doi:10.2533/chimia.2024.734 Chimia 78 (2024) 734–738

Oxidative Properties of Atmospheric Particles and their Biological Effects

Markus Kalberer[a]*, Barbara Rothen-Rutishauser[b], Kaspar Daellenbach[c], and Jean-Jacques Sauvain[d]

Abstract: Particulate Matter (PM) is the most toxic component in polluted air causing over 6 million deaths per year worldwide according to World Health Organisation estimates. Due to the highly complex composition of PM in the atmosphere, with thousands of inorganic and especially organic components, it is unknown which particle sources are responsible for their toxicity. In recent years it emerged that overall oxidising particle properties might directly link particle composition with health effects. This review summarises contributions of Swiss research groups to the chemical and biological characterisation of PM oxidising properties and identification of biological responses such as oxidative stress due to PM exposure.

Keywords: Aerosol particle · Air pollution · Health effects · Oxidative stress

Prof. Dr. Markus Kalberer did his graduate studies at the University of Bern, Switzerland. After postdocs at Caltech, USA, and ETH Zürich, Switzerland, he was a Professor of Atmospheric Sciences at the University of Cambridge, UK, for over twelve years before moving to Basel in 2018. His research interest and experience spans across Atmospheric, Physical and Analytical Chemistry with an emphasis on novel instrument development, *e.g.* mass spectrometry and fluorescence spectroscopy, to investigate organic and metal aerosol particles with a focus on potentially toxic particle components.

Prof. Dr. Barbara Rothen-Rutishauser received her PhD in 1996 in cell biology at the Swiss Federal Institute of Technology in Zurich (ETHZ). She worked as group leader at ETHZ and University of Bern, Switzerland. She is an expert in cell-nanoparticle interactions, focusing on human 3D tissue models to assess the effects of emission exhausts. Since 2011, she has been the co-chair in BioNanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland.

Dr. Kaspar R. Daellenbach earned a PhD in Environmental Sciences from ETH Zurich in 2018, followed by a postdoc at the University of Helsinki. Now a group leader at the Paul Scherrer Institute's Laboratory of Atmospheric Chemistry. His research focus is on particulate air pollution, identifying harmful aerosol sources and their impact on health. He uses techniques like mass spectrometry and air quality modelling to assess the toxicity of aerosols and predict their global health effects.

Dr. Jean-Jacques Sauvain got his PhD in chemistry at the University of Neuchâtel in 1990. He then studied environmental chemistry at EPFL where he continued as a research associate. Since 2000, he has been working in the field of occupational hygiene and health. His expertise relates to exposure characterisation to particulate matter and their health effects, measuring oxidative stress biomarkers in biological matrices like exhaled breath condensate or urine.

1. Introduction

Over the last three decades a large number of epidemiological studies consistently indicated that particulate matter (PM) in polluted air is causing a wide range of adverse health outcomes such as pulmonary and cardiovascular diseases but also lung cancer and neurological disorders.[1] The World Health Organisation (WHO) estimates that over 6 million deaths per year are attributed to outdoor and indoor PM exposure, representing by far the most significant cause of disease due to environmental pollution.[2] Despite this clear epidemiological evidence, it is largely unknown which physical particle properties or chemical particle components are responsible for their toxicity. Particles can be directly emitted into the atmosphere *e.g.* by combustion sources, industrial activity, or natural processes such as desert dust or sea spray, but the largest PM fraction in polluted areas is formed within the atmosphere from gaseous precursors in chemical oxidation reactions. Ambient PM is a complex mixture of tens of thousands of compounds, including metals, minerals, insoluble graphite-like soot, inorganic salts, and organic compounds, which are often highly oxidised.[3]

The main particle property regulated in legal frameworks aiming to reduce PM exposure in Switzerland and the EU is the total particle mass with average annual limit values for Switzerland of 10 μg $PM_{2.5}$ *per* m^3 ambient air (*i.e.* mass concentration of PM with diameters smaller than 2.5 μm). Based on recent scientific evidence, the WHO recently revised their guidelines and recom-

*Correspondence: Pof. Dr. M. Kalberer, E-mail: markus.kalberer@unibas.ch,
[a]Department of Environmental Sciences, University of Basel, Basel, Switzerland; [b]Adolphe Merkle Institute, University of Fribourg, Fribourg, Switzerland; [c]PSI Center for Energy and Environmental Sciences, Paul Scherrer Institute, Villigen-PSI, Switzerland; [d]Department of Occupational and Environmental Health, Center for Primary Care and Public Health (Unisanté), University of Lausanne, Epalinges, Switzerland.

