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Comparison of Genotoxicity of Exhaust from a Diesel, Biodiesel and Rapeseed Oil Powered Engine – pilot study

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Introduction

It is generally accepted that genotoxic effects of the combustion generated particles are mainly connected with carcinogenic polycyclic hydrocarbons (c-PAHs) and their derivatives, constituents of the organic fraction of the particulate matter (PM) emissions. These compounds are present in exhaust gases of internal combustion engines and originate from the combustion of fuel and engine lubricating oil. 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^3 of exhaust sampled. DNA adducts were analyzed by ^{32}P-postlabelling method in cell free assay consisting of calf-thymus DNA. 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 ^{32}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 B[a]P was performed. The results suggest that on ISO-8178 non-road engine test cycle, C-2 schedule, representing low engine loads, 100 μg/ml of the organic extract from standard diesel particulate emissions induces highest DNA adduct levels (10.5 adducts/10^8 nucleotides), while rapeseed oil and methyl esters of rapeseed oil induce 3.2 and 0.5 adducts/10^8 nucleotides, respectively. These results correlate with the content of carcinogenic 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 ^{32}P-postlabelling of DNA adducts by test described in the previous paragraph. The results are summarized in Table 1.

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

Engine fuel injection	Test fuel	Test cycle	PM mass [mg/kWh]	B[a]P ng/kWh	DNA adducts/ 10^8 nucleotides/kWh +S9	DNA adducts/ 10^8 nucleotides/kWh –S9	DNA adducts/ 10^8 nucleotides/mg +S9	DNA adducts/ 10^8 nucleotides/mg –S9
Cummins ISBe4 Common Rail	Diesel	2 x WHSC	**6.9**	**3.5**	**217**	**96**	**31.5**	**13.9**
	Rapeseed oil	2 x WHSC	**7.2**	**4.9**	**159**	**13**	**22.1**	**1.8**
	Diesel	4xESCmod*	**14.1**	**<2.5**	**541**	**140**	**38.2**	**9.9**
	Rapeseed oil	4xESCmod*	**23.8**	**11.1**	**378**	**145**	**15.9**	**6.1**
	B-100	2xESCmod*	**20.2**	**7.3**	**433**	**145**	**21.4**	**7.2**
	Diesel	2xESCmod*	**30.7**	**2.5**	**517**	**228**	**16.8**	**7.4**
Zetor 1505 inline pump	Rapeseed oil	1 x NRSC	**202**	**1.36**	**2351**	**874**	**11.7**	**4.3**
	Diesel	1 x NRSC	**185**	**<0.37**	**2932**	**828**	**15.9**	**4.5**

*] ESCmod cycle: 13-mode on-road engine ESC cycle with duration of each mode proportional to its weight and 20-s transitions between modes, with a total length of 1000 s

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 (data not shown).

These results should be taken as preliminary and more detailed study is going on to verify these preliminary findings.

Acknowledgements: Supported by the Czech Ministry of the Education, Youth and Sports grants #1M0568 (engine tests) and #2B08005 (toxicology) and by the Czech Science Foundation grants #101/08/1717 (rapeseed oil combustion) and P503/11/0142 (toxicology).

Comparison of Genotoxicity of Exhaust from a Diesel, Biodiesel and Rapeseed Oil Powered Engine (Pilot Study)

