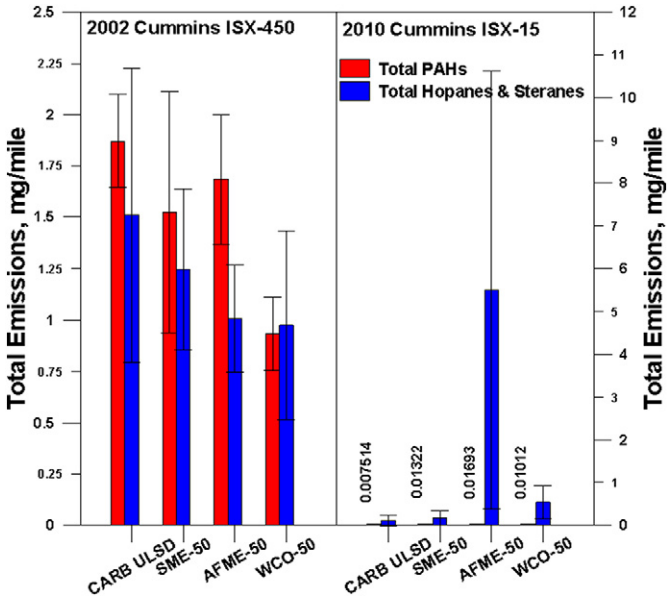

Fig. 4. Emissions of total PAHs and total hopanes and steranes for the 2002 Cummins ISX-450 and the 2010 Cummins ISX-15 vehicles.

The use of biodiesel blends resulted in some PAH emissions reductions compared to CARB ULSD, which could be a consequence of the lower aromatics and the oxygenated nature of biodiesel blends. Most biodiesel blends led to statistically significant decreases relative to CARB ULSD in phenanthrene, fluoranthene, and pyrene emissions. The reduction of light PAHs with biodiesel is a desirable result since previous studies have shown that light molecular-weight PAHs are important in terms of their potential to generate oxidative stress (Li et al., 2003). On the other hand, benzo(b)fluoranthene, benzo(k)fluoranthene, and chrysene showed higher emissions for most biodiesel blends than CARB ULSD, with the AFME-50 blend showing statistically significant increases in these pollutants compared to the CARB ULSD. Other PAH compounds, which are considered to be carcinogens or suspected carcinogens to humans, exhibited some decreases with the use of biodiesel blends including benzo(a)pyrene, benzo(a)anthracene, indeno(1,2,3-cd)pyrene, and benzo(ghi)perylene.

Hopanes and steranes are mainly lubricant oil tracers that are commonly used as organic tracers of mobile source emissions in source apportionment of $PM_{2.5}$, and which are virtually undetectable in the fuel (Kleeman et al., 2008). Fig. 4 presents the total hopanes and steranes emissions for both vehicles over the UDDS, while Table SM3 (Supplementary Material) presents the individual hopanes and steranes species. For the DPF-equipped vehicle, hopanes and steranes emissions were at substantially lower concentrations than those of the uncontrolled vehicle. As might be expected, the very low concentrations of hopanes and steranes in our samples for the controlled vehicle could be due to the presence of the DPF. The fuel effect on a PM mass per mile basis was not particularly strong, although the AFME-50 blend showed surprisingly high hopane and sterane emissions relative to the other fuels, but with large measurement variability. While hopane and sterane emissions are exclusively related to the rate of lubricant oil consumption, it is possible that this type of feedstock contained some of these species from a contamination by mineral oil materials, which were retained in the exhaust as unburned fuel fractions. For the uncontrolled vehicle, the use of biodiesel blends resulted in some reductions in hopane and sterane emissions relative to CARB ULSD, somewhat following the PAH profile for this vehicle. It is likely that hopanes and steranes are precursors for the formation of some PAHs in petroleum diesel and biodiesel combustion reactions.

