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Prevalidation of in vitro continuous flow exposure systems as alternatives to in vivo inhalation safety evaluation experimentations: Outcome from MAAPHRI-PCRD5 research program

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Abstract

Diesel engine emission aerosol-induced toxicity patterns were compared using both in vitro (organotypic cultures of lung tissue) and in vivo experimentations mimicking the inhalation situation with continuous aerosol flow exposure designs.

Using liquid media resuspended diesel particles, we show that toxic response pattern is influenced by the presence of tensioactive agent in the medium which alter particle-borne pollutant bioavailability.

Using continuous aerosol exposure in vitro, we show that with high sulfur fuel (300 ppm) in the absence of oxidation catalysis, particulate matter was the main toxic component triggering DNA damage and systemic inflammation, while a very limited oxidant stress was evidenced. In contrast, with ultra-low sulfur fuel in the presence of strong diesel oxidation catalysis, the specific role of particulate matter is no longer evidenced and the gas phase then becomes the major component triggering strong oxidant stress, increased NO_2 being the most probable trigger.

In vivo, plasma tumor necrosis factor alpha (TNFalpha), lung superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) activity levels varied in agreement with in vitro observations. Diesel emission treatment with oxycat provokes a marked systemic oxidant stress. Again NO_2 proved to account for a major part of these impacts. In conclusion, similar anti-oxidant responses were observed in in vitro and in vivo experiments after diesel emission aerosol continuous flow exposures. The lung slice organotypic culture model-exposed complex aerosol appears to be a very valuable alternative to in vivo inhalation toxicology experimentations in rodents.

Keywords: In vitro alternatives; Inhalation toxicology; Cell cultures; Lung; Aerosol exposure; Diesel exhausts; Oxidant stress; Inflammation

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Introduction

Air quality is now among the population one of the very first concerns about environment and health. In addition to pollution due to industrial activities, the population has recently been sensitized to the problem of pollution due to vehicles. Among the material emitted by vehicle engines and more precisely by diesel engines, diesel exhaust particles (DEPs) are considered to be one of the main airborne pollutants in urban areas. DEPs consist of a carbonaceous core with a large surface area to which chemicals are absorbed. These include organic chemicals such as polycyclic aromatic hydrocarbons (PAHs), nitro-derivatives of PAH, heterocyclic compounds, aldehydes and long-chain aliphatic hydrocarbons. DEPs have an aerodynamic diameter of 0.01–0.8 μm (mean 0.1 μm), a size that renders them easily respirable and capable of reaching and efficiently depositing deep in the lung. The underlying mechanisms by which DEP exposure leads to health effects are under active investigation but remain unclear, and especially the differential roles of carbon core and organic DEP chemicals remain a source of debate. Besides particulate matter, diesel engine emissions contain a series of gas-phase pollutants which may also contribute to their toxicological impact (Rudell et al., 1999). These gas-phase pollutant impacts have been under-evaluated from a toxicological point of view due to the difficulties encountered in sampling and delivering them to potential biological targets.

The overall objective of the MAAPHRI program was to develop new screening tools to assess environmentally related health issues derived from airborne pollutants by a multidisciplinary approach involving native complex aerosol toxicology, cell biology, chemistry and physics. To address the impact of particle size distribution and soluble organic fraction and gas-phase pollutants on the toxicity response profile using both in vitro screens and in vivo models for validating in vitro screens. To assess the potential benefit of reformulated fuels and engine technologies with respect to health-related modeled endpoints. Attempts to better mimic the nature of the aerosol/lung tissue interactions occurring after aerosol inhalation led to experiments performed with exposure setups allowing a continuous flow of drawn and diluted diesel engine emission aerosols. Under these conditions, animals and more recently cultured cells (Abe et al., 2000; Cheng et al., 2003; Knebel et al., 2002) or rat lung organotypic cultures (Morin et al., 1999; Le Prieur et al., 2000; Bion et al., 2002) are directly exposed to a complex atmosphere, the physico-chemical properties of which are perfectly preserved. A brief overview of the literature on diesel emission toxicity, which is too frequently restricted to diesel soot toxicology, led us to the following methodological considerations to be potential confounding factors or artifacts for pertinent aerosol toxicological impact descriptions. Most of the discussion below might apply to tobacco smoke as well.

Particulate matter sampling on filters

Potential artifacts due to aerosol component sampling procedures and delivery to biological targets

When impacted on filters, diesel particulate matter, the mean mobility diameter or aerodynamic diameter of which in native emissions ranges 80–100 nm, will form aggregates which will be extremely difficult to dissociate afterwards as shown on Fig. 1. With the best technology to date for resuspending solid material into aerosol, we have been unable to improve diesel soot size distribution better than one order of magnitude mean size compared to freshly prepared diluted diesel engine emission aerosol. A second consideration is that when sampling diesel engine emission aerosol, filter-impacted particles may act as a gas-phase component adsorbant and/or as the site of chemical reaction with a high amount of gas-phase pollutant like volatile organic compounds or nitrogen oxides (Risby and Lestz, 1983). This may lead to inappropriate chemical composition of the filter-sampled particles compared to their actual composition in the emitted aerosol. This remark may be of particular importance in terms of soluble organic fraction quantity and quality, and on associated nitro-PAH contents for instance.

Liquid suspensions of particulate matter

Filter-harvested diesel particles are very frequently resuspended into aqueous liquid media for either in vivo instillation or for in vitro cell culture exposure. Two potential sources of artifacts may occur using this methodology: dissociation of hydrophobic diesel soot aggregates into aqueous media remains a real technical challenge since very large soot aggregates are usually obtained which even resist to ultrasound treatment for dispersion. The use of tension-active agents like Tween lecithins is frequent in order to improve liquid soot dispersion by ultrasound treatment. Fig. 2 shows an image of diesel soot liquid suspensions obtained by these procedures. Size of the best-dispersed soot aggregates is largely above 2 μm, which again is much larger than soot mean diameter in raw emission aerosols (80–100 nm mean aerodynamic diameter). Furthermore, soot re-aggregation occurs quite shortly after plating. This is of great importance since recent studies report a size-dependent impact on target cells, for instance for macrophage activation (Rothen-Rutihauser et al.,