Truck emissions under severe congestion conditions: Part 2 - Effects of extended low-load operation of a non-DPF diesel engine on the relative toxicity of particulate matter organic extracts

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Introduction

The present study was performed to identify possible genotoxicity induced by organic extracts from particulate matter in the exhaust of diesel engine Zetor 1505 running on diesel and biodiesel (B100) fuels at various operating conditions typical for transit truck traffic in large cities. High ammount of emmissions originates from relatively low number of vehicles and relatively short time intervals of the engine operation. Analysis of the toxic effects of emissions should not rely on standardized test cycles only, but should better reflect real traffic conditions. This study aimed to identify possible toxicity induced by organic extracts from particulate matter in the exhast of diesel engine Zetor 1505 (details in the previous presentation of M.Vojtisek-Lom),to compare toxicity at various operating condiotions typical for transit truck traffic in large cities and to compare toxicity of diesel and biodiesel (B100) fuels.

Experimental

Technical details on the engine, collection of samples and chemical analysis are described in the previous paper of Vojtisek-Lom et al. Diluted exhaust was sampled with high-volume samplers on Teflon coated filters. Filters were extracted with dichlormethane (DCM) and DNA adduct levels and DNA oxidative damage (8-oxo-dG) induced by extractable organic matter (EOM) in an acellular assay of calf thymus DNA coupled with ^{32}P-postlabeling in the presence and absence of rat liver microsomal S9 fraction were employed. Moreover, model of human lung epithelial cells (A549) was used to study cytotoxicity, genotoxicity (DNA adducts and micronuclei) and oxidative damage of lipids and proteins. Calf thymus DNA was treated with EOM corresponding to 0.01 m^3 of the undiluted exhaust gas and A549 cells with 0.001 m^3 of undiluted exhaust gas per ml cell media.

Results and Discussion

The results of an acellular assay of DNA adducts suggest strong genotoxic effects of both types of fuels varying depending on the engine operating conditions (Figure 1).

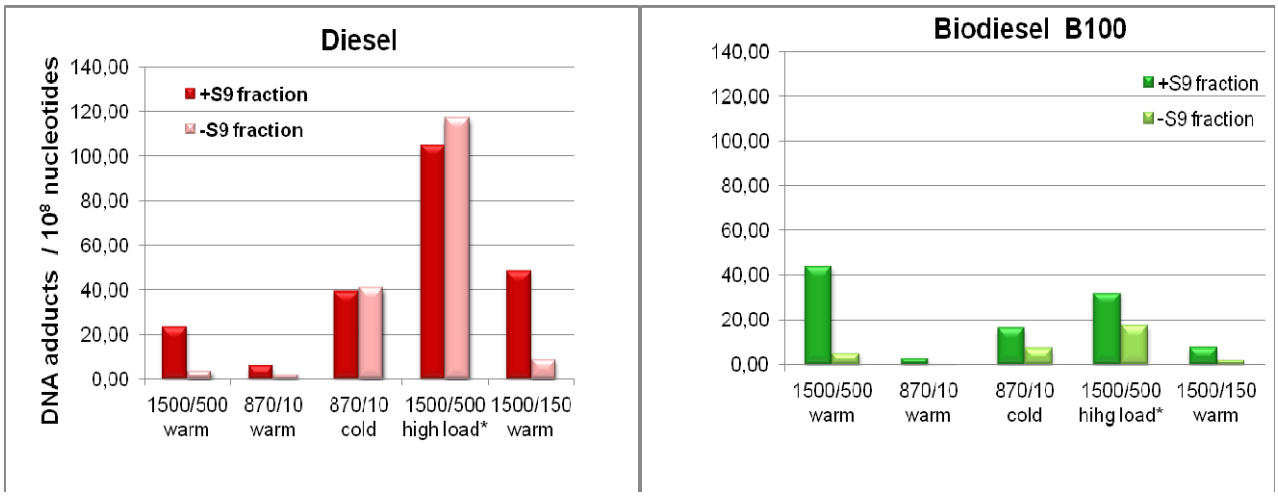

Fig.1: DNA adducts induced by organic extracts from diesel and biodiesel particulate emissions. The dose of extracts corresponds to 0.01m^3 of the undiluted emissions/ml

The DNA adduct levels varied between 3 – 44 adducts/10^8 nucleotides and 7-117 adducts/10^8 nucleotides for biodiesel and diesel fuel, respectively. The highest DNA adduct levels were observed for high load immediately following a period of extended low-load operation on diesel fuel – 105 adducts/10^8 nucleotides in the presence of PAH metabolizing enzymes (+S9). Interestingly, without PAH metabolic activation were the DNA adduct levels slightly higher (117 adducts/10^8 nucleotides) suggesting substantial genotoxicity of non-PAH components of the exhaust gas.
To confirm the data of the acellular test and to find appropriate subtoxic EOM concentrations to measure other toxicity markers, we further tested cytotoxicity of EOMs in A549 cells (Figure 2).