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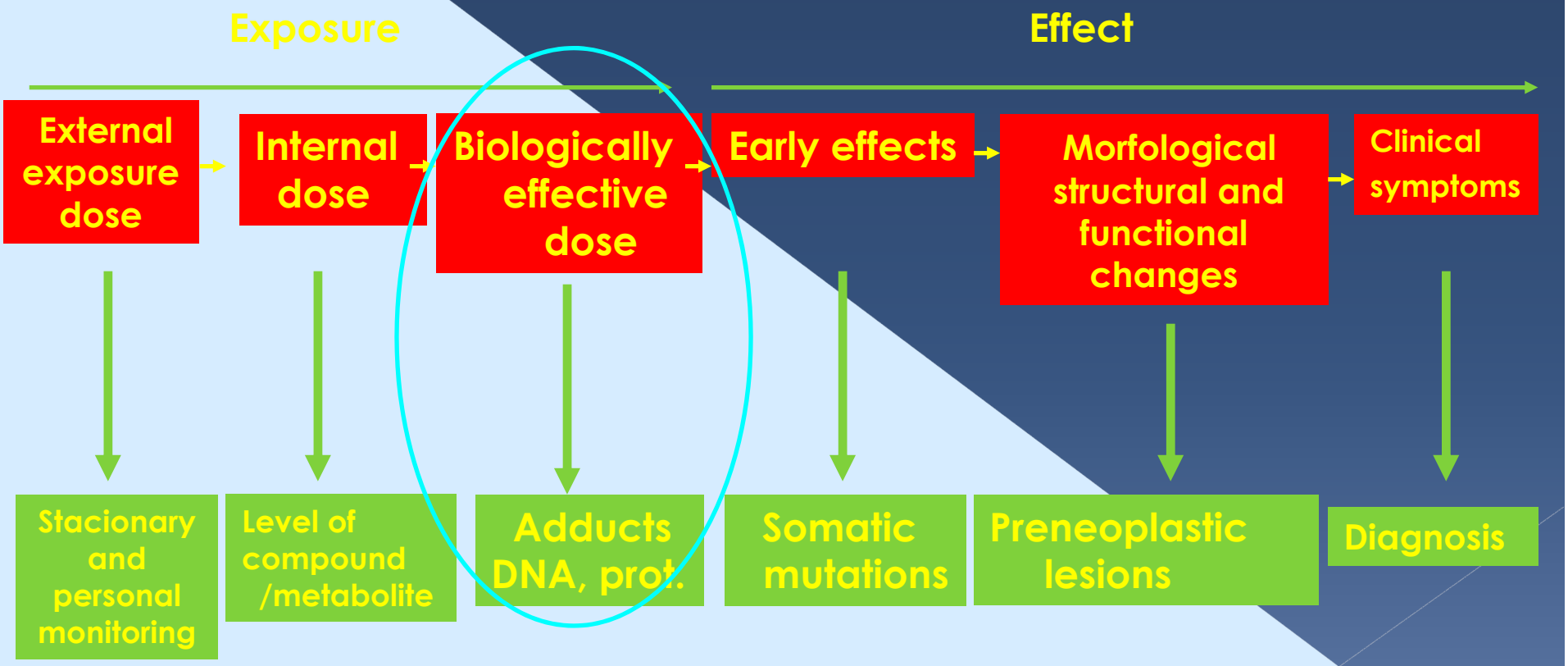
Why to study toxicity of exhaust from biofuels?

- "bio"- in general public implicates something natural, ecological and harmless to human health…
- The extensive use of biofuels might be connected with some risks to human health…These risks are difficult to assess without corresponding toxicity testing of biofuel exhaust…
- Knowledge of chemical composition of exhaust from biofuels is important, but not sufficient precondition to assess the risk connected with use of some biofuels.

What is genotoxicity ?

- Genotoxicity is defined as ability of specific factor to damage, mainly chemically, DNA.
- Most frequent genotoxic event is covalent binding of the chemical or its metabolite with nucleotides in DNA – DNA adduct…
- Genotoxic effect is the first event of the multistep process of chemical carcinogenesis.

Multistep process of chemical carcinogenesis
Significance of DNA adducts



How to analyze genotoxicity of complex mixtures ?

Number of different approaches…

Our approach to measure genotoxicity:

DNA reactivity of organic compounds bound on particulate emissions collected on filters

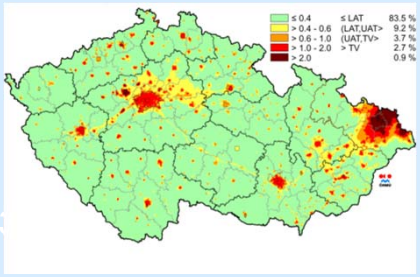
Acellular assay coupled with ^{32}P-postlabelling based on the DNA adduct forming activity of the mixtures in native DNA with/without metabolic activation by rat liver microsomal S9 fraction

Why we analyze organic extracts and PAHs ?

