EMPIR Call 2018 – Health, SI Broader Scope, Normative and Research Potential

Selected Research Topic number: **SRT-h09**
Version: 1.0

Title: Measurements for mitigating adverse health effects from atmospheric particulate pollutants

Abstract

Atmospheric particulate pollution has been linked to a broad spectrum of adverse effects including respiratory and cardiovascular diseases, lung cancer and premature death. Although these effects are related to the physical and chemical properties of the airborne particulate matter (PM) it has not been possible to relate the relative contribution of the PM's constituents to the reported population-level health effects. Therefore, synthetic reference aerosols, high-resolution optical imaging and state-of-the-art cell analysis methods need to be developed and used to study the cytotoxic effects of airborne PM in vitro. A systematic and consistent approach should be taken so that PM metrics can be correlated with specific health effects.

Keywords

Atmospheric aerosols, cell cultures, chemical models, cytotoxicity, lung organoids, optical microscopy, oxidative potential, particulate matter, inflammatory response

Background to the Metrological Challenges

Atmospheric aerosols vary significantly in their size, composition and spatiotemporal distribution, but they are only regulated by the mass concentration of the size fractions PM_{10} and $PM_{2.5}$. This is too crude a metric to characterise their potential to cause adverse health effects. In addition, most epidemiological studies have not investigated the health effects of isolated aerosol components. Thus far, it has therefore been impossible to clearly identify which metrics (e.g. particle size, number concentration, surface properties, mass concentration, chemical composition) dominate the onset and manifestation of detrimental health effects. Additional air quality particle metrics such as black carbon concentration, number concentration, oxidative potential and particle surface area have also been used for evaluating the health effects of ultrafine particles. This situation is further complicated by the fact that atmospheric aerosols can become coated with primary or secondary organic matter and this may also cause detrimental health effects.

Experiments with lab-generated reference aerosols have mostly been limited to studies with single-component aerosols, such as clean carbon particles without metal or organic coatings. Therefore, a stable and reproducible laboratory source of well-controlled and chemically defined synthetic reference aerosol mixtures needs to be developed that mimics real ambient aerosols at high concentrations. Similarly, very few studies have been undertaken on the pulmonary effects of aged organic aerosol and these have either been conducted with real ambient aerosols or using a smog chamber. There is therefore a need for the physicochemical properties of the particles to be tuneable and the aerosol source needs to be coupled to a micro-smog chamber to enable the particles to be 'aged' by coating them with secondary organic matter. This would enable the impact of atmospheric 'ageing' on the oxidative and inflammatory potentials of the aerosol mixtures to be investigated.

Many of the microscopy techniques used to image 2D, scaffold and 3D organoid cultures are either unsuitable for imaging live specimens or they have limited depth penetration, spatial resolution and imaging speed. Another issue is that most light microscopy techniques produce high levels of illumination causing phototoxic reactions that can lead to spurious cell responses. To resolve this, a combination of high-resolution optical microscopy techniques need to be used in order to quantify the effects of particle uptake by single cells at different spatiotemporal scales. State of the art computational image analysis techniques need to be developed to derive unbiased response metrics from complex biological images.

3D multicellular lung organoid models have been developed to provide a more physiologically relevant model for measuring the biological effects of in vivo exposure and toxicity. This model needs to be used to understand, define and measure the effects of aerosol particles at cellular and subcellular scales and to quantify the

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phenotypic effects of particle uptake. The measurements need to be minimally invasive to permit long-duration sampling without damaging the culture. The portability and reproducibility of this and other models, such as spheroids and organ-on-a-chip technologies, needs to be characterised and proven before assay validation.

The traceability of EU reference methods for the physical and chemical characterisation of aerosols needs to be improved using synthetic sources. The uncertainties in mass concentration and major chemical component measurements need to be reduced and they need to be quantified for emerging techniques. New approaches are also needed to ensure reproducibility and quality assurance.

Studies of the in vitro toxicology of aerosol particles were traditionally performed using simplistic model particles in submerged cell-systems. Although, it has been difficult to determine the effects of particle size, shape or surface area, previous studies have suggested that the surface area may describe their toxicity more coherently than their mass. In addition, significant experimental artefacts can be caused by the overlying media and from the atmospheres in which the cells are challenged. To resolve this and to improve in-vitro to in-vivo correlations, biocompatible respiratory tract lining fluid simulants have been used and the cell cultures have been grown under physiologic pulmonary oxygen tensions. Based on these studies, novel methods need to be developed for exposing cells at the air-liquid interface in order to mimic and quantify the effects of in vivo aerosol inhalation. High-volume samplers need to be used as ambient concentrations of aerosol particles are too low to observe acute cell responses.

