Health effect assessment of hybrid gasoline vehicle emissions by *in-vitro* cell exposure experiments in air-liquid interface

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Towards vehicles' electrification, the trade of hybrid cars in the EU registered a significant increase holding the 27% of the current EU market in 2022 [1]. Although hybrid vehicles are clean enough to mitigate air pollution and improve air quality, their emissions still contain ultrafine particles (UFPs) [2] that are harmful [3], maintaining the need to investigate the health effects of their emissions under different driving conditions.

In the current study, the health effects of emissions from a state-of-the-art gasoline plug-in hybrid electric passenger vehicle were assessed by performing Air-Liquid Interface (ALI) *in-vitro* cell exposure experiments. The tested vehicle was equipped with an emission control system consisted of a three-way catalyst (TWC) and a gasoline particulate filter (GPF) and assessed under transient driving at cycles that correspond real driving conditions of different dynamics i.e. mild dynamics for mainly urban driving (moderate-RDE driving cycle) and more dynamic driving at highway (Combined driving cycle), during cold and hot start. The cell exposure to vehicle emissions was sustained by the Multiculture *in-vitro* Cell exposure Chamber (MEC) [4] where particulates are deposited due to diffusion achieving doses equivalent to human inhalation during realistic daily exposure [5]. A549 human epithelial cells were exposed and subjected to Alamar Blue staining and lactate dehydrogenase (LDH) assays to assess cell viability, as well as to IL-1β and TNFa assays to assess production of cytokines and inflammatory response. Despite the advanced vehicle technology and emission control systems applied, a measurable biological effect of decreased viability and increased cytokine release was observed in all driving scenarios with more intense one the m-RDE driving cycle under cold start.

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