Mutation Research 777 (2015) 33–42

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

# 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

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ARTICLE INFO

*Article history:*
Received 15 July 2014
Received in revised form
27 November 2014
Accepted 29 November 2014
Available online 2 December 2014

*Keywords:*
Diesel exhaust
Aftertreatment devices
Mutagenicity
Genotoxicity
Oxidation catalysis
Particulate filter

ABSTRACT

Diesel exhausts are partly responsible for the deleterious effects on human health associated with urban pollution, including cardiovascular diseases, asthma, COPD, and possibly lung cancer. Particulate fraction has been incriminated and thus largely investigated for its genotoxic properties, based on exposure conditions that are, however, not relevant for human risk assessment. In this paper, original and more realistic protocols were used to investigate the hazards induced by exhausts emitted by the combustion of standard (DF0) *vs.* bio-diesel fuels (DF7 and DF30) and to assess the impact of exhaust treatment devices (DOC and DPF). Mutagenicity and genotoxicity were evaluated for (1) resuspended particles ("off line" exposure that takes into account the bioavailability of adsorbed chemicals) and for (2) the whole aerosols (particles + gas phase components) under continuous flow exposure ("on line" exposure). Native particles displayed mutagenic properties associated with nitroaromatic profiles (YG1041), whereas PAHs did not seem to be involved. After DOC treatment, the mutagenicity of particles was fully abolished. In contrast, the level of particle deposition was low under continuous flow exposure, and the observed mutagenicity in TA98 and TA102 was thus attributable to the gas phase. A bactericidal effect was also observed in TA102 after DOC treatment, and a weak but significant mutagenicity persisted after DPF treatment for bio-diesel fuels. No formation of bulky DNA-adducts was observed on A549 cells exposed to diesel exhaust, even in very drastic conditions (organic extracts corresponding to 500 μg equivalent particule/mL, 48 h exposure). Taken together, these data indicate that the exhausts issued from the bio-diesel fuels supplemented with rapseed methyl ester (RME), and generated by current diesel engines equipped with after treatment devices are less mutagenic than older ones. The residual mutagenicity is linked to the gas phase and could be due to pro-oxydants, mainly for RME-supplemented fuels.


## 1. Introduction

Urban air pollution has deleterious effects on human health, including cardiovascular diseases, asthma, COPD, and lung cancer [1–4]. Vehicle exhausts are responsible for a large part of this urban pollution, and diesel engines are more specifically incriminated, since they produce higher amounts of inhalable particles than gasoline engines, associated with toxic gases such as $NO_x$ and

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http://dx.doi.org/10.1016/j.mrgentox.2014.11.007
1383-5718/

aldehydes. Moreover, genotoxic and carcinogenic combustion by-products such as PAHs and nitro-PAHs are adsorbed onto particles surface. Diesel exhausts carcinogenicity was recently re-evaluated by IARC, and classified as Group1, *i.e.*, carcinogenic to humans, based on "sufficient evidence that exposure is associated with an increased risk for lung cancer" [5]. This new classification is mainly based on occupational exposure to heavy-duty engines, especially among a large cohort of underground miners, *i.e.*, in a confined environment [6,7]. Even if this decision sparked a controversy [8], it has been estimated that public health could also be compromised, particularly in developing countries, considering the many industrial applications of diesel engines [5].

Policies aiming at air quality improvement in more developed countries have led to the strengthening of the legislation through the setting-up of successive standards [9]. Diesel-fuel quality, engine technologies and exhaust after treatment devices (diesel oxidation catalysis–DOC and diesel particulate filters–DPF) were thus significantly improved over the last two decades. As a consequence, toxic pollutant emissions associated with diesel exhaust gases have generally been declining as observed by Pronk et al. [10], despite the continuous increase in the use of diesel engines. In parallel to these technical evolutions, blends of fossil fuels and renewable fuels are now used, both for economic and ecological purposes. However, the introduction of these new fuels and new after treatment devices requires a comprehensive investigation of the possible current hazards linked to their use, and an accurate evaluation of their impact on human health.

Bacterial mutagenicity and DNA adducts formation have been frequently used as a paradigm of the carcinogenic potential of diesel engine emitted particles (DEP). However, data have been mainly obtained after exposure to organic extracts (OE), which are not representative of the real and current exposure of European urban populations.