Traditional cell viability and more complex assays, which allow the exposure of human cells at the air-liquid interface, have been developed using human airway cells. This approach mimics the inhalation of aerosol nanomaterial under near physiological conditions. Simple chemical models which simulate fluids from the lining of the human respiratory tract can be used to improve the relevance of the initial interaction of the aerosols at the cellular interface. These approaches need to be used to assess how the composition of collected aerosols, and their ageing, impacts on their oxidative characteristics. A range of ‘omics’ technologies have been used to identify the molecular-level changes that underlie responses to chemical stressors. These and other cell analysis technologies need to be investigated after the tissues have been exposed to aerosol particles. Standardised validated protocols are needed at a European level and ‘omics’ data needs to be to quantitatively connected to phenotypic outcomes.

Objectives

Proposers should address the objectives stated below, which are based on the PRT submissions. Proposers may identify amendments to the objectives or choose to address a subset of them in order to maximise the overall impact, or address budgetary or scientific / technical constraints, but the reasons for this should be clearly stated in the protocol.

The JRP shall focus on the traceable measurement and characterisation of atmospheric particulate pollutants, and their effects on biological models, in order to mitigate their adverse health effects.

The specific objectives are

1. To develop a stable and reproducible laboratory source of, well-controlled and chemically defined, synthetic reference aerosol mixtures that mimic real ambient aerosols at high concentrations (e.g. at around the limit values for the EU Air Quality Directive). The physicochemical properties of the particles should be tuneable and the source should be coupled to a micro-smog chamber to enable the particles to be “aged” by coating them with secondary organic matter. The impact of atmospheric ‘ageing’ on the oxidative and inflammatory potentials of the aerosol mixtures should be investigated. In addition, the reference aerosols should be used to quantify the effects of particle uptake by single cells using state of the art high-resolution optical microscopy techniques (e.g. DIC/phase, confocal, structured illumination, light sheet). Lung organoids should also be used to investigate and quantify the phenotypic effects of particle uptake.
2. To improve the traceability of EU reference methods for the physical and chemical characterisation of aerosols using this synthetic source. The uncertainty in mass concentration measurements should be reduced to 15 % and in number concentration to < 20 %, the uncertainty in the analysis of major chemical components (e.g. elemental carbon, organic carbon, metals and ions) should be reduced to < 15 %. In addition, the uncertainties should be quantified for emerging techniques, such as Aerosol Mass Spectrometry, the Aerosol Particle Mass analyser and Brunauer–Emmett–Teller surface area measurement. In particular, new approaches should also be developed and validated to ensure reproducibility and quality assurance.

3. To mimic and quantify the effects of in vivo aerosol inhalation using novel methods for exposing cells at the air-liquid interface. The aerosol particles required for this study should be collected using high-volume samplers.

4. To assess how the composition of collected aerosols, and their ageing, impacts on their oxidative characteristics. This should be investigated in simple chemical models, which simulate fluids from the lining of the human respiratory tract, and in representative cell lines maintained under near physiological conditions. Different methods for performing cell analysis, after exposure of the tissues to aerosol particles (e.g. pro-/anti-inflammatory response, cytotoxicity/genotoxicity tests, and "omics" technologies) should be investigated. Improved validated protocols should be proposed for harmonising/standardising cell analysis studies at a European level.

5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (accredited laboratories, instrumentation manufacturers), standards developing organisations (CEN, ISO) and end users (e.g. hospitals and health centres).

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes, and it is expected that multidisciplinary teams will be required. To enhance the impact of the research, the involvement of the appropriate user community such as medical practitioners, hospitals and industry is strongly recommended, both prior to and during methodology development.

Proposers should establish the current state of the art, and explain how their proposed project goes beyond this.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 1.8 M€, and has defined an upper limit of 2.1 M€ for this project.

EURAMET also expects the EU Contribution to the external funded partners to not exceed 35 % of the total EU Contribution across all selected projects in this TP.

Potential Impact

Proposals must demonstrate adequate and appropriate participation/links to the "end user" community, describing how the project partners will engage with relevant communities during the project to facilitate knowledge transfer and accelerate the uptake of project outputs. Evidence of support from the "end user" community (e.g. letters of support) is also encouraged.

You should detail how your JRP results are going to:

- Address the SRT objectives and deliver solutions to the documented needs,
- Feed into the development of urgent documentary standards through appropriate standards bodies,
- Transfer knowledge to the health sector.

You should detail other impacts of your proposed JRP as specified in the document "Guide 4: Writing Joint Research Projects (JRPs)"

You should also detail how your approach to realising the objectives will further the aim of EMPIR to develop a coherent approach at the European level in the field of metrology and include the best available contributions from across the metrology community. Specifically, the opportunities for:

- improvement of the efficiency of use of available resources to better meet metrological needs and to assure the traceability of national standards
- the metrology capacity of EURAMET Member States whose metrology programmes are at an early stage of development to be increased
- organisations other than NMIs and DIs to be involved in the work

Time-scale

The project should be of up to 3 years duration.