3.5. Water-soluble metals

A total of 49 metals and elements were identified and quantified in the exhausts of both vehicles including redox active transition metals (Mn, V, Ni, Cu, Fe, and Cr), divalent transition metals (Zn, Cd, and Co), heavy metals (Tl, Pb, U, Th, and W), alkaline earth metals (Sr, Mg, Ba, and Ca), platinum-group elements (Rh, Pt, and Pd), rare earth (Eu, Pr, Ce, Nd, La, Sm, Y, Dy, Ho, Yb, and Lu), semi-metals (As, and Sb), and higher valent/hydrolyzed metals (Ti, Sc, and Al). As shown in Table

SM4 (Supplementary Material), the elemental matrix for both vehicles is composed largely of alkaline earth metals and divalent transition metals, followed by redox active transition metals. The first two metal categories, primarily originate from the lubricant oil and its additive package components. For the controlled vehicle, the fraction of alkaline earth metals was significantly lower than that of the uncontrolled vehicle. The use of aftertreatment reduced most metal fractions, suggesting a high removal efficiency of these elements in the DPF. In addition to the effect of the aftertreatment, the use of biodiesel blends led to reductions in most metal fractions for both vehicles, consistent with the overall reductions in PM mass. It should be stressed that all biodiesel blends showed reductions in the fraction of redox-active metals for both vehicles. Looking at the individual distribution of redox-active metals, iron (Fe) showed decreases with most biodiesel blends relative to CARB ULSD for both vehicles with the exception of WCO-50 blend, which was systematically higher than the other biodiesel blends and CARB ULSD, especially for the 2002 vehicle.

3.6. Oxidative stress and cellular markers

The oxidative activity, as measured by the prooxidant content determined from the DTT assay, for the particle-phase components of the uncontrolled vehicle is shown in Fig. 5. The DTT values for the particle phase components of the DPF-fitted vehicle were well below the filter blank levels due to the very low PM mass adsorbed on the Teflon filters, and thus are not shown in the results. Table 3 shows the vapor-phase DTT activity for both vehicles. The effects of the particles on a mouse macrophage cell line (Raw 264.7) were determined by exposing them for 16 h and determining the expression levels of tumor necrosis factor alpha (TNFα), a proinflammatory cytokine limited to those from the uncontrolled vehicle, and hemeoxygenase-1 (HO-1), an adaptive protein that generates CO and bilirubin that are anti-inflammatory and anti-oxidative, respectively (Gozzelino et al., 2009; Guastadisegni et al., 2010). Although analysis for both cellular markers was made for all vehicles/fuel combinations, results are only shown for the uncontrolled vehicle (Table 3).

The DTT consumption rate of the uncontrolled vehicle decreased with the biodiesel blends relative to CARB ULSD, which produced the most potent exhaust for redox activity. In the presence of the metal chelator, diethylenetriaminepentaacetic acid (DTPA), the CARB ULSD sample retained about 60% of the total prooxidant activity whereas the prooxidant activity of the remaining biodiesel samples was completely blocked, suggesting the biodiesel fuel prooxidants are metal-associated. The oxidative activities of the particle-phase components also showed a feedstock dependency, with the WCO-50 blend exhibiting higher redox activity than the other biodiesel blends. The major contributing factor was primarily the high content of redox-active species present in the WCO-50 blend, which could form ROS (superoxide, HOOH, and OH) and oxidize DTT. Analogous to the particle phase components of PM, the oxidative activity of the vapor phase components showed some marked reductions with the biodiesel blends relative to CARB ULSD. The prooxidant content in the vapor phase contrasted with observations made in an earlier study of ambient particles, where the prooxidant content in the vapor phase components was between 10 and 20% of that in the particle phase (Eiguren-Fernandez et al., 2015). Overall, our results agree with earlier studies showing reductions in oxidative potential with biodiesel (Gerlofs-Nijland et al., 2013; Kooter et al., 2011).

