Lung cell responses to air-liquid-exposure to exhaust from an automobile engine running on conventional gasoline and biofuel (E85)

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Combustion aerosols such as car engine emissions are an important contributor to overall air pollution and their negative impact on public health especially in big cities is tremendous. In terms of sustainability it seems reasonable to change from fossil fuels to renewable energy sources. Therefore the usage of bioethanol as a biofuel component is promoted. But the impact on the environment and human health of these new emissions are widely unknown.

Our study aims to compare lung cell responses towards the exposure to diluted car engine emissions on the level of a comprehensive transcriptome analysis. Emissions from operation with conventional fuel and biofuel as well as different driving cycles are considered. The type and regulation strength of the cellular reactions are used as indicators for hazardous effects of biofuel emissions.

A 2.0 liter flexi-fuel engine with 132 kW and a maximum torque of 320 Nm was taken as the aerosol emission source for the experiments. The engine was operated with either gasoline containing 7 % ethanol (E10) or with a mixture of 82 % ethanol and 18 % gasoline (E85). Besides the fuel usage also the speed engine operations were varied. In the European Union, fuel consumption and emission factors of regulated pollutants are determined using a standardized car driving cycle, the "New European Driving Cycle" (NEDC) that does not exceed a speed of 120 km/h. We simulated chassis dyno tests by operating the test engine on a test bench with correlating speed/torque patterns. A second self-invented driving cycle was introduced for operating the engine in a more realistic way, especially for countries with no or high-lying highway speed limits such as Germany. The cycle was named "High-speed Driving Cycle" (HSDC). It starts with one NEDC equivalent cycle followed by equally long sections of driving with constant speed. The equivalent car speed in these sections was varied between 80 and 180 km/h in a predetermined order. The exhaust for all experiments was diluted by a combination of a porous-tube dilutor and an ejector dilutor. The dilution ratios of 1:10 and 1:40 were used for the cell exposure experiments. A cell line of the lung epithelium (A549) was directly exposed at the air-liquid-interface (ALI) in a self-contained cell exposure system (Mülhopt, Dilger *et al.*, 2016) towards the diluted car engine emissions. After 4h of exposure cells were lysed and total RNA was extracted and analyzed on a whole-genome gene expression array (Agilent). Using clean air treated cells as control group, aerosol-induced gene regulation was calculated. Gene ontology (GO) analysis was performed using at least 1.5-fold regulation and $p<0.05$ in T-test with Benjamini-Hochberg FDR multiple testing correction.

The cells reacted to the E85 emissions with a 2-fold increase in regulated genes compared to conventional fuel emissions. In the high-speed cycle (gasoline) the number of affected genes increased by 28% with respect to the standard NEDC cycle. All tested aerosols caused general cell stress and affected the cell cycle as it was observed in related enriched GO terms. Response to organic substances and changes in steroid metabolism were only observed in the gasoline operated engine exhaust treated cells but not the E85 exposed cells. In contrast DNA damage responses were stronger after exposure to E85 emissions. When gasoline was used DNA damage was only induced in the high-speed mode (HSDC). We conclude that the usage of biofuel does not result in less severe effects in lung cells. Additionally, we showed that the engine operation range has strong influence on the results. Thus, it is necessary to test also alternative cycles such as the here applied high-speed conditions to be able to identify in a more realistic way the possible hazards of car emissions.

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