

# Multidisciplinary Approaches to Airborne Pollutant Health Related Issues MAAPHRI

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## FINAL SUMMARY REPORT

### Overall objectives of the project :

#### Scientific and social Objectives :

- \* to assess the potential benefit of reformulated fuels and engine technologies on combustion engine emission safety with respect to health related modeled endpoints
- \* to address the impact of particle size distribution and soluble organic fraction on the toxicity response profile using both in vitro screens and in vivo models of allergy and cardiac insufficiency
- \* to deliver biological in vitro safety evaluation tools allowing to assess industrial engine exhaust depollution strategies based on the quantification of actual health beneficial impacts.
- \* To provide policy-makers with health-related impact based knowledge for elaborating future regulatory decisions based more on scientific experimental data than on the precaution principle

#### Working methods, Results and Recommendations

We established the technical requirements for accurate sampling and dilution procedures to provide complex atmospheres made of diluted engine exhaust driven on test benches suitable for mimicking the in vivo inhalation situation in terms of deposition mechanisms and rates on in vitro biological reporter systems.

**Deliverable 1 :** design of continuous flow in vitro chambers for homogeneous exposure of multiwell culture plates to complex aerosols. A series of CFD simulations were performed and the one that best improved the uniformity of the velocity distribution is based on a geometry with a 40° closing of the outflow. These chambers proved their efficiency for simultaneous exposure of 4 multiwell plates, and were used by P1 and P2 and P6 for the completion of deliverable 6

**Deliverable 2 :** Refined unrestraint rodent inhalation exposure chambers suitable for wireless continuous telemetry recordings

State of the art Computational Fluid Dynamics (CFD) simulation tools were employed to study and optimise the flow inside the inhalation device chamber. The geometric details and inlet/outlet port boundary conditions of the inhalation chamber was studied. The uniformity of the concentration of particulate matter, as well as velocity variations in the inhalation chamber, were examined by three-dimensional simulations employing accurate unstructured grid discretizations of the governing (Navier-Stokes) equations for the flow. An axisymmetrical design is chosen to comply with the defined constraints. Indeed, this geometry give the optimal ratio between volume and inner side surface. These chambers proved their efficiency and were used by P1 and P6 for completion of deliverable 6.

**Deliverable 3 :** Selective particle sizer SPS

We designed, built and evaluated a selective particle size (SPS) sampler able to provide constant flow selection of particulate matter of different size ranges in the overall range of diesel particle size distribution (from about 10 to 400 nm) with adequate flow rates, sample conditions (e.g. temperature, pressure) and particulate matter (PM) concentrations for subsequent use for biological assessment. Two different scales of SPS were produced. Successful simultaneous and continuous separation of two particle size classes have been achieved with multiple hour stability with mean mobility diameters of 58 and 150 nm. Isonumber exposure have been performed with PM concentrations up to 10<sup>5</sup>/cm<sup>3</sup> for completion of Deliverable 6

**Deliverable 4 :** Tailored immune modulated cell lines

A series of clones expressing the luciferase reporter /cytokine constructs (IL8, IL4, NFkB, IFN $\gamma$ ) were obtained and tested for expression stability and inducibility with time and selected according to their performances for subsequent use for direct aerosol exposure and safety evaluation after adaptation to bi-phasic Air/liquid culture conditions. Thanks to the use of SPS

we have for the first time evidenced variable cytokine response patterns to size selected particles in cytokine reporter gene cell lines thus pointing out to either variability of chemical composition and/or size related triggers contributing to ultrafine combustion emitted particles impacts.

**Deliverable 5 :** Physicochemical emission characteristics from Euro3 compliant engine according to fuel sulfur and after-treatment strategy :

Regulated emissions namely Non methane hydrocarbons, CO<sub>2</sub>, nitrogen monoxide, nitrogen dioxide, carbon monoxide and particulate matter have been measured both on engine raw emissions as during both in vivo and in vitro exposures. Results showed limited differences between P6 and P2 sites where identical engines were run on test benches. Main results consisted in an unexpected increase of NO<sub>2</sub>/NO<sub>x</sub> ratio and NO<sub>2</sub> emission at the tailpipe of low sulphur fuel after-treated emissions, in the absence of total NO<sub>x</sub> emissions. This variation was tightly correlated with oxidant stress injuries reported in deliverable 6.

A new measurement technique of engine emission oxidant potential has been developed using electron spin resonance (ESR) apparatus and CPH as a spin probe dissolved in liquid media, Interestingly, we could draw tendencies according to fuel composition and after-treatment devices : fuel sulphur reduction induced increased reactive species occurrence, Diesel oxidation catalyst (DOC) induced increased oxygen reactive species occurrence, and finally, the combination of DOC + DPF (Diesel particle filter) induced intermediate increase of oxygen reactive species between untreated emission and post-treated emissions. This increase in oxygen reactive species appears to be highly correlated with NO<sub>2</sub> concentrations in the emission.