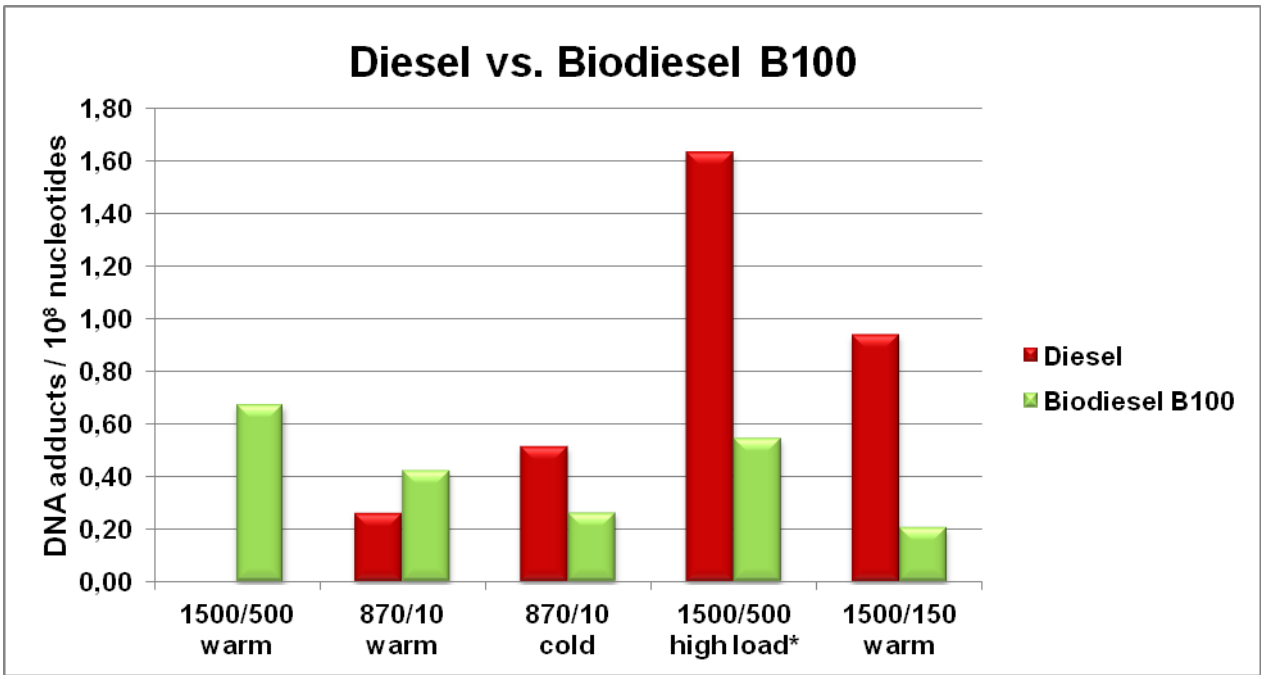

Fig.2: DNA adducts induced by organic extracts from diesel and biodiesel particulate emmissions. The dose corresponds to 1 dm^3 of the undiluted emmissions/ml media.

The results of genotoxicity received with A549 cells for subtoxic dose EOM corresponding to 0.001 m^3 of undiluted exhaust gas per ml of cell media indicate low DNA adduct levels in A549 cells confirming mostly the results of acellular test: The results suggest highest adduct levels for high load immediately following a period of extended low-load operation on diesel fuel (1.63 adducts/10^8 nucleotides), 3-fold higher than for biodiesel B100 (0.54 adducts/10^8 nucleotides). The results of oxidative damage of biomolecules and micronucleus test did not indicate clear toxic effects of EOMs and will be further investigated..

