Aerosols and health: a challenge for chemical and biological analysis

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In the industrialized countries, air pollution represents the most severe environmental impact on human health. Since the early studies on the health effects of the London smog episode in 1952 [2, 24], an enormous amount of epidemiological, toxicological, medical, chemical, physical, and technological research was conducted in order to understand and prevent the effects of air pollution, especially with fine particulate matter, on human health [1, 7, 9, 12, 13, 17, 19, 20, 25]. The legislation and abatement measures based on this work have considerably improved the air quality in many industrialized countries. However, despite all the undertaken measures, air pollution still is the most potent environmental (pollution) health threat in the first world [20]. Based on this paradigm, a scientific session at the *Analytica Conference* 2014 in Munich and a workshop at *Helmholtz Zentrum* on the topic *Aerosols and Health: A challenge for chemical and biological analysis* were organized and conducted. Thereafter, this topical issue was planned and compiled, similarly to a previous topical issue “*Aerosol Analysis*” on *Organic*

compounds in fine and ultrafine dust – Analysis and impact on human health, which was previously published in *Analytical and Bioanalytical Chemistry* [26]. The present topical issue consists of a feature article, a critical review, and seven research papers, with four contributions coming from the consortium *Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health – Aerosols and Health* (HICE, www.hice-vi.eu), which tries to combine a comprehensive analysis of aerosol properties and composition with a multi-omics comprehensive analysis of the biological effects of the aerosol onto human lung cells to improve the knowledge on the effect mechanisms [14]. Below, the contributions of this topical issue on *Aerosol and Health: A challenge for chemical and biological analysis* are briefly introduced.

The feature article of Gilmour, Kim, and Hays from the *US Environmental Protection Agency* addresses the importance of the organic composition of particulate matter (PM) from diesel engines and wildfires (i.e., bio mass burning) for health effects, in particular for (allergic) lung inflammation. They conclude that a deeper knowledge on the organic PM composition as well as the implementation of new, more efficient approaches for toxicity testing, including cell-based methods, is required. It is suggested to address several challenges in the future for improving the knowledge of the health impacts of combustion emissions. This includes the generation of combustion emission standards (see in this context also the contribution of Müller et al. on the characterization of a CAST combustion reference aerosol generator in this issue), developing validated approaches for toxicity prediction, identifying common health outcomes, and extrapolation of chronic exposure effects, including adaptive and recovery responses. Furthermore, the authors propose to better study the influence of susceptibility factors (e.g., illnesses or genetic variability) and the effects of complex mixtures (synergistic and antagonistic

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effects). A critical review included in this topical issue by Parshintsev and Hyötyläinen from *Helsinki University* and *Steno Inc.* summarizes the state-of-the-art in analytical methodology for characterization of organic compounds in atmospheric aerosol particles. They distinguish off-line and on-line methods and discuss advantages and disadvantages of the individual techniques, concluding that further method development and combination of different on-line and off-line techniques is needed for a more thorough comprehension of the composition and dynamics of organic compounds in the atmosphere. The following five research papers report on new applications and approaches for characterizing organic compounds in aerosols. The on-line real-time analysis of the particle composition by mass spectrometry is, in particular, an important aspect for understanding atmospheric transformation processes. Although the technology of Aerosol Mass Spectrometry (AMS, Aerodyne Inc., USA) [10] had a strong impact on atmospheric chemistry research in the last years, a renaissance of the classic laser-based single particle aerosol time of flight mass spectrometry (ATOFMS) [18] could be foreseen. This trend is fueled also by the recently established possibility to obtain quantitative ATOFMS data [8], similarly as by the AMS approach. The ATOFMS approach has also the capability to detect sum values for elemental and organic carbon (EC/OC) [6] and specific organic compounds. The contribution of Healy et al. from *University of Toronto* reports in this context on single-particle resolved speciation of alkylamines in ambient aerosol at five European cities. The authors could detect large fractions of alkylamine-containing aerosol particles in all five cities, with trimethylamine being the most prominent species. In some places, however, other alkylamine species also occur because of -in one case external and in another case internal- mixing. The article of Müller et al. from *Helmholtz Zentrum München* (HICE contribution) focuses on the investigation of the chemical composition (PAH) and physical properties (i.e., light absorption at different wavelengths for detection of organic-rich “brown carbon” and rather soot-like “black carbon”) of a standard reference aerosol generated by a flame soot generator (CAST, Jing-Cast Ltd., Switzerland). In addition to off-line chemical analyses (GC-MS for PAH), on-line mass spectrometry (AMS) and further on-line aerosol monitoring techniques are also applied. As discussed before, a reliable and reproducible on-line combustion aerosol standard is important for e.g., toxicological tests, and the CAST system is a reasonable candidate for such a standard system with tunable properties: depending on the air-fuel mixing ratio the physical properties and the composition of organic compounds (mainly PAH) can be adjusted. Addressing the vast complexity of combustion aerosol particles is a particular challenge. Still a large fraction of the organic inventory of emission- and ambient-PM is unidentified [3]. As described in the review within this topical collection, several high-end analytical chemical approaches based on