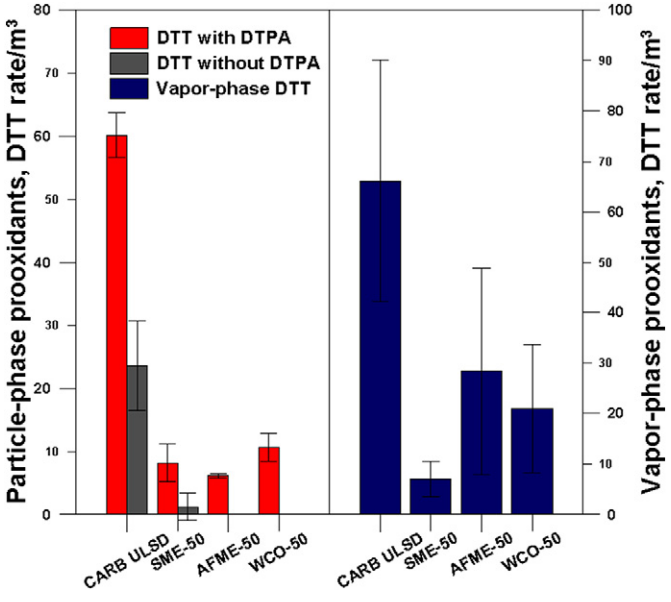

Fig. 5. Oxidative potential of the particle-phase and vapor-phase components of PM for the 2002 Cummins ISX-450 vehicle.

Table 3
Cellular markers indicating inflammation (TNFα) and adaptive response (HO-1) for the 2002 Cummins ISX-450 vehicle over the UDDS cycle.

	Particle-phase	Vapor-phase
HO-1 expression (DEP units/m^3)		
CARB ULSD	1.700 ± 0.927	909.9
SME-50	0.682 ± 0.691	76.1
AFME-50	−0.264 ± 0.185	296.94
WCO-50	0.697 ± 0.127	11.6
TNFα expression (LPS units/m^3)		
CARB ULSD	0.301 ± 0.360	−193.4
SME-50	0.057 ± 0.061	51.61
AFME-50	−0.048 ± 0.015	−182.82
WCO-50	0.059 ± 0.057	−63.5

Values shown are the averages ± SD with N ≥ 2. Protein expression for each sample was normalized to the expression by a standard stimulant to correct for variability between cell exposures. The standard stimulants were lipopolysaccharide (LPS) and a diesel exhaust particle preparation from Japan (DEP). Values are the means ± SD (N ≥ 2). The negative values for AFME indicate the expression levels were lower than those observed for a blank filter. For the vapor-phase samples, the HO-1 and TNFα were averaged for the replicates (3 for CARB ULSD and SME-50 and 2 for AFME-50 and WCO-50).

The cytokine TNFα was increased for some biodiesel particle phase samples for the uncontrolled vehicle compared to the activity of CARB ULSD, as shown in Table 3. Of interest were the high negative values of the uncontrolled vehicle for the vapor phase samples, which could reflect suppression of the TNFα response. In micro array studies with ambient vapor samples from the Los Angeles basin, a suppression of the mRNA for TNFα in a bronchial epithelial cell line was observed (Shinkai et al., 2013). The negative TNFα response to the vapor phase samples may reflect the effects of the actions of the vapor components on the antioxidant-antielectrophile response element (ARE) (Li et al., 2000). This element is associated with a protective response, and micro array studies with vapor phase samples in Riverside showed that vapor phase components suppressed TNFα mRNA by 40% (Shinkai et al., 2013). Table 3 also shows the particle phase samples for the WCO-50 blend increased the expression of HO-1 at greater levels than those exhibited by the other biodiesel blends, which could be a result of the higher concentrations of redox-active species in the blend. It is interesting to note that the SME blend appeared to be more reactive with greater cellular effects, whereas the AFME blend elicited the lowest cellular response. The vapor phases from the test fuels caused greater expression of HO-1 than their corresponding particle phases, which may reflect a different distribution of the causative chemical species between the two phases. Based on the cellular data reported here, it can be hypothesized that the HO-1 responses appear to reflect the prooxidant content of the samples, suggesting that the basis for ARE activation may be prooxidant, not electrophilic activity.

Although the sample size was relatively limited, a Pearson-based correlation analysis was performed to assess the relationships between likely contributors to the cellular responses observed for the

Table 4
Pearson correlation analysis between the heath effect assays and the redox active metals (Fe and Cu), and WSOC for the 2002 Cummins ISX-450 vehicle.