Conclusions:

- This preliminary data indicate relatively high genotoxicity of exhaust produced during high load immediately following a period of extended low-load operation operating condition, compared to other operating conditions, for both fuels in an acellular assay as well as in human lung cell line A549 (model of the human lung epithelial cells).
- Under this operating condition, diesel fuel emissions exhibit approximately 3-fold higher genotoxicity compared to biodiesel.
- High load immediately following a period of extended low-load operation was found to be most toxic engine regimen for both diesel and biodiesel B100
- The most sensitive toxicity marker is DNA adduct analysis – both in cell-free and human lung cell model.
- Other toxicity markers (oxidative stress, micronuclei) provide equivocal results (low sensitivity?)
- Diesel emissions are more genotoxic than emissions from B100
- Other compounds than PAHs seem to play main role in genotoxicity diesel emissions (nitro-PAHs?).

This work was supported by the EU LIFE+ project MEDETOX (LIFE10 ENV/CZ/651) and Czech Science Foundation Grant 13-01438S.

Truck emissions under severe congestion conditions:
Part 2: Effects of extended low-load operation of a non-DPF diesel engine on the relative toxicity of particulate matter organic extracts

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LIFE10: 17th ETH-Conference on Combustion Generated Nanoparticles , 23.-26.6 . 2013, Zurich

MEDETOX

Background

- **Traffic emissions represent one of the main sources of the air pollution by UF particles in urban agglomerations.**

- **High amount of emissions originates from relatively low number of vehicles and relatively short time intervals of the engine operation.**

- **Analysis of the toxic effects of emissions should not rely on standardized test cycles only, but should better reflect real traffic conditions.**

Aims

- To identify possible toxicity induced by organic extracts from particulate matter in the exhast of diesel engine Zetor 1505 (details in the previous presentation of M.Vojtisek-Lom)

- To compare toxicity at various operating condiotions typical for transit truck traffic in large cities

- To compare toxicity of diesel and biodiesel (B100) fuels

Methods

•Organic extracts (EOMs) from PM2.5 engine emission particles were prepared
•Priority PAHs were analyzed in EOMs (reported in the previous presentation)
•Toxic effects of EOMs were analyzed in:

Acellular tests

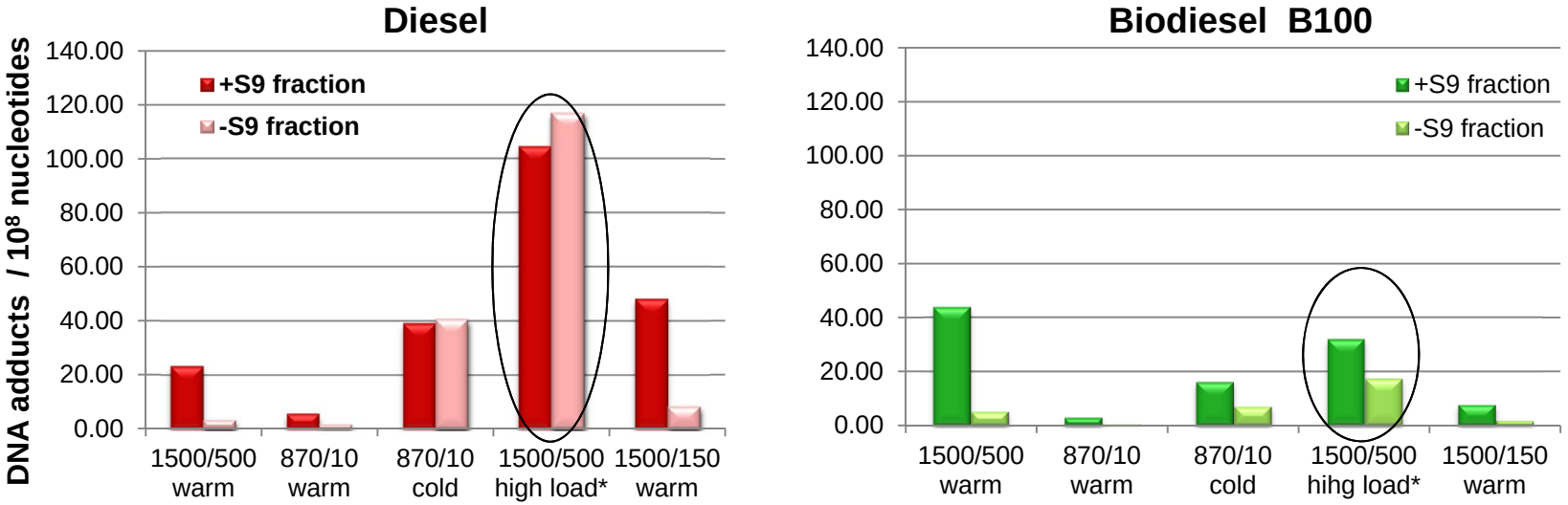
- **Genotoxicity:** DNA adducts and oxidative DNA damage in cell-free assay with ctDNA

