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

Jan Topinka[1], Alena Milcová[1], Jana Schmuczerová[1], Martin Mazač[2], Martin Pechout[2], Michal Vojtíšek-Lom[2]

[1]Institute of Experimental Medicine AS CR, Prague, Czech Republic
[2]Technical University Liberec, Czech Republic

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

Exposure → **Effect**

External exposure dose → Internal dose → Biologically effective dose → Early effects → Morfological structural and functional changes → Clinical symptoms

- External exposure dose ↓ Stacionary and personal monitoring
- Internal dose ↓ Level of compound /metabolite
- Biologically effective dose ↓ Adducts DNA, prot.
- Early effects ↓ Somatic mutations
- Morfological structural and functional changes ↓ Preneoplastic lesions
- Clinical symptoms ↓ Diagnosis

How to analyze genotoxicity of complex mixtures ?

Number of different approaches…

approach to measure genotoxicity:

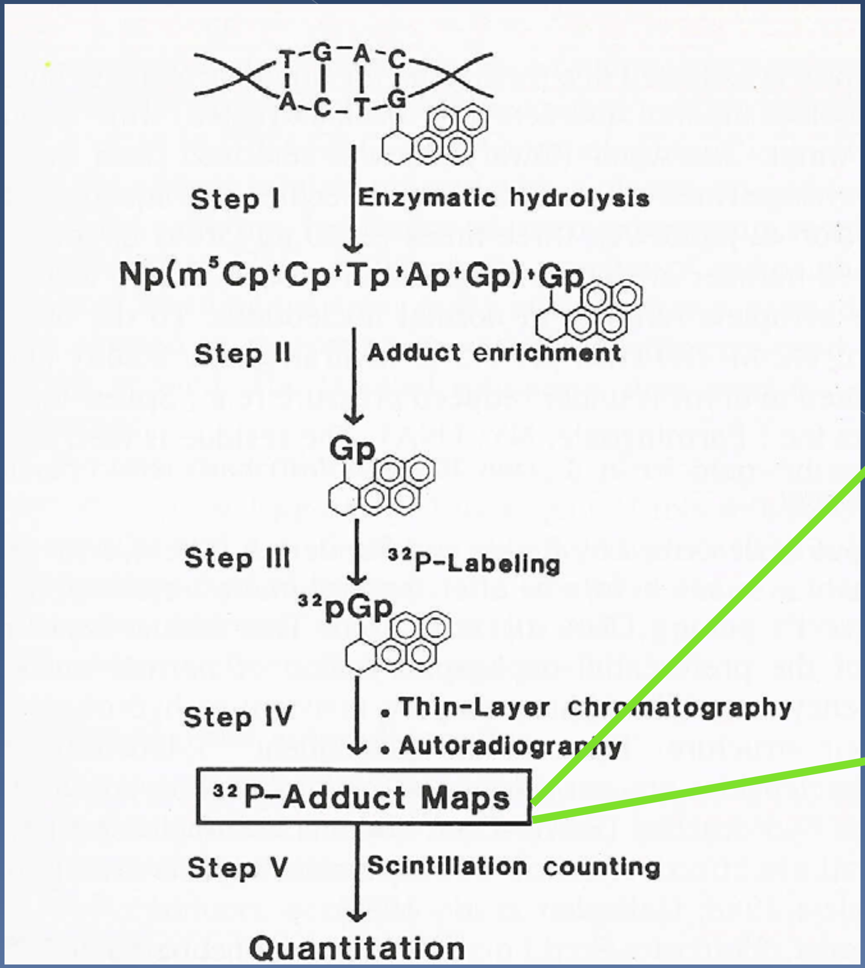
DNA reactivity of organic compounds bound on particulate emissions collected on filters

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

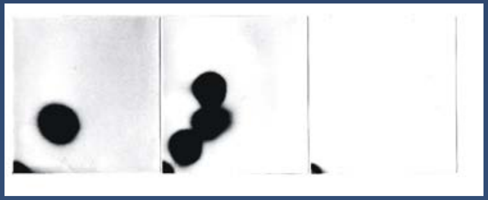
Acellular assay of genotocity

- Calf thymus DNA (1 mg/ml) was incubated with organic extracts from filters with collected exhaust particles (100 μg extract/ml) 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 were used as positive and negative controls, respectively.
- DNA was isolated by phenolic extraction and the DNA samples were kept at −80 °C until analysis of DNA adducts by ^{32}P-postlabelling.