	DTT	Fe	Cu	WSOC	TNFα (avg)	HO-1 (avg)
DTT		0.99 (p = 0.01)	1.00 (p = 0.00)	0.83 (p = 0.17)	0.96 (p = 0.04)	0.86 (p = 0.14)
Fe	0.99 (p = 0.01)		1.00 (p = 0.00)	0.87 (p = 0.13)	0.95 (p = 0.05)	0.86 (p = 0.14)
Cu	1.00 (p = 0.00)	1.00 (p = 0.00)		0.83 (p = 0.17)	0.95 (p = 0.05)	0.84 (p = 0.16)
WSOC	0.83 (p = 0.17)	0.87 (p = 0.13)	0.83 (p = 0.17)		0.92 (p = 0.08)	0.95 (p = 0.05)
TNFα (avg)	0.96 (p = 0.04)	0.95 (p = 0.05)	0.95 (p = 0.05)	0.92 (p = 0.08)		0.97 (p = 0.03)
HO-1 (avg)	0.86 (p = 0.14)	0.86 (p = 0.14)	0.84 (p = 0.16)	0.95 (p = 0.05)	0.97 (p = 0.03)	

uncontrolled vehicle. As shown in Table 4, the analysis included the water soluble organic carbon (WSOC), and water soluble iron and copper. The analysis suggested the redox active metals were associated with the TNFα response but not the HO-1 response, which correlated with the WSOC content of the PM. Thus, the results reported here show that the inflammatory responses (indicated by TNFα) are associated with the redox active components of the particles, i.e., the DTT activity and the major transition metals. The correlation of DTT activity with WSOC has been reported by others (Verma et al., 2009), but its correlation with the TNFα response by the cells has not. Although the correlation is based on a very limited sampling, the results suggest that the DTT-based prooxidants include a group of chemical species, such as iron and copper, that promote inflammatory responses (Guastadisegni et al., 2010). In contrast, HO-1, is associated with the organic material in the PM in promoting this response. These results are consistent with previous studies of vapor phase components from ambient air, in which HO-1 expression was promoted and TNFα expression was suppressed (Shinkai et al., 2013). Note that the DTT did not correlate with total PAHs, since PAHs are not redox-active. Possible correlations between the DTT and PAHs are due to the correlation of PAHs and quinones, some of which can oxidize DTT (Kumagai et al., 2002).

4. Conclusions

This study showed that most harmful emissions, including PM mass and number, carbonyl compounds, and PAHs reduced with the addition of biodiesel, with the exception of NO_x. Emissions of NO_x showed some feedstock dependency, especially for the controlled vehicle. Overall, the use of robust aftertreatment systems results in significantly lower emissions on a mass and number basis and generate PM emissions with lower hazard potential. Our findings showed that PM emitted from biodiesel obtained from waste cooking oil could be more biologically active than other feedstocks due to the higher concentrations of redox-active metals, such as Fe and Cu that oxidized DTT and showed a strong correlation with TNFα. The results of this study are useful in understanding the effects of different biodiesels on the exhaust emissions from vehicles with and without aftertreatment systems operating in California and the rest of the country and also for developing more extensive sampling and testing procedures when evaluating the biological properties of PM from current technology vehicles.

Acknowledgments

The authors thank Mr. Don Pacocha, Mr. Eddie O'Neal, and Mr. Joe Valdez of the University of California, Riverside for their contribution in conducting the emissions testing for this program. We thank the staff of the Wisconsin State Laboratory of Hygiene for assistance with the analysis of the particulate matter samples for organic compound and trace metals. We acknowledge funding from the South Coast Air Quality Management District (SCAQMD) under contract 12197.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.01.187.

References

Andre, V., Barraud, C., Capron, D., Preterre, D., Keravec, V., Vendeville, C., Cazier, F., Pottier, D., Morin, J.P., Sichel, F., 2015. Comparative mutagenicity and genotoxicity of particles and aerosols emitted by the combustion of standard vs. rapeseed methyl ester supplemented bio-diesel fuels: impact of after treatment devices: oxidation catalyst and particulate filter. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 777, 33–42.