mends a $PM_{2.5}$ limit value of 5 μg/m^3,[4] causing considerable challenges for clean air policies worldwide. Although legal limit values focus on easy to monitor parameters at air monitoring stations such as PM mass, it is unlikely that the overall PM mass is the most suitable metric to describe particle toxicity, considering the vast physical and chemical complexity of atmospheric particles. The lack of a mechanistic understanding of air pollution toxicity largely hinders more focused policy strategies to minimise PM-related burden of disease and mortality and to identify and minimise the most toxic particle sources.

Particle size is clearly an important toxicity-relevant factor because different particle sizes reach different parts of the respiratory tract. Upon inhalation, larger particles (1-10 μm) preferentially deposit in larger conducting airways (trachea, bronchi), whereas the smallest particles (*i.e.* ultrafine particles < 100 nm) penetrate to and deposit efficiently in peripheral lung regions (alveoli). Once deposited, particles interact with pulmonary surfactant and are displaced by wetting forces into the aqueous hypophase,[5] where interaction with pulmonary cells may occur. Besides mass and size, there are many other particle properties that could explain and contribute to their toxicity, *e.g.* surface charge, acidity, (in-)solubility or the presence of individual components such as metals or polycyclic aromatic hydrocarbons (Fig. 1).

Overall oxidising properties of particles emerged in recent years as a possible summarising metric that could explain many observed health effects, by causing oxidative stress in lung cells but also systemically. Oxidative stress may occur when cellular antioxidant defences are overwhelmed by reactive oxygen species (ROS, *i.e*. organic and inorganic peroxides and radicals) present in atmospheric PM or generated by PM components in the lung upon particle deposition.[6] The ROS concentration in PM and the capability of particles to produce ROS with simultaneous reduction of anti-oxidant concentrations in the lung is defined as oxidative potential (OP) of particles (Fig. 1). ROS can react with DNA, proteins, or lipids. The antioxidant defence system in the lung is mainly based on enzymatic components, such as superoxide dismutase, glutathione peroxidase and small antioxidants such as ascorbic acid to protect against ROS-induced cellular damage.[7] Inhaled particles such as combustion-derived diesel particles can provoke oxidative stress, causing inflammation in the epithelium and increased levels of oxidative stress biomarkers that can contribute to carcinogenesis.[8,9]

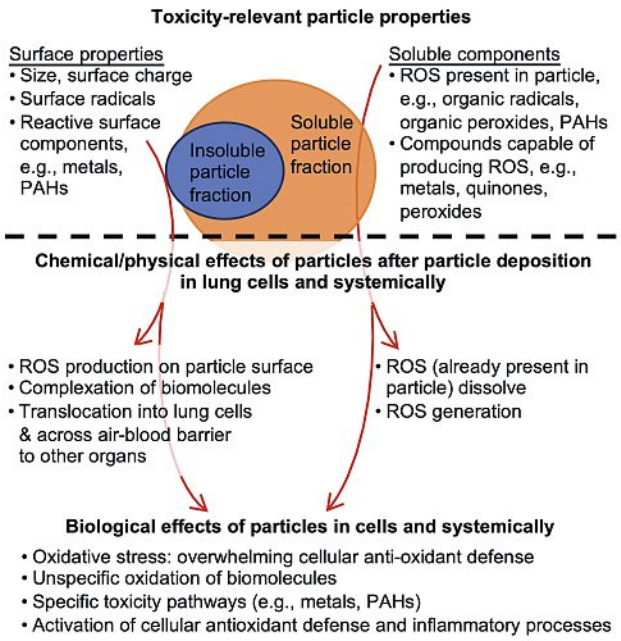

Fig. 1. Schematic of particle components and interactions relevant for biological effects after particle inhalation and deposition in the lung (adapted from ref. [10]).

In this review research activities at Swiss universities and institutes are summarised, which aim to establish if ROS and/or OP could be an integrating novel metric to describe atmospheric PM toxicity. Research projects tackle this highly interdisciplinary question from a chemical as well as from a cell biology/toxicity perspective.