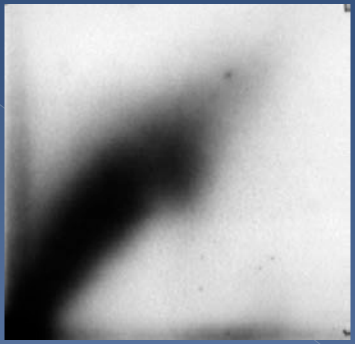
Analysis of DNA adducts (^{32}P-postlabelling)



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

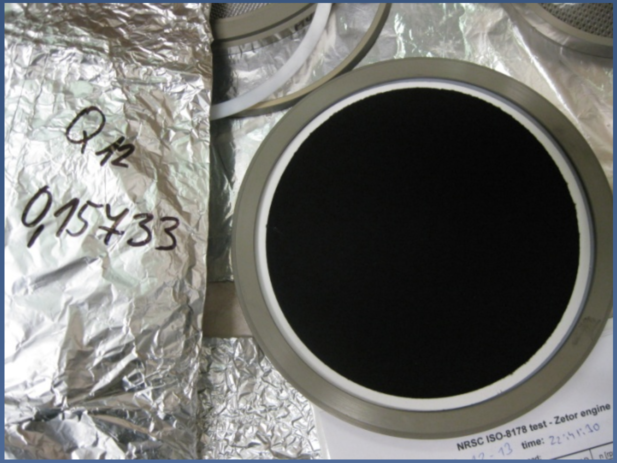
Sampling of emissions

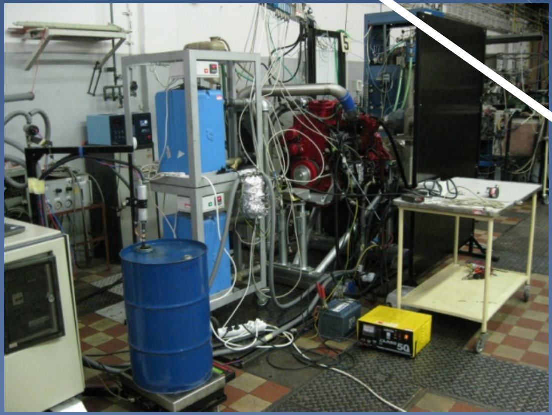
Engines: Cummins ISBe4 (on-road, Common Rail) and Zetor 1505 (off-road, mechanical injection pump)

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

Fuels: diesel, biodiesel, heated rapeseed oil

Exhaust gases (80-600 m^3/h, 100-550º 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 [μg/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

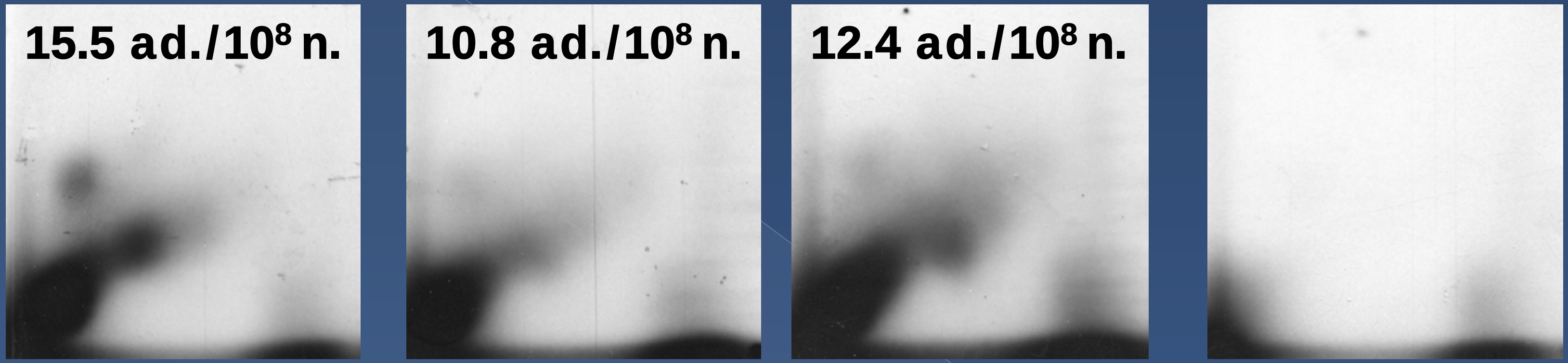
Samples of particulate emissions collected on filters were extracted by DCM, evaporated to propandiol a dissolved in DMSO