Model of human lung epithelial cells (A549)

- **Cytotoxicity:** WST1
- **Genotoxicity:** DNA adducts by ^{32}P-postlabelling and Comet assay
- **Oxidative damage:** DNA (Comet assay), proteins (carbonyl groups) a lipids (15-F_{2t}-isoprostane)
- **Cytogenetics:** micronucleus test

Results – DNA adducts in acellular tests

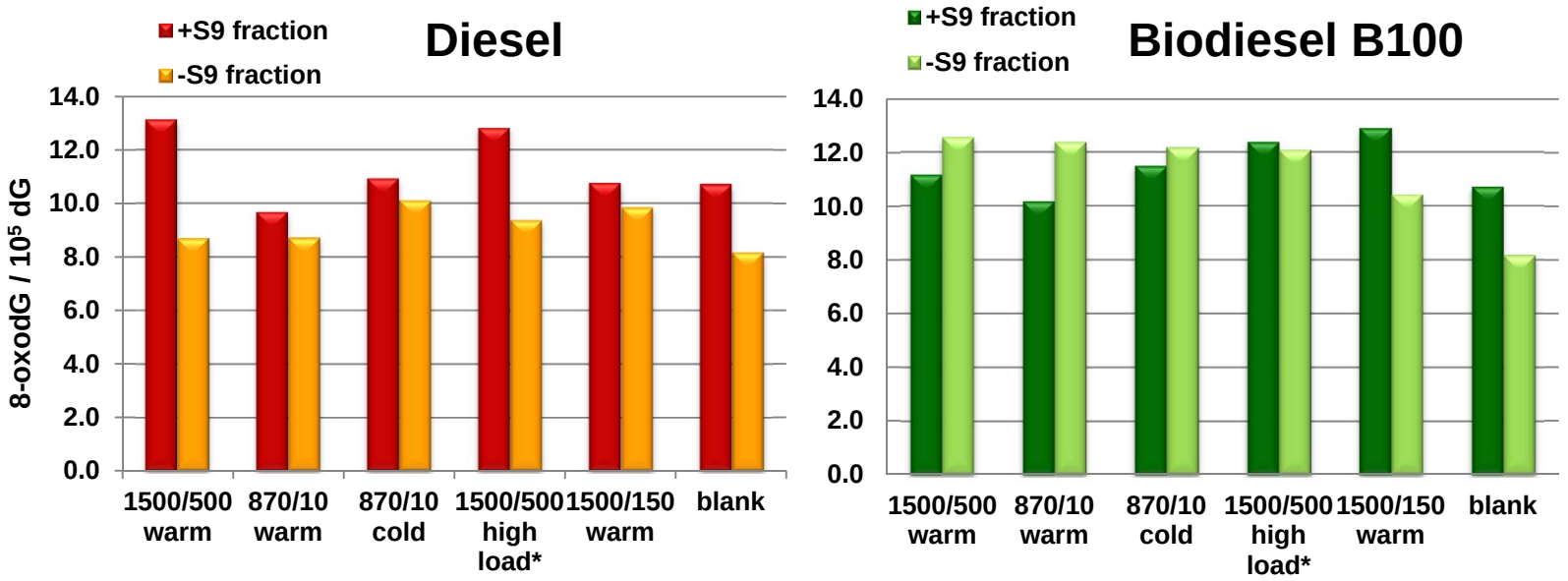
Sampling in laboratory



*high load immediately following a period of extended low-load operation
Mean from two independent experiments with variability < ±15%