2. Experimental Approaches

2.1 *Characterization of ROS and OP in PM*

Studies quantifying particle-induced OP or ROS use chemical redox reactions (often termed 'assays') between ROS and a reducing compound, such as dithiothreitol (DTT), ascorbic acid (AA), iron(II) (FOX) or dichlorofluorescein (DCFH).[11] For most studies quantifying OP or ROS in PM in field campaigns or laboratory studies, samples are collected on filters, followed by sample work-up and analysis in the laboratory using one or several OP and ROS assays. Filter-based so-called 'offline' methodologies offer the advantage of easy sample collection at many different locations but usually allow only for a poor time resolution of about a day. Many ROS and OP-active components such as radicals and peroxides are reactive and thus short-lived and therefore might decay before offline analysis, potentially resulting in an underestimation of particle OP and ROS.

To address these potential shortcomings, different so-called online OP and ROS quantification instruments were developed and tested by research groups in Switzerland where PM collection and analysis are combined into a single field-deployable instrument and where OP or ROS is quantified within seconds to an hour after collection.[12-16] A recent study by Campbell *et al*. demonstrated that 60% to 99% of OP and ROS decay within hours in a wide range of aerosol types such as secondary organic aerosol (SOA), diesel and gasoline car exhaust, biomass burning and in the ambient atmosphere, emphasising the advantages of online measurement concepts.[17]

The first step in online OP or ROS analysis instruments is the transfer of aerosol particles into a liquid phase, followed by OP or ROS quantification in the liquid phase. Online instruments with continuous or batch flow detection using DCFH or DTT allow time resolutions of 5 minutes[12,13] to an hour.[14] A third instrument recently developed measures the kinetics of Fe(II) oxidation by ROS in the presence of orange xylenol (FOX assay).[15] A fourth instrument was developed to quantify aerosol OP using AA, an anti-oxidant naturally occurring in the human lung.[16]

2.2 *Biological Effects of Lung-Deposited PM*

The oxidative stress effects of PM on lung cells *in vitro* can be studied by either collecting particles on filters and preparing extracts or suspensions for cell exposures or exposing cells directly to aerosol particles out of a continuous air flow.[18] The advantage of exposing lung cell cultures at the air-liquid interface directly to aerosol particles is to (i) realistically mimic particle deposition during inhalation and (ii) study the combined and separate toxicity effects of particles and gaseous fractions of an aerosol (for a review see refs. [19,20]). ROS is not only present in or generated by PM, but also in cells as a measure of oxidative stress. ROS detection kits based on, *e.g.* small-molecule fluorescent probes that can permeate into living cells, can determine ROS concentrations inside cells (Fig. 2a).[21]

As ROS are short-lived and challenging to analyse in cells, the oxidative stress response is often assessed by the effects of ROS on lipids, proteins, and DNA or by analysing the antioxidative response. The level of lipid peroxidation can be evaluated *e.g. via* malondialdehyde concentration and DNA oxidation by the oxi-

dised derivative 8- hydroxy-2'-deoxy-guanosine as biomarkers for oxidative stress.[22] In addition, the cellular antioxidative response can be assessed by glutathione (GSH), a major antioxidant in the lung, usually present in the reduced form. Changes in the ratio between reduced GSH and oxidised GSSG can be quantified as a measure of increased oxidative stress. The transcriptional response to oxidative stress can also be determined by real-time reverse-transcriptase polymerase chain reaction (rt-PCR) using cell lysates. An increased gene expression of enzymes such as *heme oxygenase 1* (*HMOX1*) and *superoxide-dismutase* (*SOD1*) indicates an activated antioxidant response.

3. Results

3.1 *Quantification of ROS and OP in PM*

As mentioned above, 60% to 99% of particle-bound ROS and OP from a wide range of sources have a lifetime of only minutes to hours, illustrating the advantage of online instruments for ROS and OP characterisation.[17] In recent years we synthesised and quantified atmospherically relevant compounds that could account for this dominant short-lived fraction of OP and ROS in PM. The lifetime of hydroperoxides, and peroxy acids,[23,24] or organic radicals[25] formed during the oxidation of organic compounds like terpenes (an important compound class forming PM) was characterised in matrices typical for atmospheric PM and was found to be in the range of minutes to hours, similar to ROS and OP in aerosols.[17]

