

# Comparison of genotoxicity of exhaust from a diesel, biodiesel and rapeseed oil powered engine – pilot study

J. Topinka<sup>1</sup>, A. Milcova<sup>1</sup>, J. Schmuczerova<sup>1</sup>, M. Mazac<sup>2</sup>, M. Pechout<sup>2</sup>, M. Vojtisek-Lom<sup>2</sup>

<sup>1</sup>Department of Genetic Ecotoxicology, Institute of Experimental Medicine AS CR, Prague, 142 20, Czech Republic

<sup>2</sup>Department of Vehicles and Engines, Technical University of Liberec, Liberec, 461 17, Czech Republic

Keywords: diesel exhaust, biofuels, particulate matter, genotoxicity.

Presenting author email: jtopinka@biomed.cas.cz

## Introduction

Last decades are characterized by massive use of alternative fuels, including biofuels. Since the reports on the toxic effects of exhaust from engines powered by biofuels are often contradictory, it might be of great interest to compare genotoxicity of standard diesel particulate emissions with that of the most frequently used biofuels. For this purpose we performed the pilot study with the aim to identify possible genotoxicity induced by organic extracts from the samples of exhaust of engines running on diesel fuel, biodiesel (neat methylester of rapeseed oil) and neat heated, fuel-grade rapeseed oil. The engines were tested in a laboratory using engine dynamometers.

## Methods and Results

In one set of tests, a Zetor tractor engine with an inline mechanical injection pump and no exhaust gas aftertreatment device was tested using the NRSC cycle (also the ISO-8178 test with C-1 weighing, normally used for certification of non-road engines) and the ISO-8178 test with C-2 weighings, representing low-load operation. A sample of undiluted exhaust was drawn through a cartridge with a fluorocarbon-coated filter and two polyurethane foam plugs, with 2.0-3.5 m<sup>3</sup> of exhaust sampled. As a marker of the genotoxic potential, DNA adduct levels induced by extractable organic matter (EOMs) in an acellular assay of calf thymus DNA coupled with <sup>32</sup>P-postlabeling in the presence and absence of microsomal S9 fraction (contains enzymes for metabolic activation of genotoxic compounds such as PAHs) were employed. Simultaneously, chemical analysis of 16 priority PAHs in EOMs, including 7 carcinogenic PAHs (US EPA) was performed. The results suggest that on ISO-8178 non-road engine test cycle, C-2 schedule, representing low engine loads, the organic extract from standard diesel particulate emissions induces highest DNA adduct levels (53 adducts/10<sup>5</sup> nucleotides/ kWh), while rapeseed oil and methyl esters of rapeseed oil induce 5.8 and 2.4 adducts/10<sup>5</sup> nucleotides/ kWh, respectively. These results correlate with the content of carcinogenic PAHs (c-PAHs) and B[a]P in the corresponding EOMs.

In a second set of tests, the exhaust was routed to the laboratory main exhaust duct, which has served as an improvised full-flow dilution tunnel, with dilution ratio of approximately 1:100 at idle to 1:15 at full load. From this duct, diluted exhaust was sampled with high-volume samplers (Digitel) on the Teflon coated filters (Pallflex) normally used for ambient air quality measurements, at rates 500-1000 litres per minute,

with a target accumulation on the order of 10 mg of particulate mass. Two engines were tested. One was a Cummins ISBe4 engine with a Common Rail fuel injection system and no exhaust gas aftertreatment device, tested using the World Harmonized Stationary Cycle (WHSC) and modified Engine Stationary Cycle (ESC). The ESC cycle was modified by altering the length of each of the 13 modes and including transitions between modes to facilitate continuous sampling. The other engine was the Zetor engine described above, which was tested using the NRSC cycle. Filters were extracted by dichlormethane and genotoxicity of extracts was analyzed by <sup>32</sup>P-postlabelling of DNA adducts by test described in the previous paragraph. Major results are described in Table 1.

Table 1. Genotoxicity of the organic extracts from particulate emissions of selected fuels

Engine fuel injection	Fuel /cycle	PM mg/kWh	7c-PAH µg/kWh*	DNA add. /10 <sup>8</sup> n./kWh**	
				+S9	-S9
Cummins ISBe4	D <sup>1</sup> /WHSC	6.9	0.59	271	119
	R <sup>2</sup> /WHSC	7.2	0.77	198	16
Common Rail	D <sup>1</sup> /ESC	14.1	2.05	648	175
	R <sup>2</sup> /ESC	23.8	1.76	473	182
	B <sup>3</sup> /ESC	20.2	1.63	542	182
Zetor 1505	D <sup>1</sup> /NRSC	185	18.33	3678	1038
	R <sup>2</sup> /NRSC	202	11.39	2950	1096

\*7 carcinogenic PAHs: benzo(a)anthracene, chrysene, benzo(b)fluoran-thene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene; \*\*DNA adducts/10<sup>8</sup> nucleotides/kWh; <sup>1</sup>D-diesel; <sup>2</sup>R-rapeseed oil; <sup>3</sup>B-biodiesel B-100

## Conclusions

1. The emissions of classic diesel contain more of total PAHs, but much less B[a]P and other carcinogenic PAHs.
  2. Genotoxicity of particulate emissions of selected biofuels is comparable with a classic diesel.
  3. Metabolic activation (+S9) resulted in several fold higher genotoxicity suggesting major contribution of PAHs to the DNA adduct levels. However, directly acting genotoxicants (-S9) are also significant.
  4. Genotoxicity is highly dependent on the test cycle (ESC vs. WHSC).
  5. Genotoxicity of the emissions is dose dependent.
- These results should be taken as preliminary and more detailed study is going on to verify these preliminary findings.

**Acknowledgements:** Supported by the LIFE+ project MEDETOX (LIFE10ENV/CZ/651) and by the Czech Science Foundation grants #101/08/1717 (rapeseed oil combustion) and P503/11/0142 (toxicology).