Results – oxidative DNA damage in acellular test

Sampling in laboratory



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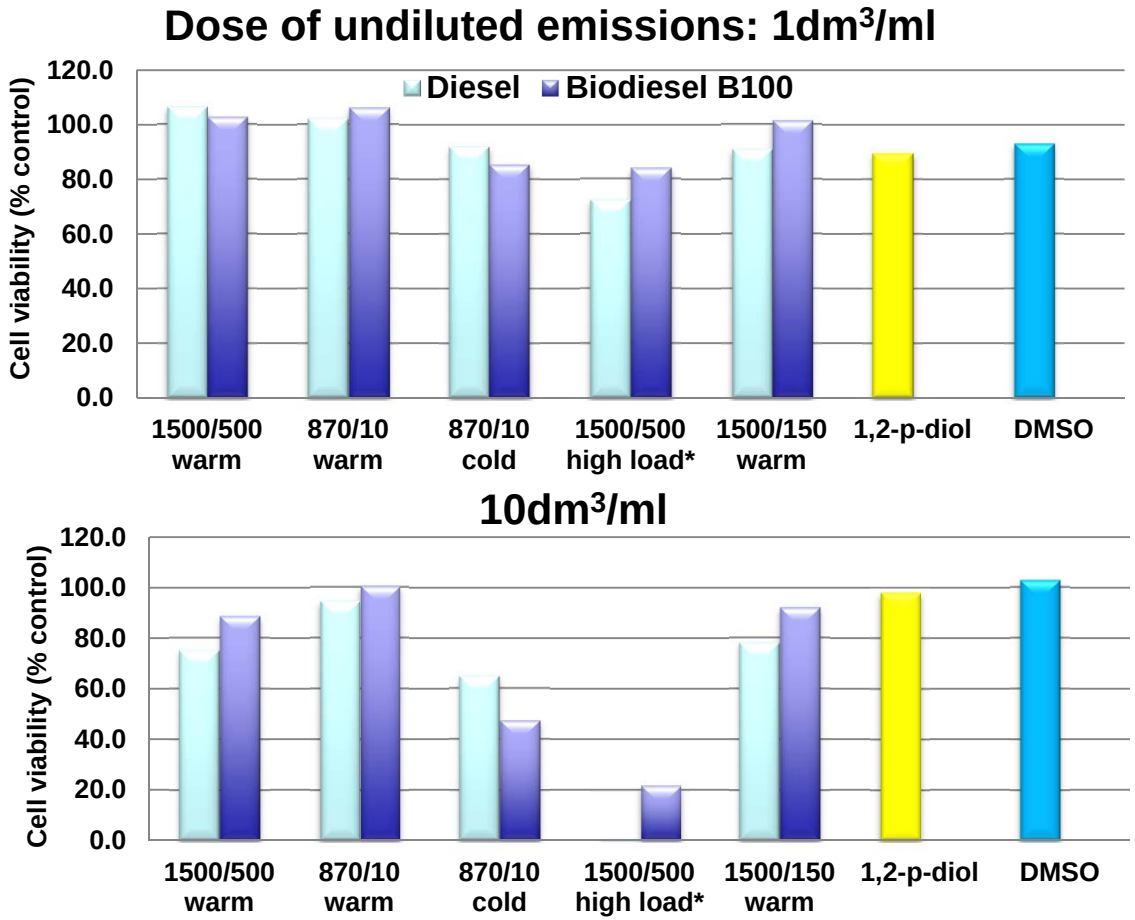
Mean from two independent experiments with variability < ±15%

