Most of the motorized road vehicles are powered by internal combustion engines. As these engines use fossil fuels, they represent a major source of air pollution, particularly in metropolitan areas. Recently, various fuel alternatives, both for diesel and gasoline, have been introduced. However, their effect on concentrations of air pollutants and toxicity of emissions is not well characterized. We studied chemical composition and genotoxicity of organic extracts from extractable organic matter (EOMs) obtained from emissions from various blends of biodiesel with diesel and gasoline. For diesel engines, we studied emissions from standard diesel (BO), diesel fuel with 30% biodiesel (B30), biodiesel only (B100) and a new generation biodiesel (NEXBTL100). We tested two types of gasoline engines: Direct Injection Spark Ignition (DISI; operated using standard gasoline, gasoline with 15% ethanol (E15), 25% n-butanol and 25% iso-butanol) and Multi-Point injection engine (MPI; operated using standard gasoline and E15). In acellular calf thymus DNA system we analyzed induction of bulky DNA adducts and oxidative DNA damage.

**Aims of the Study**

We aimed to compare the following parameters for diesel and gasoline engines that used standard and alternative fuels:

1. emissions of polycyclic aromatic hydrocarbons (PAHs) and PAH-derivatives
2. levels of bulky DNA adducts induced by organic extracts from particulate matter (PM) generated by the engines in the acellular calf thymus (CT) DNA system in the presence of microsomal S9 fraction
3. levels of oxidative DNA damage (8-oxodG) induced by organic extracts from PM in the acellular system

**Methods**

**Engines**

- Disi: brake tractor 5.91, 176 kW
- Gasoline: Direct Injection Spark Ignition (DISI; Ford EcoBoost 1.0, 92 HP)

**Fuels**

- Diesel: B0 – standard diesel, B30 – diesel fuel with 30% biodiesel, B100 – biodiesel only, NEXBTL100 – a new generation biodiesel
- Gasoline: DISI engine: standard/gasoline, E15 – gasoline with 15% ethanol, gasoline with 25% n-butanol, gasoline with 25% iso-butanol. MPI engine: standard/gasoline, E15
- Diesel: World Harmonized Transient Cycle (WHTC)
- Gasoline: Arteria Urban, Arteria Rural, Arteria Motorway 130 Cycles

**Sampling**

- Raw exhaust were diluted by constant-volume sampler to 50 m³/min for diesel and 11 m³/min for gasolines. Emissions were sampled by 110/14 sampler Ecotech 3000 (7.0 m³/h flow rate). Organic extracts (EOMs) were prepared from PM using dichloromethane.

**Test cycle**

- Calf thymus DNA + rat liver microsomal S9 fraction + EOMs (50, 100, 250 µg PM/ml), incubation at 37 °C for 24 h.
- Bulky DNA adduct levels: β-erythroid bone marrow with nuclease P1 extraction.
- Oxidative DNA damage: 8-oxodeoxyguanosine (8-oxodG) analyzed using competitive ELISA with N4,S1 primy antibody; expressed as relative level of DNA oxidation (relative DNA oxidation level of the control sample = 1.0).

**Conclusions**

1. Blends of alternative and standard fuels may result in production of EOMs containing high concentrations of PAHs.
2. The highest levels of bulky DNA adducts were observed for EOMs generated by the DISI engines regardless the fuel used.
3. Oxidative potential of tested EOMs was relatively low and mostly comparable for the engines and fuels tested. EOMs from the MPI engines induced highest oxidative DNA damage levels.

**Results**

1. EOMs from biodiesel fuels B30 and B100 contained significantly more PAHs and their derivatives than standard diesel or NEXBTL100 (Fig 1-A-D).
2. The DISI engines generated mostly comparable amount of PAHs and their derivatives per mg PM regardless fuel used (Fig. 1-A-D). The MPI engines generated highest levels of carcinogenic PAHs when gasoline + 15% ethanol was used (Fig. 1-A).
3. Levels of bulky DNA adducts induced by EOMs from diesel and biodiesel fuels were comparable (Fig. 2). 4. EOMs from emissions from the DISI engines induced several-fold higher levels of bulky DNA adducts when compared with the MPI engines (Fig. 2). 5. Oxidative DNA damage was comparable in samples from diesel and biodiesel, with the exception of B100 where no induction of oxidative damage was observed (Fig. 3). 6. EOMs from most emissions from the DISI engines induced low levels of oxidative DNA damage; the MPI engines generated EOMs with slightly higher oxidative potential (Fig. 3).

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