In laboratory experiments, the online AA- and DCFH-instruments mentioned above[12,16] were used to quantify OP and ROS in SOA, which is one of the most important types of PM in the atmosphere. SOA from anthropogenic sources was found to contain much higher concentrations of ROS, than SOA from natural sources, indicating that anthropogenic SOA sources might have a higher toxicity[26]. Metals present in PM (often emitted by anthropogenic sources) were shown to alter OP and ROS in SOA.[27] Combustion emissions are another important particle source containing not only directly emitted particles but also contributing to SOA due to the large amount of gaseous organic and nitrogen oxides emissions. Using our novel online instruments, we could show that direct tail pipe PM exhaust has little OP activity whereas the exposure of the exhaust to UV light and oxidising gases such as ozone and OH radicals (mimicking atmospheric oxidation conditions) can increase ROS and OP levels by orders of magnitude.[28] A DCFH online instrument was used to characterise OP in ambient PM in Bern and Beijing[29] identifying SOA as important contributors to OP, in addition to residential heating and traffic exhaust. An online DTT instrument was also deployed in Beijing and found that both primary combustion related PM and SOA substantially contribute to OP.[14] An online FOX instrument deployed at an urban air quality station in Lausanne (Switzerland) indicated that O_3 strongly contributes to overall OP, notably more than $PM_{2.5}$, while NO_2 does not appear to play a role.

Such online measurements provide high time resolution OP and ROS concentration profiles showing a highly dynamic diurnal structure of OP activity with maxima in the morning and afternoon, which are distinctly different from total PM diurnal pattern and thus suggest that not all PM sources are equally contributing to OP and ROS.[14,30]

As mentioned above, offline ROS and OP analysis allows the characterisation of OP across a wide range of locations due to the abundant availability of PM filter samples worldwide. Daellenbach *et al*. performed offline analyses using DTT, DCFH, and AA in selected Swiss and European locations and found that OP levels vary strongly between seasons and environments (rural, urban, urban-traffic).[31] This study used air quality modelling to identify traffic exhaust and biomass burning as significant contributors to OP. A follow-up study[32] assessed offline OP levels in North India, a region with severe pollution and a large population, and found that OP levels in North India, like in China, are strongly enhanced compared to those in Europe. In North India, OP is predominantly driven by organic aerosols from the incomplete combustion of biomass and fossil fuels, including traffic emissions.

The interpretation of ROS and OP quantification (from online and offline studies) in the ambient atmosphere requires advanced statistical methods to identify the importance of different PM sources, *e.g.* using receptor models relying on variations in the particle chemical composition and multilinear regression methods to identify PM sources relevant for OP. Overall, these field studies showcase the important role of anthropogenic activities on OP levels and highlight that particle composition might be critical to describe particle toxicity.

All of the above OP and ROS assays were used in a number of field and lab studies and it has become evident that the different OP and ROS reaction systems are sensitive to different fractions of OP- and ROS-active components in PM.[33-35] In addition, specific reaction conditions and experimental procedures make comparability between studies difficult. This illustrates that more lab studies are needed to characterise the different OP reaction system in more detail.

Besides ambient air pollution, exposure to particles indoors has often been neglected in recent years. At workplaces, specifically, PM concentrations are often significantly higher than in ambient air. Applying this OP approach to occupational hygiene and health gives the opportunity to gain new insights on worker's exposure. Sauvain *et al*.[36] showed in a pilot study that although tunnel workers were exposed to higher PM mass than bus workers, their exposure based on OP was similar. Workers in the metal industry using different cutting fluids (MWF) are often exposed to oil mist aerosols. Using the FOX OP assay, the potentially highest hazard for workers exposed to water based MWF was shown to correspond to the particulate fraction, while for workers using straight oils, it was the gaseous fraction. suggesting that different exposure reduction strategies are needed to protect worker's health, depending on the MWF type.[37]

3.2 *Oxidative Stress in Cells*

Advanced highly standardised 3D human lung tissue models have been developed, which can be used in combination with particle sources such as vehicle exhaust systems for exposure of lung cells. Emissions from an engine can be taken directly at the exhaust outlet and brought into contact with lung cell surfaces with exhaust characterisation being performed on-line. Such a system was established for scooter exhaust,[38] diesel,[39] and gasoline cars operated with gasoline[40] and ethanol-gasoline blends.[41] This system can also test the effects of different exhaust filters or fuels.[42]