mass spectrometry, such as comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry [23] or ultra-high resolution mass spectrometry [21] can be used to address the yet unknown chemical space of aerosol PM. An important aspect for all mass spectrometric technologies, however, in this context is the ionization method used for the mass spectrometric detection. Different ionization methods such as electrospray ionization (ESI), atmospheric pressure photo ionization (APPI), electron ionization (EI), or matrix assisted laser desorption ionization (MALDI) can address different molecules, depending on the molecular weight, structure, elemental composition, and physical chemical properties and the type of sample and, furthermore, all have specific strengths and weaknesses. Thus the development, evaluation, and comparison of different ionization methods for PM analysis is very important for the research field. The publication of Rüger et al. from *University of Rostock* (HICE contribution) discusses the characterization of primary emission particulate matter from a ship diesel engine at the molecular level by means of laser desorption ionization (LDI)-ultra-high resolution mass spectrometry. The developed method could be directly applied to PM deposits collected by an impactor-based PM-sampling device. The high mass resolution and accuracy of the used FT-ICR mass analyzer allows a quick profiling using only a minimal sample size. By using an imaging approach for characterization of the structures formed by the deposited PM, LDI-artifacts such as fullerene formation from soot at higher laser fluences could be studied and avoided. In the publication, lastly the composition of ship engine emission particles are compared with the composition of the fuel (heavy fuel oil), showing that a large fraction of the compounds in the aerosol particles is actually stemming from unburnt fuel. An aerosol is defined as solid or liquid micro- and nanoparticles dispersed in a gaseous phase. If the health effects of aerosols are addressed, an important but often neglected aspect is the impact of the gas-phase composition. Combustion effluents and ambient aerosols (e.g., during photochemical smog conditions) often contain quite large amounts of reactive or toxic gases, such as butadiene, aldehydes, ozone, or benzene. Often the mass concentrations of toxic gas-phase constituents are higher than the PM concentration. The synergy of PM and toxic gases in the emission and ambient aerosols might thus be particularly relevant for health effects as the small, inhalable particles supposedly act as carrier for adsorbed toxic gas-phase species, potentially transporting them through the denuding sections of the airway system in the vulnerable deeper regions of the lung. This scenario motivates the study of relevant gaseous compounds in emissions and the ambient environment. The highly dynamic changes of gas-phase concentrations in many emission sources suggest the use of real-time on-line analysis methods. Recently, several on-line mass spectrometric analysis systems based on soft ionization by either chemical ionization (CI) or

photo ionization (PI) became available (e.g., IONICON, Austria; Photonion, Germany). The contribution of Radischat et al., *University of Rostock* (HICE contribution) focuses on the real-time mass spectrometric analysis of organic compounds in ship engine aerosol emissions using the PI and CI methods of resonance-enhanced multiphoton ionization-mass spectrometry and proton transfer-mass spectrometry. The ship engine was operated either with diesel fuel (DF) or heavy fuel oil (HFO) at different engine parameters (e.g., load or fuel injection parameters). The on-line real-time approach allows the coverage of dynamic processes (e.g., change of the type of fuel). It was observed that the emission of most gas-phase compounds is considerably higher in case of HFO use. This was also observed for the organic compounds in the particulate matter [14]. The performed on-line gas-phase exhaust measurements furthermore depict that low loads and the operation of the engine in idling mode cause particularly high emissions of carboxylic and aromatic compounds.

In order to understand the aerosol-induced biological effects and, in the long run, also the human health effects, in addition to the chemical information on known toxicants or potentially toxic compounds in the aerosol, as well as the toxicological or biological response information from aerosol-exposed biological systems need to be analyzed. Only as a first approximation, a rough evaluation of the toxicity potential of PM or gases can be performed by chemical target analysis of relevant known toxicants in the PM, using known toxicity- or toxicity-equivalence values of well-known compounds such as PAH [15]. Another possibility is the use of chemical assays using indicator reactions (e.g., for the oxidative potential of emission particles [5], which is believed to be connected with induction of inflammatory processes in lung and other target organs [22]. The application of lung cell-based assays in this context promises to be a reproducible tool for accessing initial biological responses of the primary target tissue on collected PM [9] or, if adequate exposure technologies are used, directly on emission- or ambient aerosols [16]. However, the study of systemic effects finally needs animal- [4] or human patient studies. The latter approaches, however, are largely limited by cost and ethical restrictions. Cell-based test systems thus are considered as a good option for standard testing of the initial biological effects induced by aerosol- or PM-exposure. This topical issue reports on three cell-based studies on the PM or aerosol toxicity.