**Deliverable 6 :** Health related safety issues

During this program a variety of health related endpoints have been addressed as being representative of Inflammation, Allergy, cardiovascular, oxidant stress and DNA alterations when varying fuel and lubricant compositions and emission after-treatment strategies in a representative way comparable to technical strategies developed to reach the attainment dictated by the evolution of the regulation. All exposures of biological systems were performed directly to continuous flows of continuously sampled and conditioned diluted combustion aerosols. These studies thus allowed us to evaluate the respective contributions of both gaseous and particulate phase of the emissions.

To summarize the results : Our in vitro experimental models showed that with high sulphur fuel (300ppm) 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. At the opposite, with ultra-low sulphur fuel (0ppm) and lubricant (Low SPAsh) in the presence of strong Diesel oxidation catalysis, with or without Diesel particulate filter, the specific role of particulate matter was no more evidenced and the gas phase becomes the major component triggering strong oxidant stress,

We have for the first time evidenced variable cytokine response patterns to size selected particles in cytokine reporter gene cell lines.

Using a direct exhaust gas exposure system for Ames test, we could not evidence mutation occurrence with two strains of Salmonella typhimurium (TA98 and TA100) up to high emission concentrations (dilution 1/2) of combustion emitted aerosols while the system was clearly responsive to Benzylchloride vapors used as a positive control. These observations question the bioavailability of combustion emitted airborne pollutants for exerting their known genotoxic potential.

In our in vivo inhalation experimental situations, we could not identify urinary excretion of PAH related DNA adducts, nor of 8-oxoguanine. We could not identify any significant immune modulation using the KLH test, nor any alteration in bronchoalveolar lavage fluids through TNF alpha assay, nor inflammatory cell counts. However, we could clearly identify

increased plasma levels of TNF $\alpha$ , and a strong oxidant stress not only restricted to respiratory organ, but transmitted through systemic compartment to heart, liver and kidneys. In healthy rats, we evidenced a clear alteration of heart rate variability concomitant of a marked systemic oxidant stress we could relate to increased emissions of NO<sub>2</sub> and increased occurrence of reactive oxygen species. In chronic insufficient rats, we evidenced a clear alteration of heart rate variability, concomitant of a marked pro-arrhythmic effect of diluted Diesel engine emission which again correlates with NO<sub>2</sub> concentrations.

We suggest that increased NO<sub>2</sub> emissions raise a new health safety concern that should be addressed by regulatory authorities since recent epidemiologic studies point out to an important role of NO<sub>2</sub> for triggering airborne pollutant acute cardiorespiratory impacts.

***Deliverable 7*** : Recommendations to meet emission and health effects reduction

Screening for Health related issues of combustion emissions should consider :

1 Global aerosol : Particles and vapour phase (Solid, liquids and gaseous components) for inhalation related experiments.

2 Particulate Matter : Preserving Native aggregate Size distribution : size dependent impacts)/ On demand selection and control of aggregate size for exposure of both in vitro and in vivo biological reporter test systems

3 Gas phase component preservation : Avoid as much as possible uncontrolled aged samples : concentrated combustion aerosols are potential chemical reactors, condensation processes may occur. ROS trigger mechanisms (Gas/particles) in biological fluids and tissues

4 Multidisciplinary approaches :

Intradisciplinary (cells, tissues, whole body) Inflammation, oxidant stress, DNA alterations, mutation. Interdisciplinary : Physicochemical metrology, Sampling and delivery to reporter test systems

Prospective : The Commission should encourage Systematic biological safety studies of combustion engine emissions

Fuel composition and additives/Engine technology/After-Treatment Strategies

Representative of existing market system (DOC, DPF, SCR, additives ...)

Prospective Biofuels, DeNox,

HCCI, Diesel IDI, DI, Common rail, 2 strokes engines (2-wheelers, gardening tools, ...)

Studies with optimised native emission Sampling, diluting, conditioning and delivery procedures

Specificity of Particle sampling : size, chemistry, morphology

Specificity of gas phase sampling,

Geometry of deposition/exposure design for biological reporter tests

Future Regulations

To consider systemic interactions as well as single components

To anticipate the complex and the unexpected by a wholistic approach of complex aerosols

***These statements are not restricted to combustion emissions and may be applied to other nanoaerosols, especially for future assessment strategy of manufactured nanoparticles which could benefit of techniques developed in the frame of MAAPHRI program.***