Locality	Sampling period	Air volume [m³]	$PM_{2,5}$ [µg/m³]	B[*a*]P [ng/m³]	c-PAHs* [ng/m³]
Ostrava-Bartovice	03/2009	29 900	36.7	**13.6**	**81.6**
Ostrava-Poruba	03/2009	35 200	25.8	4.28	27.2
Karvina	04/2009	47 400	n.a.	1.88	12.1
Trebon	11-12/2008	44 700	11.4	1.11	7.92

*c-PAHs include: benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene

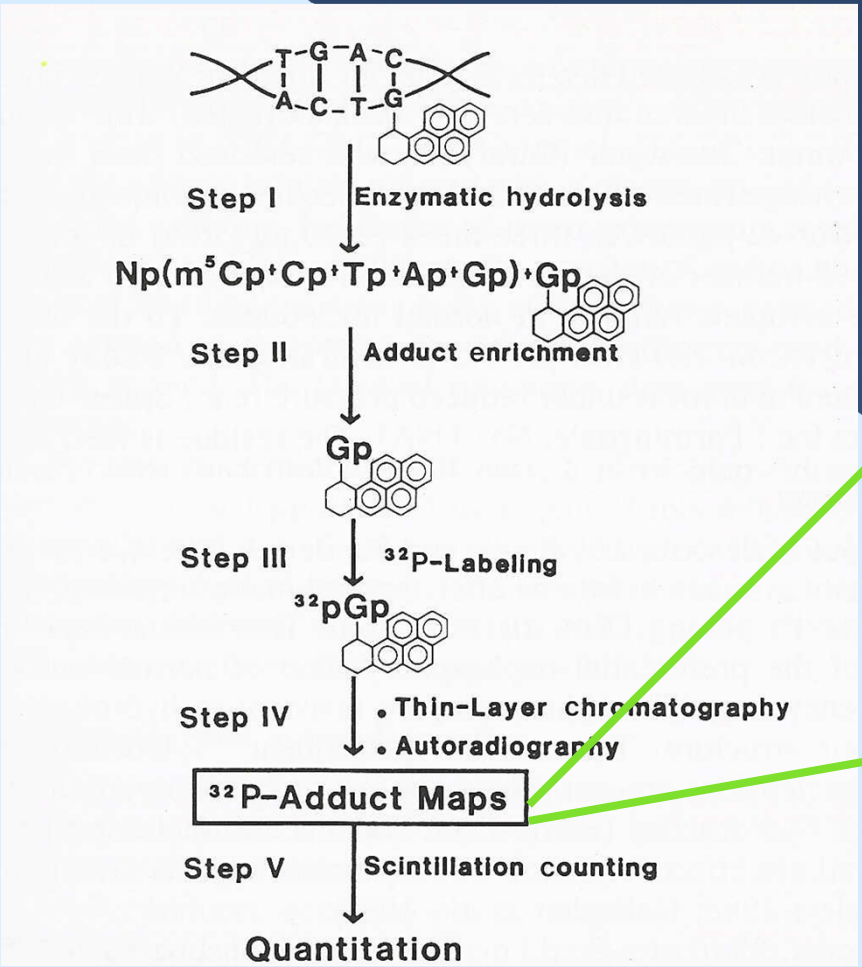
Winter inversion in January 2010
- **BaP: 30-100 ng/m³**
- **PM2.5: 500-700 µg/m³**



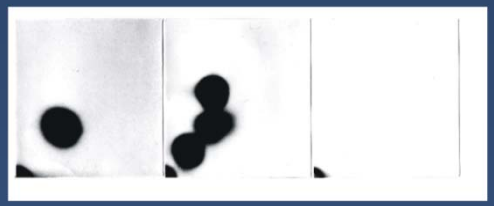
Acellular assay of genotocity

- Calf thymus DNA is incubated with with various doses of organic extracts from filters with collected exhaust particles for 24 h at 37 °C with and without metabolic activation using the rat liver microsomal S9 fraction (1 mg protein/ml).
- B[a]P and DMSO treated calf thymus DNA samples are used as positive and negative controls, respectively.
- DNA is isolated and ^{32}P-postlabelling is performed.