Therefore, the present study is a contribution to the *in vitro* re-evaluation of the mutagenicity and genotoxicity assessment of diesel exhausts emitted by a current diesel engine fuelled with standard diesel fuel and RME-blends, using some more realistic exposure conditions. Hence, particles alone were first resuspended in an aqueous culture medium, in order to take into account the bioavailability of compounds adsorbed onto particles. Then, the whole aerosol was studied in dynamic conditions (continuous flow of particles associated with the gas phase), using various biological models (*Salmonella typhimurium*, A549 human cells and organotypic rat lung slices) exposed in biphasic conditions (air-liquid interface, ALI).

Comparisons with data obtained from classical exposure to the corresponding OE were performed in order to demonstrate the relevance of these protocols for the evaluation the genotoxic impact after inhalation of aerosols.

The genotoxicity of a standard diesel fuel and RME-blends (7% and 30% RME) was compared, and the effects of exhaust after treatment devices (DOC and DPF) on the aerosol toxicity were also investigated.

All together, these data could contribute to the health risk assessment of current diesel exhausts.

## 2. Materials and methods

### 2.1. Diesel particles (DEPs) sampling

A 2 L direct injection turbocharged intercooled diesel engine (corresponding to Euro3 standard), representative of the majority of the French car park in 2010, was placed on a test bench. It was used according to the urban section of the ARTEMIS cycle in order to mimic emissions produced in urban driving conditions. The fuels used were a reference diesel fuel (DF0) containing less than 50 ppm of sulphur (current quality in station), and two diesel fuels supplemented with rapeseed methyl ester (RME) at 7% (DF7) and 30% (DF30), respectively. DEPs were sampled directly downstream of the diesel engine or downstream of the oxidation catalyst.

### 2.2. Preparation of organic extracts

Organic extracts were obtained from 200 mg of DEPs, using a soxhlet apparatus and 100 mL dichloromethane (DCM) as a solvent over 24 h. The organic phase was either concentrated under a nitrogen stream for GC–MS analysis, or evaporated. The dry residue was dissolved into DMSO for mutagenic and genotoxic potential evaluations.

### 2.3. Chemical analysis of organic extracts

OE were analysed with GC–MS (VARIAN 1200 TQ model). The materials used for compound identification and quantification were purchased from Sigma–Aldrich (St Quentin Fallavier, France). Hydrocarbons identifications were assigned by comparing the retention times of chromatographic peaks from samples with those from standard mixtures and by comparing mass spectra with those contained in NIST and/or WILEY libraries. The estimated quantification limit (QL) was 0.1 μg/g particles.

### 2.4. Mutagenicity of organic extracts

The mutagenicity of OE was evaluated in overnight cultures of 3 *Salmonella typhimurium* tester strains TA98, YG1041, and TA102, without (−S9mix) and with (+S9mix) Aroclor-induced S9 addition, using the preincubation method previously described [11]. Revertant colonies were automatically counted (Ames software, Noesis, France) after 48-h incubation at 37 °C. For each sample, four concentrations (2, 10, 50, and 200 μg equivalent-particles/plate) were tested in triplicate. Toxicity was evaluated in parallel through microscopic observation of the background lawn density. Results were expressed as the mean of at least two independent experiments.

Positive controls were 2-nitrofluorene (2NF) 2.5 μg/plate (−S9mix) and 2-aminofluorene (2AF) 0.5 μg/plate (+S9mix) for TA98; 2NF 1.25 μg/plate (−S9mix) and 2AF 0.1 μg/plate (+S9mix) for YG1041 and terbutylhydroperoxyde 2 μmol/plate for strain TA102 (−S9mix).

### 2.5. Mutagenicity of resuspended particles ("off line" mutagenicity)

The DEPs were resuspended in either DMSO or PBS with 0.04% tween, in order to limit the formation of aggregates in this aqueous solvent. The mutagenicity of these suspensions was evaluated using the procedure described above, in a range of concentrations from 5 to 75 μg/plate for DEPs in DMSO and from 5 to 100 μg/plate for DEPs in PBS.

### 2.6. Mutagenicity of whole aerosols ("on line" mutagenicity)

The Ames test procedure was adapted for a 6-well plate protocol to evaluate the mutagenicity of the whole aerosols. For TA98 and YG1041 strains, a VBE medium was complemented with an Histidin + Biotin (0.5 mM) solution (2.5 mL in 100 mL), and introduced in 6-well plates (8 mL/well). Fifty micro liter (TA98) or 40 μL (YG1041) of overnight cultures were then poured onto the agar surface. For the TA102 strain, a VBE medium was first distributed into wells, then 7.5 μL of a (Histidin + Biotin) 0.5 mM solution was