Environmental Pollution 235 (2018) 514–524

Contents lists available at ScienceDirect

Environmental Pollution

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

Comparative study of diesel and biodiesel exhausts on lung oxidative stress and genotoxicity in rats☆

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ARTICLE INFO

Article history:
Received 17 July 2017
Received in revised form
13 December 2017
Accepted 21 December 2017

Keywords:
Diesel exhaust
Biodiesel
Particles
Lung toxicity
Gene expression

ABSTRACT

The contribution of diesel exhaust to atmospheric pollution is a major concern for public health, especially in terms of occurrence of lung cancers. The present study aimed at addressing the toxic effects of a repeated exposure to these emissions in an animal study performed under strictly controlled conditions. Rats were repeatedly exposed to the exhaust of diesel engine. Parameters such as the presence of a particle filter or the use of gasoil containing rapeseed methyl ester were investigated. Various biological parameters were monitored in the lungs to assess the toxic and genotoxic effects of the exposure. First, a transcriptomic analysis showed that some pathways related to DNA repair and cell cycle were affected to a limited extent by diesel but even less by biodiesel. In agreement with occurrence of a limited genotoxic stress in the lungs of diesel-exposed animals, small induction of γ-H2AX and acrolein adducts was observed but not of bulky adducts and 8-oxodGuo. Unexpected results were obtained in the study of the effect of the particle filter. Indeed, exhausts collected downstream of the particle filter led to a slightly higher induction of a series of genes than those collected upstream. This result was in agreement with the formation of acrolein adducts and γH2AX. On the contrary, induction of oxidative stress remained very limited since only SOD was found to be induced and only when rats were exposed to biodiesel exhaust collected upstream of the particle filter. Parameters related to telomeres were identical in all groups. In summary, our results point to a limited accumulation of damage in lungs following repeated exposure to diesel exhausts when modern engines and relevant fuels are used. Yet, a few significant effects are still observed, mostly after the particle filter, suggesting a remaining toxicity associated with the gaseous or nano-particular phases.

1. Introduction

Several works, such as the multicenter study "Air Pollution and Health, a European Approach" carried out in 15 European cities on a total population of 25 million inhabitants, together with other investigations (Abbey et al., 1999; Künzli et al., 2000; Hoek et al., 2002; Aga et al., 2003), have associated atmospheric pollution with an increased mortality resulting from cardiovascular and/or

☆ This paper has been recommended for acceptance by Dr. Harmon Sarah Michele.

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https://doi.org/10.1016/j.envpol.2017.12.077
0269-7491/

respiratory causes. In addition, a study carried out on a large scale in the USA (10 years follow-up of a 4 million inhabitants population in 51 cities) demonstrated in an unambiguous way that the decrease in pollution observed between the years 1980 and 2000 was associated with a reduced mortality corresponding to an increase gain in survival of 15% (Pope et al., 2009).

Pollution produced by automobile traffic is one of the main sources of pollutants in urban atmosphere and is largely accounted for by the exhausts of the diesel engine powered vehicles. Exhausts of diesel engines are complex mixtures of toxic substances that are difficult to study from a toxicological point of view as the results of a difficult extensive characterization of the pollutants, the difficult sampling, the synergy between the different compounds and the wide variety of biological effects. Among the main components of diesel and biodiesel exhausts (Agarwal et al., 2011; Lanjekar and Deshmukh, 2016), one can find carbon monoxide and dioxide, nitrogen and sulphur oxides, volatile and particulate polycyclic aromatic hydrocarbons (PAHs) (Scheepers and Bos, 1992) and particulate matter (soot) (Tree and Svensson, 2007). Exhausts from diesel engines may account for the presence of 13% of the mass of the ambient particles exhibiting a diameter below 10 μm (PM_{10}) (CITEPA, 2017). This contribution is slightly higher (16%) for fine particles with a diameter below 2.5 μm ($PM_{2.5}$) and for ultrafine particles with a diameter below 0.1 μm ($PM_{0.1}$).

These carbon particles are small enough to be inhaled and trapped in depth in the lungs. They exhibit a large specific area that makes possible the adsorption of organic compounds such as PAHs known to be toxic and carcinogenic (Kagawa, 2002). The genotoxicity and mutagenicity of PAHs and of their nitrated derivatives present in diesel exhausts have been extensively studied. Such effects have been linked, at least in part, to PAHs binding to the cytoplasmic aryl hydrocarbon receptor (AhR), thereby triggering its nuclear translocation and interaction to its partner, i.e., the aryl hydrocarbon receptor nuclear translocator (ARNT). The complex AhR/ARNT next binds to xenobiotic responsive elements found in the promoter of PAH-regulated genes, such as phase I enzymes (e.g. cytochrome P450 family) metabolizing PAHs. As a result, molecules such as benzo [a]pyrene (B[a]P), 1,8-dinitropyrene, 1,6-dinitropyrene and 3-nitrobenzanthrone, present in soot of diesel engine exhausts, are converted into strongly mutagenic metabolites upon cytochrome P450-mediated biotransformation, leading to the formation of DNA adducts (Hilario et al., 2002; Arlt, 2005) and induction of oxidative stress.

