Chemosphere 147 (2016) 396–403

Contents lists available at ScienceDirect

Chemosphere

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

Evaluation of the reactivity of exhaust from various biodiesel blends as a measure of possible oxidative effects: A concern for human exposure

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HIGHLIGHTS

- Reactivity of exhaust from various biodiesel blends was studied in vitro for oxidative effects.
- Glutathione (GSH) was used as a surrogate for the oxidative health effects of biodiesel exhaust.
- Three different solvents were used to extract particulate matter of biodiesel blends.
- Oxidation of GSH to the disulfide (GSSG) was confirmed using mass spectrometry.
- Decrease in GSH concentrations was observed in the presence of biodiesel exhaust extracts.

ARTICLE INFO

Article history:
Received 9 October 2015
Received in revised form
18 December 2015
Accepted 21 December 2015
Available online 15 January 2016

Handling Editor: David Volz

Keywords:
Diesel particulate matter
Biodiesel blends
Oxidative effect
Glutathione
Fluorophoric agent

ABSTRACT

Diesel exhaust particles (DEP) are a major constituent of ambient air pollution and are associated with various adverse health effects, posing a major safety and public health concern in ambient and occupational environments. The effects of DEP from various biodiesel blends on biological systems was investigated using glutathione (GSH) as a marker of possible oxidative effects, based on the decrease in the concentration of GSH at physiological pH. The fluorophoric agent 2,3-naphthalenedicarboxaldehyde (NDA) was used as a selective probe of GSH in the presence of any likely interferents via fluorescence detection. Three different polar solvents (acetonitrile, methanol and water) were used to extract DEP generated during the combustion of different biodiesel blends (5%–99%). Oxidation of GSH to the disulfide (GSSG) was confirmed using electrospray ionization mass spectrometry.

A decrease in the concentration of GSH was observed in the presence of DEP extracts from all of the biodiesel blends studied, with reaction rates that depend on the biodiesel blend. Interestingly the reactivity peaked at 50% biodiesel (B50) rather than decreasing monotonically with increased biodiesel content, as was expected. Organic solvent DEP extracts showed wider variations in reactivity with GSH, with methanol extracts giving the largest decrease in GSH concentrations. This may imply a more organic nature of the oxidants in the biodiesel exhaust. It is therefore important to consider ways of reducing concentrations of organic components in biodiesel exhaust that can cause different toxic activity before any blend is offered as a preferred alternative to petroleum diesel fuel.

1. Introduction

Diesel fuel is widely used throughout our society in both stationary and mobile applications: it powers the trucks that deliver products to our communities, the buses that carry us to school and work, the agricultural equipment that plants and harvests our food, and backup generators for emergencies. The popularity of the diesel engine, invented by Rudolph Diesel in 1892, as an alternative to the spark-ignition gasoline engine (Gilman-EPA, 2002), has greatly expanded because it offers excellent fuel economy and

Abbreviations: DEP, diesel exhaust particles; GSH, reduced glutathione; GSSG, glutathione disulfide (oxidized glutathione); B5, B25, B50, B75, B99, biodiesel/petroleum diesel blends, where the number indicates the volume percentage biodiesel; ROS, reactive oxygen species; NOx, sum of NO and NO_2 volume mixing ratios; NDA, 2,3-naphthalenedicarboxaldehyde, a fluorophoric agent for GSH; DEE, diesel exhaust extract.

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http://dx.doi.org/10.1016/j.chemosphere.2015.12.074
0045-6535/

durability. However, exhaust from earlier generations of these engines contains substances that pose a risk to human health and has been linked in numerous scientific studies to various diseases including cardiovascular disease (Brook et al., 2010; Vrijheid et al., 2011), pulmonary/respiratory disorders (Vedal, 1997; Siegel et al., 2004; Bernstein, 2012), neurodegradative disease (Cruts et al., 2008; Levesque et al., 2011) and cancer (Stayner et al., 1998; Steenland et al., 1998; IARC, 2012).

The toxicology of DEP is a matter of significant concern and debate in both the political and scientific worlds because exposure to diesel exhaust is widespread; partially due to the prevalence of the engines and partially because the particles and gaseous components can be transported from source to non-source areas. People are exposed to diesel exhaust in both public and occupational environments and exposure levels can be significant in many cases (Sauvain et al., 2003; Zhu et al., 2002; Steenland et al., 1998; Ma and Ma, 2002). The heterogeneous and chemically complex nature of these particles makes delineation of the detailed chemical mechanisms of their toxicity difficult. A large variety of compounds such as aliphatic hydrocarbons, polycyclic aromatic hydrocarbons and their derivatives, heterocyclics, and redox active semi-quinones and transition metals that are able to produce reactive oxygen species (ROS) are found in diesel exhaust (Schuetzle et al., 1981; Madden et al., 2003). The small size of the diesel exhaust particles makes them easily respirable, raising health concerns including lung cancer, allergy, and asthma (McClellan, 1987). Several lines of evidence indicate that ROS generated by diesel exhaust particles and adsorbed compounds play an important role in its adverse effect (Hiura et al., 1999; Sagai et al., 1993; Bai et al., 2001).