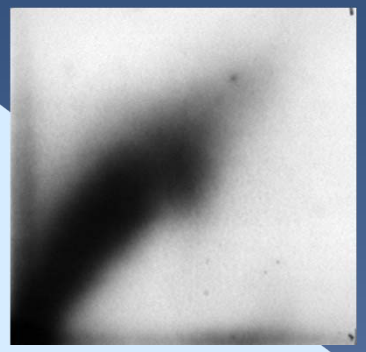
Analysis of DNA adducts (^{32}P-postlabelling)



B[a]P DB[a,l]P Control



Individual compounds



Complex mixtures
(extracts from PM)

Pilot study

Comparison of genotoxicity of particulate emissions from classic diesel and selected biofuels

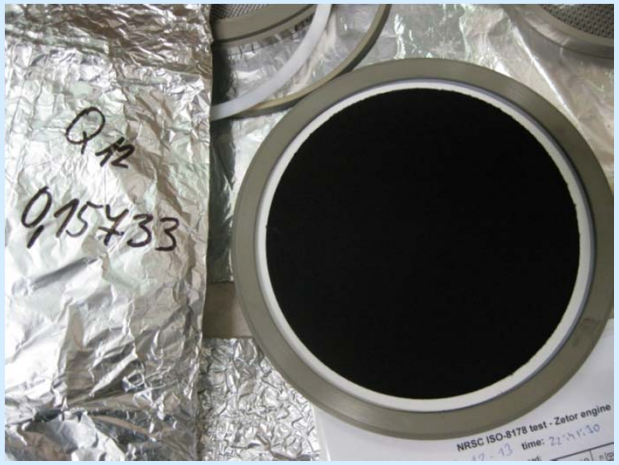
Sampling of emissions

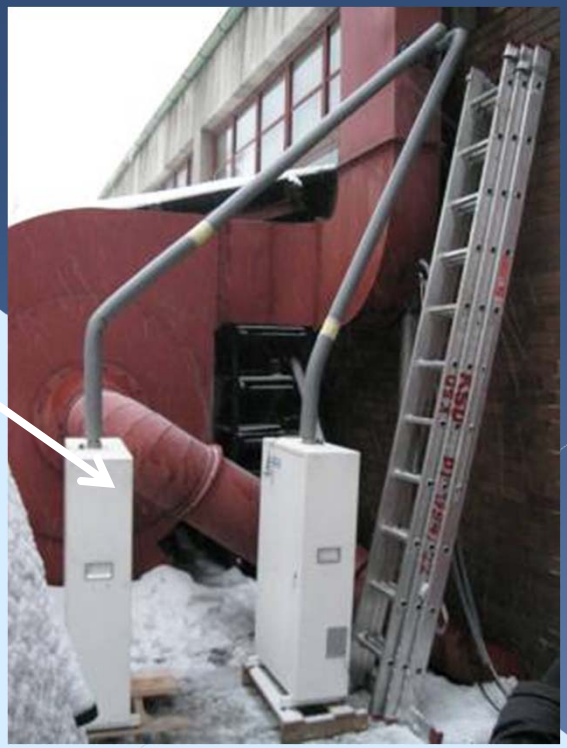
Engines: Cummins ISBe4 (on-road, Common Rail) and Zetor 1505 (off-road, mechanical injection pump)

Cycles ESC and WHSC (Cummins), NRSC (Zetor)

Fuels: diesel, biodiesel, heated rapeseed oil

Exhaust gases (80-600 m^3/h, 100-550° C) diluted by ambient air in an improvised full-flow tunnel (9000 m^3/h), from which samples are collected by Digitel high-volume samplers (30-60 m^3/h)