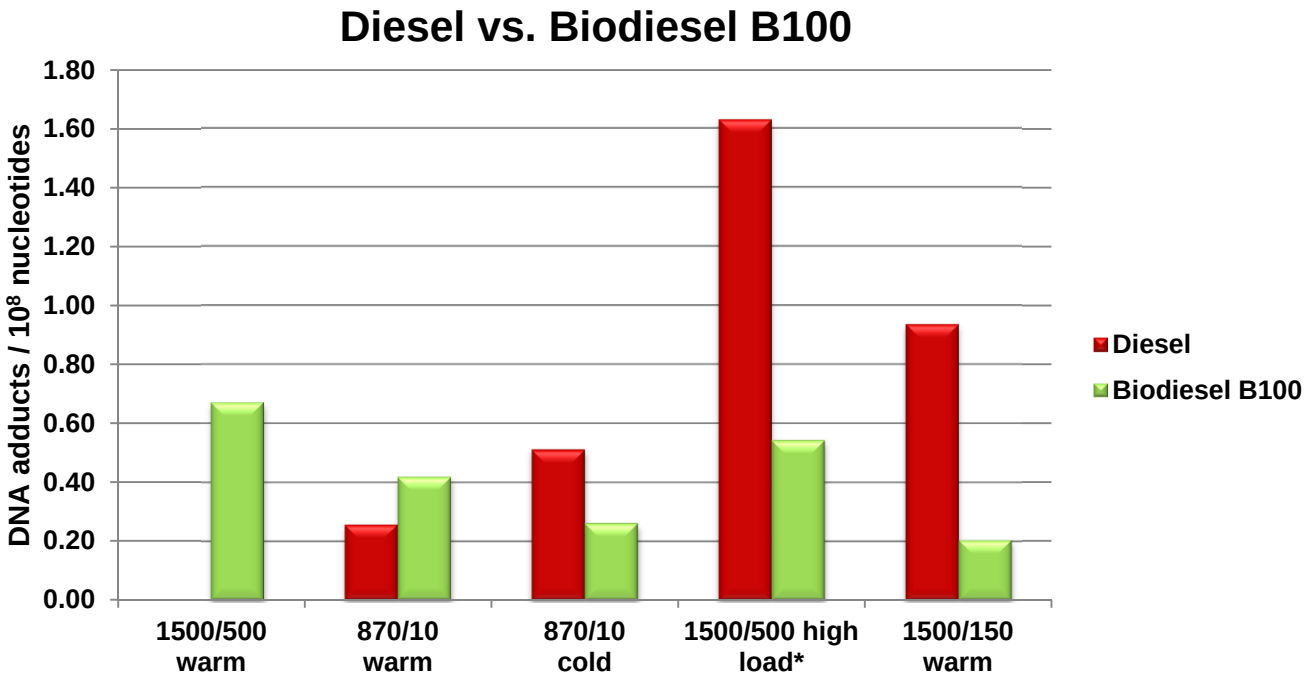
Cytotoxicity in A549 cells-WST1 test



***high load immediately following a period of extended low-load operation**
Mean from two independent experiments with variability < ±15%