*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



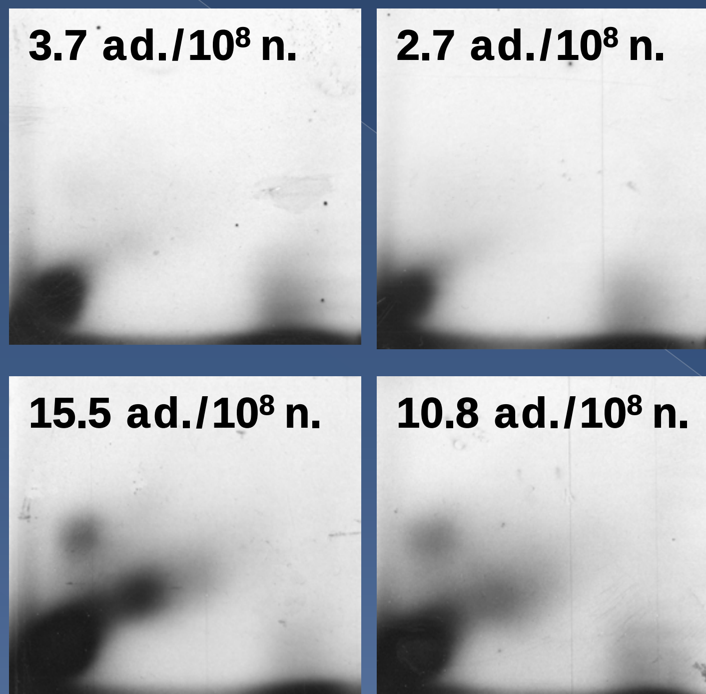
Cummins ISB engine; Cycle ESC-1; 3 m^3/sample; ctDNA (1 mg/ml +S9 + cof.)

DNA adducts – comparison of test cycles

Diesel Rapeseed oil

WHSC

ESC-1



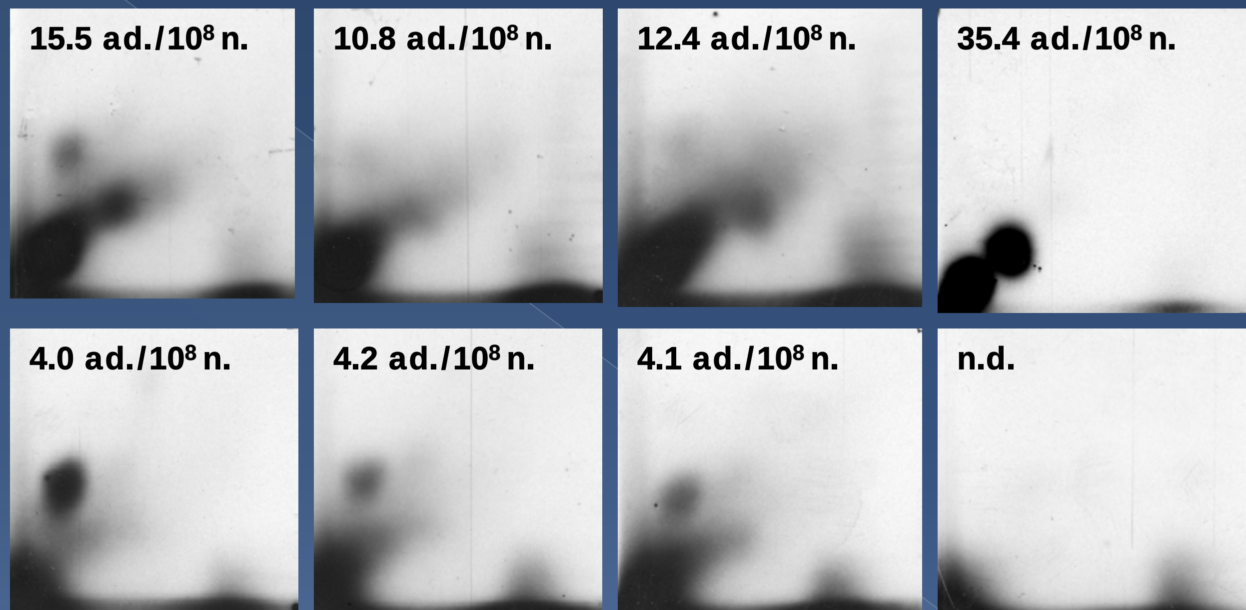
Cummins ISB engine; cycle ESC-1; 3 m^3/sample; ctDNA (1 mg/ml +S9 + cof.)

DNA adducts – effect of metabolic activation

Diesel | Rapeseed oil | Biodiesel (FAME) | BaP

+S9

-S9



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

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	–S9	DNA adducts/ 10^8 nucleotides/mg PM +S9	–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

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).
- <u>These results should be taken as preliminary and more detailed study is going on to verify these preliminary findings.</u>

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)
- <u>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…)</u>

Acknowledgements

- Institute of Experimental Medicine AS CR
  Jana Schmuczerová a Alena Milcová

- Technical University Liberec a Czech Technical University in Prague
  Michal Vojtíšek, Martin Mazač, Martin Pechout

- 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 (DNA adducts) Pilot study

Fuel	Engine fuel injection	Test cycle	BaP [ng/m³]	cPAHs [ng/m³]	DNA adducts/ 10^8 nuc. +S9; 0,3 m³	DNA adducts/ 10^8 nuc. +S9; 3 m³	DNA adducts/ 10^8 nuc. -S9; 3 m³	+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 (0,1m³)	13.23 (1m³)	4.92 (1m³)	2.7
Diesel	Zetor 1505	1 x NRSC	<0.24	7.91	3.03 (0,1m³)	16.49 (1m³)	4.66 (1m³)	3.5

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