Sampling and chemical analysis of PAHs

Engine (fuel injection)	Test fuel	Test cycle	Collected volume [m^3]	PM [$\mu g/m^3$]	BaP [ng/m^3]	cPAHs* [ng/m^3]	PAHs** [ng/m^3]
Diesel	Cummins ISBe4	2xWHSC	62,7	38,8	0,16	0,72	7,72
Rapeseed oil	Cummins ISBe4	2xWHSC	61,7	40,5	0,23	1,13	8,54
Diesel	Cummins ISBe4	4xESC-1	32,5	277,2	< 0,12	0,74	84,2
Rapeseed oil	Cummins ISBe4	4xESC-1	58,7	214,5	0,36	2,98	43,4
Biodiesel (FAME, B100)	Cummins ISBe4	2xESC-1	31,9	192,8	0,75	4,29	31,1
Biodiesel	Cummins ISBe4	2xESC-1	32,8	230,8	0,30	2,13	70,0
Rapeseed oil	Zetor 1505 in line pump	1xNRSC	17,3	1131	0,81	6,30	242,6
Diesel	Zetor 1505 in line pump	1xNRSC	16,3	1035	<0,24	7,91	256,9

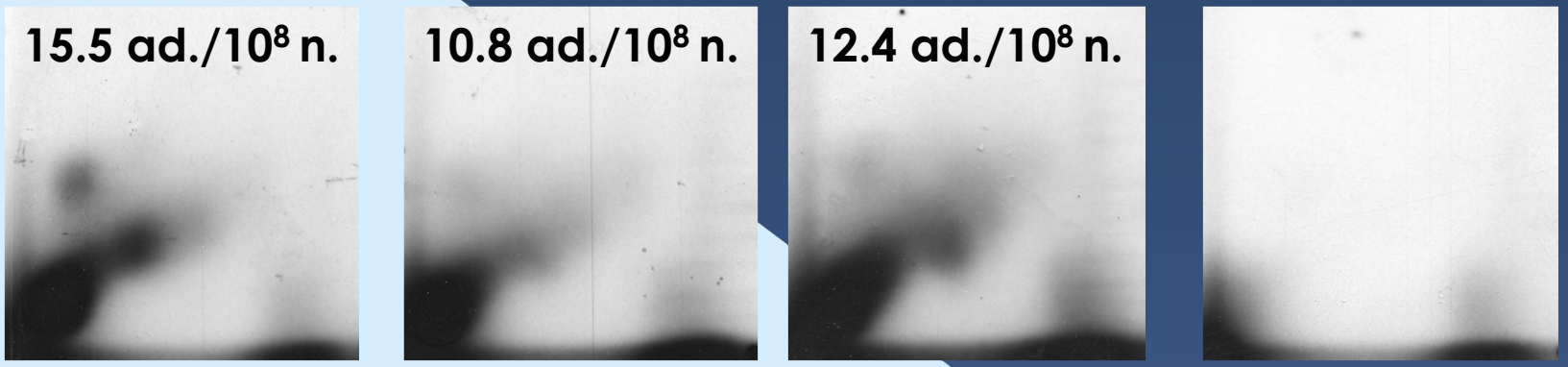
Samples of particulate emissions collected on filters were extracted by DCM, evaporated to propandiol a dissolved in DMSO