Results – DNA adducts in A549 cells

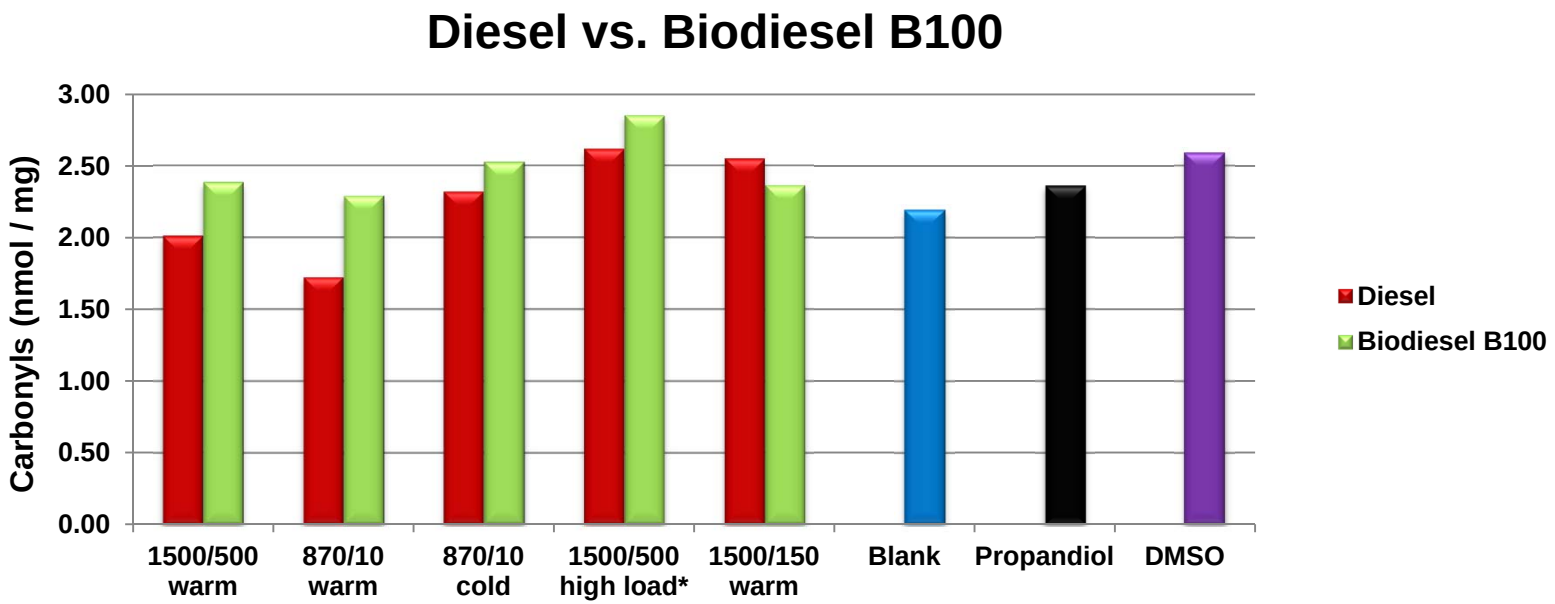
Sampling in laboratory



*high load immediately following a period of extended low-load operation
Mean from two independent experiments with variability < ±15%

Results – Oxidation of proteins in A549 cells

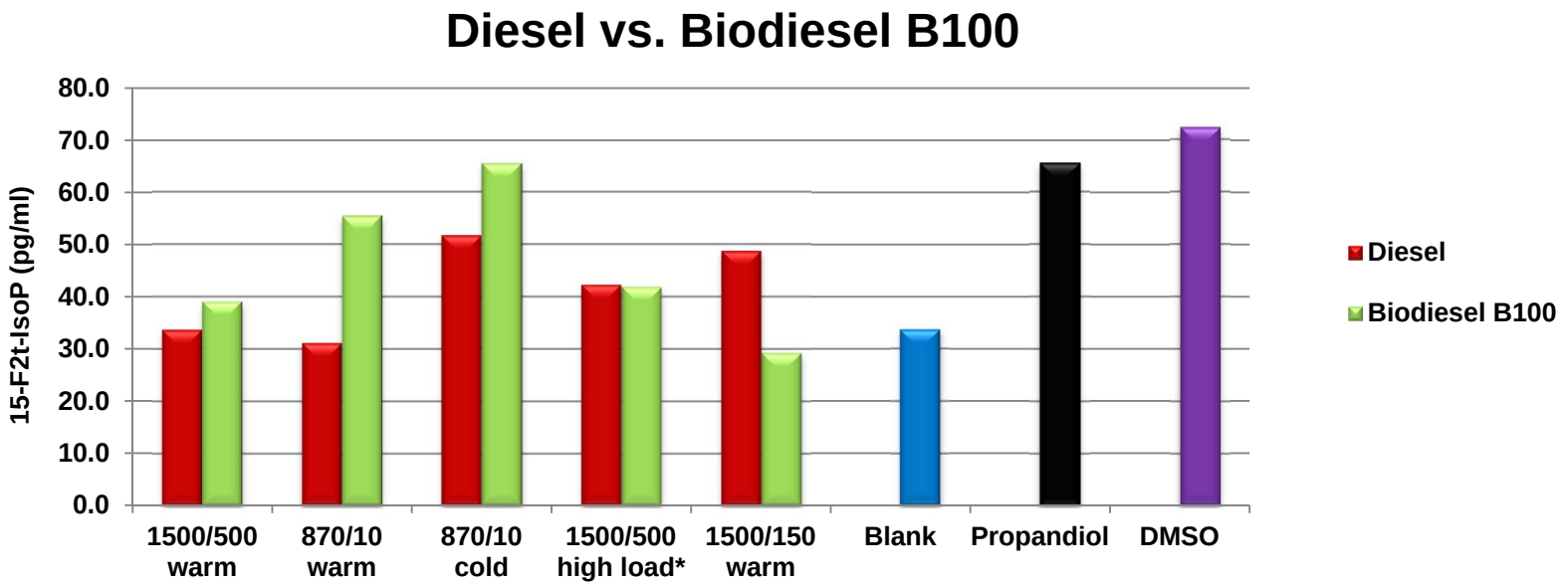
Sampling in laboratory



*high load immediately following a period of extended low-load operation
Mean from two independent experiments with variability < ±15%

