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Hazard identification of gasoline engine exhausts using a multi-cellular human lung model

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Ambient air pollution are correlated with adverse effects such as cardiovascular and respiratory diseases. Major contributors to air pollution are traffic related combustion processes, *i.e.* diesel and gasoline engines, with the majority of passenger cars in Switzerland being gasoline fueled.
Nowadays an increasing number of gasoline engines use the direct injection technology instead of the multipoint port injection technology, offering higher power and fuel-efficiency, but leading to higher particle number concentrations in exhausts compared to that of older gasoline vehicles. This change in vehicle emissions requires a re-evaluation of their toxicity, because directly correlating adverse health outcomes from vehicle exhaust compositions is currently not possible.
The aim of this PhD thesis was to compare the (adverse) outcomes of different gasoline engines by *in vitro* exposures of human lung cell cultures at the air-liquid interface to complete exhausts. A variety of gasoline engines was tested, using different driving cycles (or: simulations), particle filters, and fuels (e.g. ethanol addition). Effects were measured using two cell models: a multi cellular human lung model composed of a bronchial cell line supplemented by immune cells and primary MucilAir^{TM} cells representing reconstituted airway bronchial tissue. Investigated endpoints included: i) Cell viability and cell morphology by microscopy methods, ii) oxidative stress, and iii) (pro-)inflammation, whereby ii) and iii) by protein-levels (ELISA) and gene expression (qPCR).
The results show that changes in fuel or addition of particle filters lead to altered exhaust characteristics. But even with significantly altered emissions the biological effects on the bronchial cell culture models remain moderate and can be summarized as follows: i) adverse effects on human lung cell cultures depend on the vehicle and driving cycle. One vehicle caused oxidative stress effects on the multi-cellular lung cell cultures, which cannot be completely diminished by a particle filter. Another vehicle did not induce any adverse effects (no improvement was observed with a particle filter). ii) The ethanol-supplemented fuel did not have any negative impact on our cell system. iii) The simulation of old cars did not lead to increased biomarkers in both lung cell models. iv) The cell cultures with the bronchial cell line can be exposed for a prolonged period (up to three days) to gasoline exhaust. A significant increase in oxidative stress and (pro)inflammation was observed, however, this increase was small compared to effects observed with diesel vehicle exhaust. Additionally, the cell cultures were exposed for up to three days to ambient air in summer and in winter, in this study the winter ambient air significantly elevated multiple genes for (pro-)inflammation.
In conclusion, in the cell exposure system the observed gasoline exhaust toxicity was generally much lower than what was observed from diesel exhaust. However, we have shown that prolonged exposures are feasible, not only at the exhaust gas control station but also with ambient air. Future experiments could be performed in other locations, e.g. work place, nanomaterial production facility, or shooting range, and proteomics and genomics analytics could be included to gain more insight into adverse outcome pathways.

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