*BaA, chrysene, BbF, BkF, BaP, DBahA, IcdPy

**fenanthren, anthracene, fluoranthene, pyrene, BaA, chrysene, BbF, BkF, BaP, DBahA, IcdPy, BghiPe, coronene

DNA adducts – comparison of classic diesel with biofuels

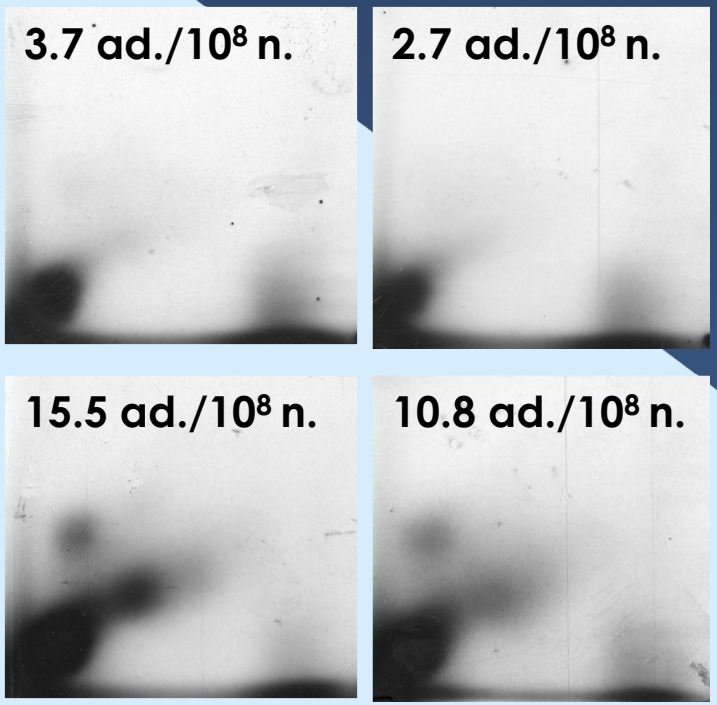
Diesel	Rapeseed oil	Biodiesel (FAME)	Control
15.5 ad./10^8 n.	10.8 ad./10^8 n.	12.4 ad./10^8 n.	



Cummins ISB engine; Cycle ESC-1; 3 m^3/sample; ctDNA (1 mg/ml +S9 + cof.)

DNA adducts – comparison of test cycles

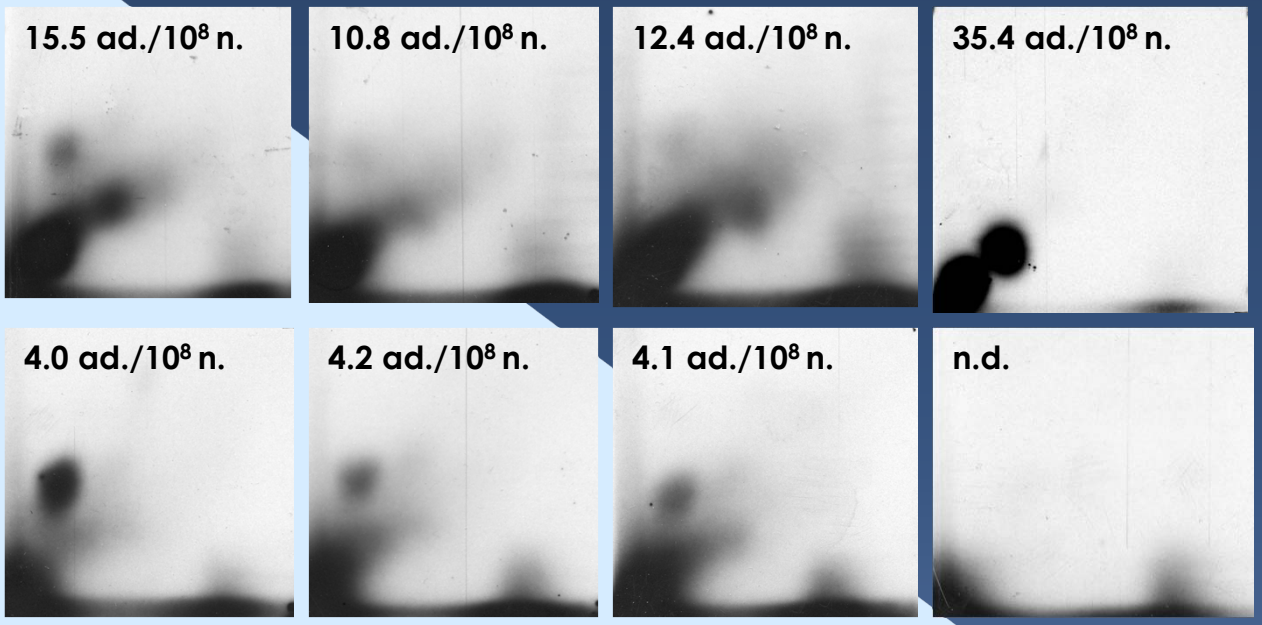
	Diesel	Rapeseed oil
WHSC	3.7 ad./10^8 n.	2.7 ad./10^8 n.
ESC-1	15.5 ad./10^8 n.	10.8 ad./10^8 n.