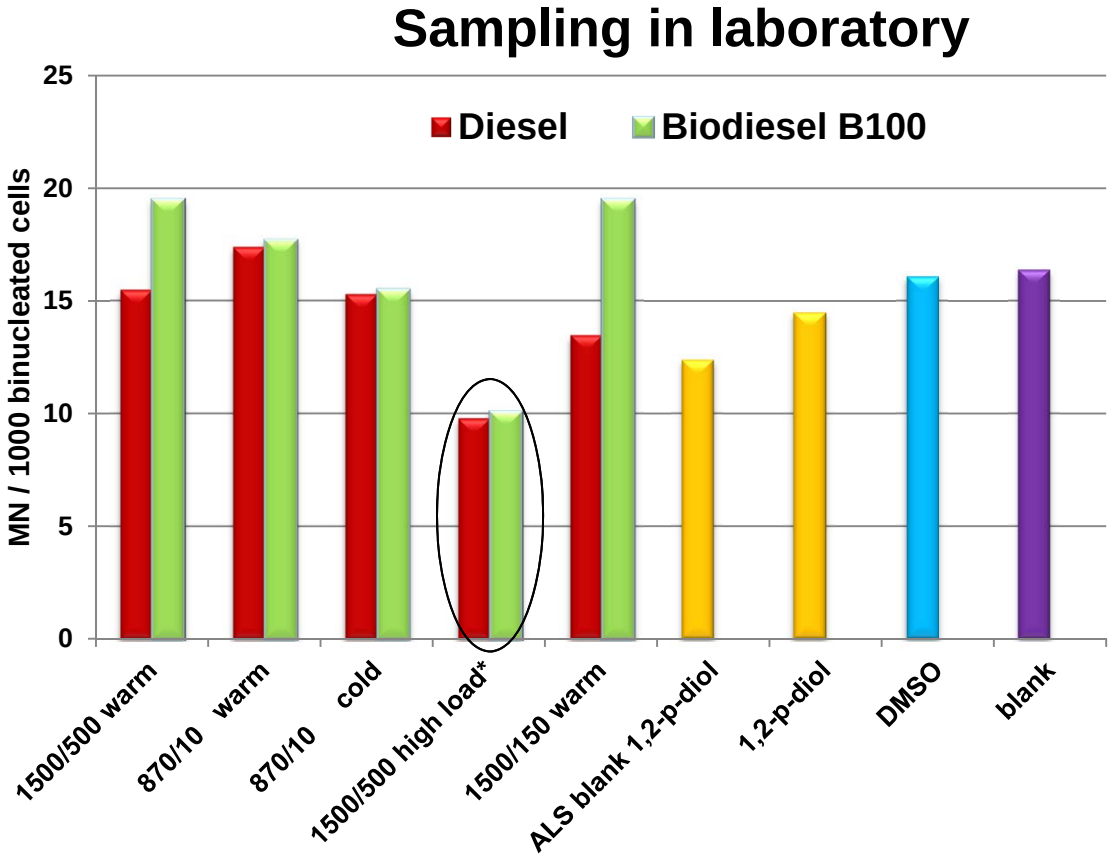
Results – lipidperoxidation in A549 cells

Sampling in laboratory



*high load immediately following a period of extended low-load operation
Mean from two independent experiments with variability < ±15%

Results – micronucleus test in A549 cells

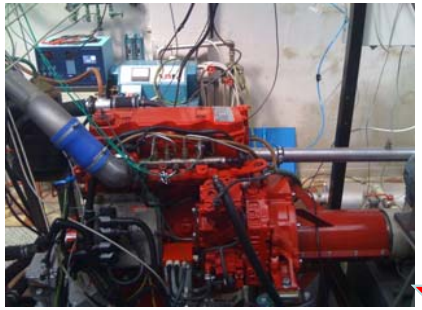


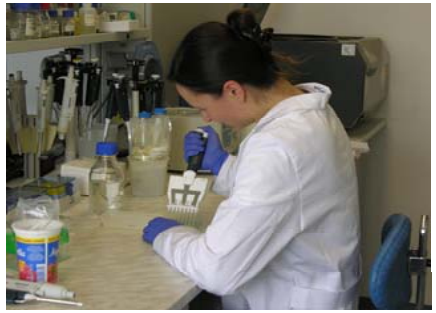
*high load immediately following a period of extended low-load operation
~5,000 cells was evaluated per sample

Conclusions

- High load immediately following a period of extended low-load operation was found to be most toxic engine regimen for both diesel and biodiesel B100
- The most sensitive toxicity marker is DNA adduct analysis – both in cell-free and human lung cell model.
- Other toxicity markers (oxidative stress, micronuclei) provide equivocal results (low sensitivity?)
- Diesel emissions are more genotoxic than emissions from B100
- Other compounds than PAHs seem to play main role in genotoxicity diesel emissions (nitro-PAHs?).

Acknowledgements





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Project MEDETOX (LIFE10 ENV/CZ/651)

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