The health effects of diesel exhaust and socio-economic concerns have led to recent interest in the use of renewable alternatives. So far, biodiesel has emerged as the most widespread alternative to petroleum-derived diesel fuel (either in full or in part) and this has led to legislation and incentives in a few countries to encourage the use of biodiesel and its blends. For example, in the USA, HR 4520, the Jobs Creation Bill of 2004 provided for more production of biodiesel by offering a federal excise tax credit. By the end of 2005, industrial production was 75 million gallons, a 300% increase in 1 year, and subsequent expansion and new plant construction lead to rapid increases in the industry's capacity (Di et al., 2009; McCormick, 2007; Payri et al., 2009).

Relative to petroleum diesel, biodiesel is often seen as safer and healthier. Its emissions have been shown to contain less particulate matter by mass. As an oxygenated fuel, it presents lower total hydrocarbon (THC) and carbon monoxide (CO) emissions than regular diesel fuel (Graboski et al., 2003; McDonald and Spears, 1997; Sharp et al., 2000) but may produce higher nitrogen oxide (NOx) emissions (Payri et al., 2009; Sendzikiene et al., 2006). The soluble organic fraction of the emitted particles is also commonly a greater percentage in biodiesel exhaust emissions (Durbin et al., 1999). However, recent studies have shown that the physical and chemical characteristics of biodiesel exhaust have certain adverse effects on the environment and may be dangerous to human health (Larcombe et al., 2015; Beer et al., 2007). There is increasing evidence that, whereas the mass of particles produced by combusting biodiesel is characteristically less than that produced from combusting of petroleum diesel, the number concentration and surface area of particles may be greater, thereby producing more severe health effects (Hawley et al., 2014; Mullins et al., 2014; Larcombe et al., 2015; Cheung et al., 2009). Also, the smaller production of particles with a greater concentration of soluble organic fraction may impact the biological effects and toxicity of biodiesel exhaust particles (Swanson et al., 2007).

Oxidative stress in cells is assumed to be one of the factors contributing to the adverse effects of DEP (Shima et al., 2006). Reactive oxygen species (ROS) are known to induce oxidation of molecules such as thiols, proteins, lipids, and DNA, which is associated with damage to cells and elicitation of various biological responses including inflammation (NIOSH, 1988; Donaldson et al., 2003). The response of a cell to oxidative stress typically involves alterations in thiol content (Dickinson and Forman, 2002), so plasma aminothiol concentrations are increasingly being used for clinical and translational research involving oxidative stress (Himmelfarb and Hakim, 2003) and for routine clinical diagnosis of metabolic disorders.

This study focused on the effects of DEP from different blends of biodiesel and petroleum diesel on biological systems using glutathione (GSH) as a marker of oxidative stress. GSH was chosen because it is a key antioxidant metabolite, it is produced in all organs, and its levels and oxidation state are sensitive indicators of cell function and viability. The GSH level in human tissues normally ranges from 0.1 to 10 mM, being most concentrated in the liver (up to 10 mM) and in the spleen, kidney, lens, erythrocytes and leukocytes. It acts as a redox buffer to prevent oxidative damage due to its reducing and nucleophilic properties (Pastore et al., 2003; Cooper and Kristal, 1997). The redox state of a cell can be measured by the relative amounts of the reduced and oxidized forms of glutathione (GSH/GSSG) (Chai et al., 1994).

Several methods have been described in the literature for GSH determination. The most commonly used assay is Ellman's method (Ellman, 1959) which uses the reaction between GSH and 5,5-dithiobis(2-nitrobenzoic acid) to generate 2-nitro-5-mercaptobenzoic acid, that can be monitored spectrophotometrically. Although this assay is inexpensive and simple, it is not sufficiently sensitive or selective for this study. High-performance liquid chromatography (HPLC) with various detection methods (usually requiring post-column derivatization (Buchberger and Winsauer, 1987; Parmentier et al., 1998; Shi et al., 1999)) including spectrophotometry (Raggi et al., 1991) and potentiometry (Compagnone et al., 1991) have also been reported. They often require extraction and pre-concentration steps (Pereira-Rodrigues et al., 2007), and are therefore not suitable in many cases where a rapid and accurate GSH determination is required. Voltammetric sensors have been developed (Wring et al., 1989; Kinoshita et al., 1999; Rover et al., 2001), but application of a large over-potential is usually required (Pereira-Rodrigues et al., 2007).

Fluorescence spectrometry provides an opportunity for sensitive, selective and versatile analytical detection of GSH. However, GSH lacks usable fluorophores so it is necessary to derivatize the compound for fluorescence detection (Koller and Eckert, 1997). Orwar et al. (1995) first reported that NDA could be used to measure GSH with high specificity, because of its ability to rapidly form a stable cyclic reaction adduct with the cysteine sulfhydryl group and glutamyl amino group of GSH (Fig. 1), with sufficient fluorescence yield to measure low concentrations of GSH, while its native fluorescence does not interfere with the detection of GSH. A cyclic compound with similar fluorescence characteristics could also result from reaction of NDA with other compounds that contain appropriately spaced cysteine sulfhydryl and glutamyl amino moieties but this is not found in other biologically-relevant thiols except GSH's precursor, γ-glutamylcysteine (γ-GC). NDA is thus confirmed to be a good fluorogenic dye for sensitive and selective determination of GSH in the presence of other thiols, including the oxidized disulfide form GSSG, and other likely chemical and spectral interferences. For this study, 2,3-naphthalenedicarboxaldehyde (NDA) was used as a specific probe for the determination of GSH via florescence detection.