In addition to its effects on metabolism and DNA adducts formation, AhR is considered to act as a regulator of the immune system. Indeed, B[a]P-activated AhR leads to cytokine secretion (Fardel, 2013) and lung inflammation (Podechard et al., 2008). PAHs-containing diesel particle extract exposure alters macrophage phenotype and their cytokine secretion ability (Jaguin et al., 2015). Moreover, pulmonary inflammation has been shown to impact B[a]P genotoxicity in the lung (Arlt et al., 2015), underlining the link between chronic inflammation and lung carcinogenesis (Tsay et al., 2013).

Yet, most of the genotoxicity studies performed to date involved either exposure of *in vitro* models to unique doses of organic extracts or *in vivo* exposure to components of these exhaust such as 3-nitrobenzanthrone (Nagy et al., 2005). A few pieces of information are available on the formation of DNA adducts and 8-oxo-7,8-dihydroguanine (8-oxoGua) following chronic or sub-chronic *in vivo* exposure by inhalation of diesel particles (Bond et al., 1990; Ichinose et al., 1997; Iwai et al., 2000). Intra-tracheal injection of diesel exhausts particles was also found to lead to the formation of 8-oxoGua in mice lungs (Ichinose et al., 1997). Other organic molecules such as aldehydes, quinones and benzene may also be emitted in large amounts and thus contribute to the toxicity of diesel exhaust.

In order to take into consideration the health impact of automobile pollution due to diesel engines, several strategies aimed at limiting the exhausts have been designed, either related to the engines technologies (injection pressure and or internal aerochemistry), the addition of fuels (for example cerium oxide) or after-treatment by oxidation catalysis and/or filtration of the emissions (Khair et al., 2009; McClellan et al., 2012). Yet, the consequences in terms of quantity and properties of the emitted pollutants as well as their toxicological impact remain largely unknown (McClellan et al., 2012; Khalek et al., 2015). It is also important in the context of these technological developments to study the influence of the use of biofuels in fossil fuels and to determine their possible impact on the toxicology of gaseous and particulate emissions. To date, few studies concerning the toxicological effects of the biodiesel emissions have been published. Soy-based biofuels have been recently studied suggesting that biodiesel and diesel emissions have comparable toxicity for many cardiopulmonary endpoints (Bass et al., 2015; Madden, 2016). Contrasted conclusions on mutagenic effects of rapeseed-methyl ester supplemented fuels have been reported, stressing the need for further toxicological assessments (Bunger et al., 2007; Westphal et al., 2013; André et al., 2015).

Through a multidisciplinary approach associating motorists and toxicologists, the present animal study was designed to extensively describe the effects of diesel exhausts in the lungs repeatedly exposed compared to biodiesel. The second aim was to verify the efficiency of the depollution systems of engines at protecting from genotoxicity and oxidative stress.

2. Experimental part

2.1. Exhaust generation

The engine used for the production of aerosols of interest was a Euro4-compliant supercharged common rail direct injection diesel engine, representative of the pool of vehicles found nowadays in the French car fleet. It was placed in a test bench cell equipped with a dynamic asynchronous chassis dyno which allows real time control of both engine speed and load, as well as the continuous recording of a series of technical parameters relevant for the characterization of the engine's functioning. Two typical depollution situations were compared on the exhaust line of the engine: either a combination of an oxidation catalyst and a diesel particulate filter (DPF) or the oxidation catalyst only. For this purpose, exhausts were collected either upstream (P1) or downstream (P2) of the DPF (Scheme 1). For these two investigated situations, the fuel used was reference gasoil (RF0) containing less than 3 ppm of sulphur (current quality in gas station). Gasoil containing 30% of rapeseed methyl ester (BD30) was also studied. The engine was used under dynamic conditions of urban parts of the New European Driving Cycle (NEDC) in order to mimic emissions produced in urban driving. Emission from internal combustion engines were drawn directly from the exhaust line. The primary dilution by a factor 10 was performed by a Dekati® Fine Particle Sampler (Dekati Finland). Table 1 shows the average concentration of regulated pollutants in raw exhausts during NEDC cycling before dilution and exposure.

2.2. Exposure

This project was reviewed and approved by a certified comity according to European legislation (authorization number 00291.01). Male Wistar rats (Janvier, France) were randomly divided into 6 groups (n = 6 per experimental group): two control