Askin, A.C., Barter, G.E., West, T.H., Manley, D.K., 2015. The heavy-duty vehicle future in the United States: a parametric analysis of technology and policy tradeoffs. Energy Policy 81, 1–13.

Bhavaraju, L., Shannahan, J., William, A., McCormick, R.L., McGee, J., Kodavanti, U., Madden, M., 2014. Diesel and biodiesel exhaust particle effects on rat alveolar macrophages with in vitro exposure. Chemosphere 104, 126–133.

Borillo, G.C., Tadano, Y.S., Godoi, A.F.L., Santana, S.S.M., Weronka, F.M., Penteado Neto, R.A., Rempel, D., Yamamoto, C.I., Potgieter-Vermaak, S., Potgieter, J.H., Godoi, R.H.M., 2015. Effectiveness of selective catalytic reduction systems on reducing gaseous emissions from an engine using diesel and biodiesel blends. Environ. Sci. Technol. 49, 3246–3251.

Cahill, T.M., Okamoto, R.A., 2012. Emissions of acrolein and other aldehydes from biodiesel-fueled heavy-duty vehicles. Environ. Sci. Technol. 46, 8382–8388.

Castranova, V., Ma, J.Y.C., Yang, H.M., Antonini, J.M., Butterworth, L., Barger, M.W., Roberts, J., Ma, J.K.H., 2001. Effect of exposure to diesel exhaust particles on the susceptibility of the lung to infection. Environ. Health Perspect. 109, 609–612.

Cocker, D.R., Shah, S., Johnson, K., Miller, J.W., Norbeck, J., 2004. Development and application of a mobile laboratory for measuring emissions from diesel engines. I regulated gaseous emissions. Environ. Sci. Technol. 38, 2182–2189.

Coronado, C.R., de Carvalho, J.A., Silveira, J.L., 2009. Biodiesel CO_2 emissions: a comparison with the main fuels in the Brazilian market. Fuel Process. Technol. 90, 204–211.

Cheung, K.L., Polidori, A., Ntziiachristos, L., Tzamkiozis, T., Samaras, Z., Cassee, F.R., Gerlofs, M., Sioutas, C., 2009. Chemical characteristics and oxidative potential of particulate matter emissions from gasoline, diesel, and biodiesel cars. Environ. Sci. Technol. 43, 6334–6340.

Di, Y., Cheung, C.S., Huang, Z., 2009. Experimeental investigation on regulated and unregulated emissions of a diesel engine fueled with ultra-low sulfur diesel fuel blended with biodiesel from waste cooking oil. Sci. Total Environ. 40, 835–846.

Eckerle, W.A., Lyford-Pike, E.J., Stanton, D.W., LaPointe, L.A., Whitacre, S.D., Wall, J.C., 2008. Effects of methyl ester biodiesel blends on NOx emissions. SAE Tech. Pap. (2008-01-0078).

Eiguren-Fernandez, A., Di Stefano, E., Schmitz, D.A., Lefol Nani Guarieiro, A., Salinas, E.M., Nasser, E., Froines, J.R., Cho, A.K., 2015. Chemical reactivities of ambient air samples in three Southern California communities. J. Air Waste Manage. Assoc. 65, 266–273.

Anon, 2007. Energy Independence Security Act of 2007. (https://www.gpo.gov/fdsys/pkg/BILLS-110hr6enr/pdf/BILLS-110hr6enr.pdf).

Fontaras, G., Kousoulidou, M., Karavalakis, G., Tzamkiozis, T., Pistikopoulos, P., Ntziachristos, L., Stournas, S., Samaras, Z., 2010. Effects of low concentration biodiesel blends application on modern passenger cars. Part 1: feedstock impact on regulated pollutants, fuel consumption and particle emissions. Environ. Pollut. 158, 1451–1460.

Fontaras, G., Karavalakis, G., Kousoulidou, M., Tzamkiozis, T., Ntziachristos, L., Bakeas, E., Stournas, S., Samaras, Z., 2009. Effects of biodiesel on passenger car fuel consumption, regulated and non-regulateed pollutant emissions over legislated and real-world driving cycles. Fuel 88, 1608–1617.