An important strategy to address the components in the aerosol, which are responsible for biological effects in cellular systems, lies in the fractionation of the sample in chemically different, less complex sub-fractions, and subject those individually to the cell test assays. This principle was applied in the work “Assessing the Role of Chemical Components in Cellular Responses to Atmospheric Particle Matter (PM) Through Chemical Fractionation of PM Extracts” by Heo et al. from *University of Wisconsin-Madison*. The authors

investigated the role of different water-soluble metals on lung cells. Water extracts from ultrafine particles (PM_{0.25}) collected on filters at different places in the Los Angeles region were generated. By using different metal-chelating agents, the authors generated different soluble metal-containing chemical fractions. A rat alveolar macrophage model (NR8383) was used in submerge cell culture to determine the formation of reactive oxygen species (ROS) and the pro-inflammatory tumor necrosis factor α (TNF α) by a fluorescence- and an ELISA-assay with the different generated fractions, respectively. The authors summarize that the transition metals Mn, V, and Fe are the most important soluble metals generating oxidative stress (ROS-formation) in conclusion with (e.g., epidemiological studies). The contribution of Kanashova et al. from the *Max Delbrück Centre of Molecular Medicine* (HICE contribution) describes a different approach of chemical fractionation in their work. Collected emission particles from a ship diesel engine operated with heavy fuel oil (HFO) were investigated. The HFO-particles contain a large fraction of semi-volatile and low-volatile compounds that were thermally stripped in a thermal analysis instrument at 280 and 580 °C, respectively. Murine macrophages (RAW 647.5) were exposed to the untreated and thermally treated fractions. The biological response of the cells was investigated by a differential proteomic analysis of the exposed mouse macrophages using an automated sample preparation workflow. An interesting aspect is the much more comprehensive bioanalytical approach for effects monitoring. Instead of investigating only a limited number of endpoints, the concentration of thousands of proteins were determined in a quantitative proteomics approach using stable isotope labeling (SILAC) for referencing. By this approach, the course of several biological pathways can be assessed, for example, results of the successive removal of the adsorbates in a decreasing activity of DNA-damage and repair. This might be explained by the removal (i.e., vaporization) of the polycyclic hydrocarbons. Many other important pathways, including the enhancement of the phagocytosis (i.e., the engulfment of particles) by ship emission particles were described. The last contribution in the topical collection by Steiner et al. from the *University of Fribourg* studies the effects of an iron-based fuel-borne catalyst and a diesel particle filter on exhaust toxicity in lung cells in vitro. An important parameter in lung cell-based test systems is the type of PM or aerosol exposition. Routinely, lung cell cultures are exposed to collected PM samples in submersed culture (see the two previous contributions). A suspension of the PM sample in a solvent is added to the medium in which the cells are growing. This approach has advantages (e.g., in simplicity and technical reproducibility) but obviously does not reflect the natural exposure situation well. Recently developed air liquid interface (ALI) technologies, in which the cells are growing in general as a confluent layer on a semipermeable membrane in contact with the liquid culturing media, allow a much more

realistic exposure approach by mimicking the situation in the lung [11, 16]. An important aspect in the study is that the more realistic exposure scenario at the air liquid interface (ALI) is applied for the exposure of the lung cell culture composed of lung cell line (16HBE14o⁻) and primary (human blood monocyte derived macrophages) cells. The cells are ALI-exposed for different times to exhaust of a diesel engine with and without diesel particulate filter. Diesel fuels with different additives are used. The cells are exposed for different times to the diluted emission aerosol and the cytotoxicity (LDH-release), oxidative potential, as well as interleukin 8- and TNF α -gene expression and secretion is measured. The outcome is that the particulate filter, depending on the additives, does not reduce all toxic effects of the exhaust gas, as reactive gases (or very small nanoparticles) are still emitted. Steiner et al. conclude that a more comprehensive aerosol characterization is required to better understand the observed biological effects in future.

Summarizing the scientific session at Analytica 2014, the workshop and the publications presented in this topical issue of *Analytical and Bioanalytical Chemistry on Aerosols and Health: A challenge for chemical and biological analysis* the state-of-the-art and research gaps have been identified and discussed. The previous workshop and special issue from 2011 [26] resulted in the proposal and finally establishment of the HICE-consortium with some of the first results now being presented and discussed within this topical issue and in other publications [14]. However, it becomes clear that a better understanding of aerosol-related health effects will remain a huge task for the decades to come.

Last but not least, I hope that the readers of *Analytical and Bioanalytical Chemistry* are interested in the contributions on the interdisciplinary field of Analytical Sciences in Aerosol and Health research compiled in this special issue.

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