Cummins ISB engine; cycle ESC-1; 3 m^3/sample; ctDNA (1 mg/ml +S9 + cof.)

DNA adducts – effect of metabolic activation

	Diesel	Rapeseed oil	Biodiesel (FAME)	BaP
+S9	15.5 ad./10^8 n.	10.8 ad./10^8 n.	12.4 ad./10^8 n.	35.4 ad./10^8 n.
-S9	4.0 ad./10^8 n.	4.2 ad./10^8 n.	4.1 ad./10^8 n.	n.d.



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Genotoxicity of the organic extracts from particulate emissions of selected fuels

Engine fuel injection	Test fuel	Test cycle	PM mass [mg/kWh]	B[a]P ng/kWh	DNA adducts/ 10^8 nucleotides/kWh +S9	DNA adducts/ 10^8 nucleotides/kWh –S9	DNA adducts/ 10^8 nucleotides/mg PM +S9	DNA adducts/ 10^8 nucleotides/mg PM –S9
Cummins ISBe4 Common Rail	Diesel	2 x WHSC	6.9	3.5	217	96	31.5	13.9
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Summary

The pilot study indicates:

- 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 (data not shown).
- These results should be taken as preliminary and more detailed study is going on to verify and extend these preliminary findings.

How to continue?

- Multiple studies were reported on the chemical composition of biofuel-derived emissions under standardized testing conditions. Much less is known on their toxicity…
- Genotoxicity is only one specific area in the whole scale of various potential adverse effects of vehicle emissions…
- Standardized testing conditions should be compared with real traffic conditions.
- Mass of emitted particles may be of limited importance – are nanoparticles more effective carriers of cPAHs causing higher toxicity?
- Those aspects will be addressed in forthcoming project MEDETOX (supported by EC within LIFE+ Program)
- Complex toxicity study focusing on the possible hazard identification and on the mechanisms of the effect of emissions from biofuels is missing (human lung cells, genomics…)

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Comparison of Genotoxicity (DNA adducts) Pilot study

Fuel	Engine fuel injection	Test cycle	BaP [ng/m^3]	cPAHs [ng/m^3]	DNA adducts/ 10^8 nuc. +S9; 0,3 m^3	DNA adducts/ 10^8 nuc. +S9; 3 m^3	DNA adducts/ 10^8 nuc. -S9; 3 m^3	+S9/-S9
Diesel	Cummins ISB	2 x WHSC	0.16	0.72	0.19	3.67	1.62	2.3
Rapeseed oil	Cummins ISB	2 x WHSC	0.23	1.13	0.51	2.69	0.22	12.2
Diesel	Cummins ISB	4xESCmod*	< 0.12	0.74	3.04	15.45	4.00	3.9
Rapeseed oil	Cummins ISB	4xESCmod*	0.36	2.98	2.67	10.80	4.15	2.6
Biodiesel (FAME)	Cummins ISB	2xESCmod*	0.75	4.29	2.04	12.37	4,14	3.0
Diesel	Cummins ISB	2xESCmod*	0.30	2.13	2.96	14.79	6.52	2.3
Rapeseed oil	Zetor 1505	1 x NRSC	0.81	6.30	5.52 (0,1m^3)	13.23 (1m^3)	4.92 (1m^3)	2.7
Diesel	Zetor 1505	1 x NRSC	<0.24	7.91	3.03 (0,1m^3)	16.49 (1m^3)	4.66 (1m^3)	3.5

Samples of particulate emissions collected on filters were extracted by DCM, evaporated to propandiol and dissolved in DMSO