Fukagawa, N.K., Li, M., Poynter, M.E., Palmer, B.C., Parker, E., Kasumba, J., Holmen, B.A., 2013. Soy biodiesel and petrodiesel emissions differ in size, chemical composition and sstimulation of inflammatory responses in cells and animals. Environ. Sci. Technol. 47, 12496–12504.

George, I.J., Hays, M.D., Snow, R., Faircloth, J., George, B.J., Long, T., Baldauf, R.W., 2014. Cold temperature and biodiesel fuel effects on speciated emissions of volatile organic compounds from diesel trucks. Environ. Sci. Technol. 48, 14782–14789.

Gerlofs-Nijland, M., Totlandsdal, A.I., Tzamkiozis, T., Leseman, D.L.A.C., Samaras, Z., Lag, M., Scwarze, P., Ntziachristos, L., Cassee, F.R., 2013. Cell toxicity and oxidative potential of engine exhaust particles: impact of using particulate filter or biodiesel fuel blend. Environ. Sci. Technol. 47, 5931–5938.

Giakoumis, E.G., Rakopoulos, C.D., Dimaratos, A.M., Rakopoulos, D.C., 2012. Exhaust emissions of diesel engines operating under transient conditions with biodiesel fuel blends. Prog. Energy Combust. Sci. 38, 691–715.

Godoi, R.H.M., Polezer, G., Borillo, G.C., Brown, A., Valebona, F.B., Silva, T.O.B., Ingberman, A.B.G., Nalin, M., Yamamoto, C.I., Potgieter-Vermaak, S., Neto, R.A.P., de Marchi, M.R.R., Saldiva, P.H.N., Pauliquevis, T., Godoi, A.F.L., 2016. Influence on the oxidative potential of a heavy-duty engine particle emission due to selective catalytic reduction system and biodiesel blend. Sci. Total Environ. 560–561, 179–185.

Gozzelino, R., Jeney, V., Soares, M.P., 2009. Mechanisms of cell protection by heme oxygenase-1. Annu. Rev. Pharmacol. Toxicol. 50, 323–354.

Grigoratos, T., Fontaras, G., Kalogirou, M., Samaras, C., Samaras, Z., Rose, K., 2014. Effect of rapeseed methylester blending on diesel passenger car emissions-part 2: unregulated emissions and oxidation activity. Fuel 128, 260–267.
Guarieiro, A.L.N., Santos, J.V.D.S., Eiguren-Fernandez, A., Torres, E.A., da Rocha, G.O., de Andrade, J.B., 2014. Redox activity and PAH content in size-classified nanoparticles emitted by a diesel engine fueled with biodiesel and diesel blends. Fuel 116, 490–497.
Guastadisegni, C., Kelly, F.J., Cassee, F.R., Gerlofs-Nijland, M.E., Janssen, N.A., Pozzi, R., Brunekreef, B., Sandstrom, T., Mudway, I., 2010. Determinants of the proinflammatory action of ambient particulate matter in immortalized murine macrophages. Environ. Health Perspect. 118, 1728–1734.
Hajbabaei, M., Johnson, K.C., Okamoto, R.A., Mitchell, A., Pullman, M., Durbin, T.D., 2012. Evaluation of the impacts of biodiesel and second generation biofuels on NOx emissions for CARB diesel fuels. Environ. Sci. Technol. 46, 9163–9173.
Hajbabaei, M., Karavalakis, G., Johnson, K.C., Guthrie, J., Mitchell, A., Durbin, T.D., 2014. Impacts of biodiesel feedstock and additives on criteria emissions from a heavy-duty engine. Fuel Process. Technol. 126, 402–414.
Johnson, T., 2016. Vehicular emissions in review. SAE Int. J. Engines 9. http://dx.doi.org/10.4271/2016-01-0919.
Kampa, M., Castanas, E., 2008. Human health effects of air pollution. Environ. Pollut. 151, 362–367.
Karavalakis, G., Bakeas, E., Stournas, E., 2010. Influence of oxidized biodiesel blends on regulated and unregulated emissions from a diesel passenger car. Environ. Sci. Technol. 44, 5306–5312.
Karavalakis, G., Johnson, K.C., Hajbabaei, M., Durbin, T.D., 2016. Application of low-level biodiesel blends on heavy-duty (diesel) engines: feedstock implications on NOx and particulate emissions. Fuel 151, 259–268.
Kawano, D., Mizushima, N., Ishii, H., Goto, Y., Iwasa, K., 2010. Exhaust emission characteristics of commercial vehicles fuelled with biodiesel. SAE Tech. Pap. (2010-01-2276).
Kleeman, M.J., Riddle, S.G., Robert, M.A., Jakober, C.A., 2008. Lubricating oil and fuel contributions to particulate matter emissions from light-duty gasoline and heavy-duty diesel vehicles. Environ. Sci. Technol. 42, 235–242.
Kooter, I.M., van Vugt, M.A.T.M., Jedynska, A.D., Tromp, P.C., Houtzager, M.M.G., Verbeek, R.P., Kadijk, G., Mulderij, M., Krul, C.A.M., 2011. Toxicological characterization of diesel engine emissions using biodiesel and a closed soot filter. Atmos. Environ. 45, 1574–1580.
Kousoulidou, M., Fontaras, G., Ntziachristos, L., Samaras, Z., 2010. Biodiesel blend effects on common-rail diesel combustion and emissions. Fuel 89, 3442–3449.
Kumagai, Y., Koide, S., Taguchi, K., Endo, A., Nakai, Y., Yoshikawa, T., Shimojo, N., 2002. Oxidation of proximal protein sulfhydryls by phenanthraquinone, a component of diesel exhaust particles. Chem. Res. Toxicol. 15, 483–489.
Lammert, M.P., McCormick, R.L., Sindler, P., Williams, A., 2012. Effect of B20 and low aromatic diesel on transit bus NOx emissions over driving cycles with a range of kinetic intensity. SAE Tech. Pap. (2012-01-1984).
Lapuerta, M., Armas, O., Rodriguez-Fernandez, J., 2008. Effect of biodiesel fuels on diesel engine emissions. Prog. Energy Combust. Sci. 34, 198–223.
Lea-Langton, A., Li, H., Andrews, G.E., 2008. Comparison of particulate PAH emissions for diesel, biodiesel and cooking oil using a heavy duty DI diesel engine. SAE Tech. Pap. (2008-01-1811).
Li, N., Venkatesan, M.I., Miguel, A., Kaplan, R., Gujuluva, C., Alam, J., Nel, A., 2000. Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element. J. Immunol. 165, 3393–3401.
Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M.Y., Oberley, T., Froines, J., Nel, A., 2003. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ. Health Perspect. 111, 455–460.
Lim McKenzie, C.H., Ayoko, G.A., Morawska, L., Ristovski, Z.D., Jayaratne, E.R., 2007. Influence of fuel composition on polycyclic aromatic hydrocarbon emissions from a fleet of in-service passenger cars. Atmos. Environ. 41, 150–160.
Magara-Gomez, K.T., Olson, M.R., Okuda, T., Walz, K.A., Schauer, J.J., 2012. Sensitivity of hazardous air pollutant emissions to the combustion of blends of petroleum diesel and biodiesel fuel. Atmos. Environ. 50, 307–313.
McCormick, R.L., Graboski, M.S., Alleman, T.L., Herring, A.M., Tyson, K.S., 2001. Impact of biodiesel source material and chemical structure on emissions of criteria pollutants from a heavy-duty engine. Environ. Sci. Technol. 35, 1742–1747.
Mills, N.L., Torngvist, H., Robinson, S.D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N.A., Donaldson, K., Blomberg, A., Sandstorm, T., Newby, D.E., 2005. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112, 3930–3936.
Mueller, C., Boehman, A., Martin, G., 2009. An experimental investigation of the origin of increased NOx emissions when fueling a heavy-duty compression-ignition engine with soy biodiesel. SAE Int. J. Fuels Lubr. 2, 789–816.
Mutlu, E., Warren, S.H., Matthews, P.P., King, C., Walsh, L., Kligerman, A.D., Schmid, J.E., Janek, D., Kooter, I.M., Linak, W.P., Gilmour, M.I., DeMarini, D.M., 2015. Health effects of soy-biodiesel emissions: mutagenicity-emission factors. Inhal. Toxicol. 27, 585–596.
Na, K., Biswas, S., Robertson, W., Sahay, K., Okamoto, R., Mitchell, A., Lemieux, S., 2015. Impact of biodiesel and renewable diesel on emissions of regulated pollutants and greenhouse gases on a 2000 heavy duty diesel truck. Atmos. Environ. 107, 307–314.
Nel, A.E., Diaz-Sanchez, D., Ng, D., Hiura, T., Saxon, A., 1998. Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system. J. Allergy Clin. Immunol. 102, 539–554.
Prokopowicz, A., Zaciera, M., Sobczak, A., Bielaczyc, P., Woodburn, J., 2015. The effects of neat biodiesel and biodiesel and HVO blends in diesel fuel on exhaust emissions from a light duty vehicle with a diesel engine. Environ. Sci. Technol. 49, 7473–7482.
Ratcliff, M.A., Dane, A.J., Williams, A., Ireland, J., Luecke, J., McCormick, R.L., Voorhees, K.J., 2010. Diesel particle filter and fuel effects on heavy-duty diesel engine emissions. Environ. Sci. Technol. 44, 8343–8349.
Rouce, P., Tsolakis, A., York, A.P.E., 2012. Speciation of particulate matter and hydrocarbon emissions from biodiesel combustion and its reduction by aftertreatment. Fuel 96, 90–99.
Shafer, F.Q., Buettner, G.R., 2001. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med. 30, 1191–1212.
Schönborn, A., Ladommatos, N., Williams, J., Allan, R., Rogerson, J., 2009. The influence of molecular structure of fatty acid monoalkyl esters on diesel combustion. Combust. Flame 156, 1396–1412.
Shinkai, Y., Nakajima, S., Eiguren-Fernandez, A., Di Stefano, E., Schmitz, D.A., Froines, J.R., Cho, A.K., Kumagai, Y., 2013. Ambient vapor samples activate the Nrf2-ARE pathway in human bronchial epithelial BEAS-2B cells. Environ. Toxicol. 25, 1222–1230.
Sun, J., Caton, J.A., Jacobs, T.J., 2010. Oxides of nitrogen emissions from biodiesel-fuelled diesel engines. Prog. Energy Combust. Sci. 36, 677–695.
Tsolakis, A., 2006. Effects on particle size distribution from the diesel engine operating on RME-biodiesel with EGR. Energy Fuel 20, 1418–1424.
US EPA, 2015. 40 CFR Part 80, Renewable Fuel Standard Program: Standards for 2014, 2015, and 2016 and biomass-based diesel volume for 2017; Proposed rule, June 10, 2015.
Verma, V., Ning, Z., Cho, A.K., Schauer, J.J., Shafer, M.M.S., Sioutas, C., 2009. Redox activity of urban quashi-ultrafine particles from primary and secondary sources. Atmos. Environ. 43, 6360–6368.
Westphal, G.A., Krahl, J., Munack, A., Rosenkranz, N., Schroder, O., 2013. Combustion of hydrotreated vegetable oil and jatropha methyl ester in a heavy duty engine: emissions and bacterial mutagenicity. Environ. Sci. Technol. 47, 6038–6046.
Wofsy, S.C., Logan, J.A., Sillman, S., 1990. The sensitivity of ozone to nitrogen oxides and hydrocarbons in regional ozone episodes. J. Geophys. Res. 95, 1837–1851.
Young, L.H., Liou, Y.J., Cheng, M.T., Lu, J.H., Yang, H.H., Tsai, Y.I., Wang, L.C., Chen, C.B., Lai, J.S., 2012. Effects of biodiesel, engine load and diesel particulate filter on nonvolatile particle number size distributions in heavy-duty diesel engine exhaust. J. Hazard. Mater. 199–200, 282–289.
Zhang, Z.H., Balasubramanian, R., 2014. Effect of oxygenated fuels on physicochemical and toxicological characteristics of diesel particulate emissions. Environ. Sci. Technol. 48, 14805–14813.