In the study of Steiner *et al*.[42] a combination of assays was applied to investigate the oxidative stress response of diesel exhaust treated with a non-catalysed diesel particle filter. The whole and particle-filtered exhaust (*i.e.* only the gaseous exhaust fraction) was exposed to a lung cell co-culture model composed of human bronchial cells, macrophages, and dendritic cells at the air-liquid interface. It could be shown that filtered diesel exhaust was highly oxidative, more than unfiltered exhaust as quantified by total reduced glutathione (Fig. 2b), while both exhaust types triggered comparable responses to oxidative stress as measured by *HMOX1* (Fig. 2c) and *SOD1* gene expression. These results allow the differentiation between the toxicity of the particulate and gaseous fractions of diesel exhaust and show that a non-catalysed particle filter is not sufficient to eliminate the induction of oxidative stress in the filtered exhaust. Other studies looked at cell responses of ambient PM from urban and rural locations and

identified stronger inflammation responses for PM samples collected in winter compared to summer.[43]

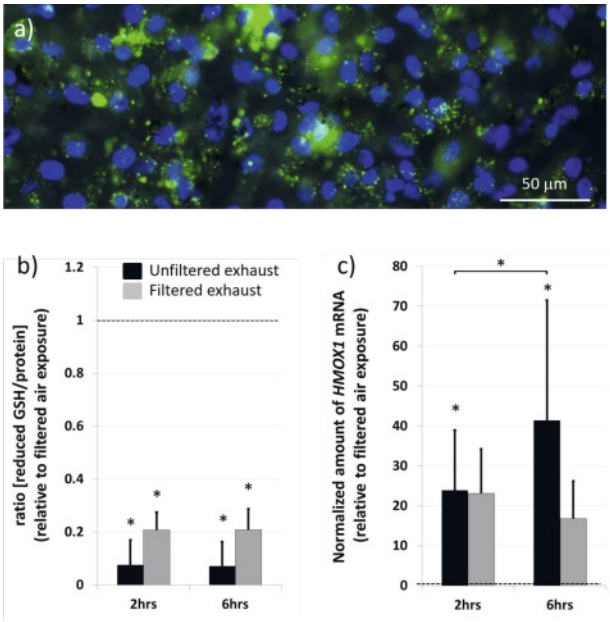

Fig. 2. Assessing oxidative stress reactions in lung cells *in vitro*. a) ROS production in A549 lung epithelial cells exposed to 125 µg/mL diesel particles for 24 h. Blue staining shows the cell nuclei and green staining shows the ROS (courtesy Loretta Müller). b) Levels of total reduced GSH, and c) HMOX-1 gene expression in human epithelial tissue (*i.e.* co-cultures of bronchial cells, macrophages and dendritic cells) exposed at the air-liquid interface to non-catalyzed particle-filtered diesel exhaust (grey bars) and compared to unfiltered exhaust (black bars). All values are reported relative to the unfiltered air exposure (dotted line). Error bars indicate standard deviations. N = 10 (unfiltered exhaust) N = 6 (filtered exhaust, except for gene expression data, where N = 3). Asterisks indicate statistical significance ($p < 0.05$). (Reprinted with permission from ref. [39]). Copyright **2013**, Elsevier Science & Technology Journals).

4. Conclusions and Outlook

4.1 *ROS and OP Characterisation*

Emerging evidence suggests that OP and ROS of PM might be directly linked to the drivers and severe health effects of air pollution. Characterising and quantifying the effects of oxidising components in PM with a single parameter, as attempted by the assays described above, is desirable because ultimately such a particle property would need to be monitored at large numbers of air monitoring stations with automated instruments. As a new metric, OP and ROS measurements are not yet standardised to the same extent as other metrics, such as organic carbon/elemental carbon analyses or total PM mass. First prototypes of OP and ROS instruments have been developed over the last years with Swiss research groups leading this worldwide effort. A key aspect in the development of new instruments will be a thorough characterisation of their performance, especially compared to traditional offline filter analyses, which is still used in most studies today.

First attempts of such online/offline comparisons have been made[17] but more standardisation studies will be necessary to advance this research field and assure intercomparability of OP assays and studies. Such standardisation efforts are also needed for mechanistic biological effect studies of particle OP and epidemiological studies to elucidate the link of OP and acute and chronic health effects.

Initial attempts at modelling OP are ongoing but are limited to specific regional areas. In addition, the identification of driving contributors to OP relies on detailed laboratory studies and multilinear regression statistical analyses of ambient data sets, for which more studies are urgently needed.

4.2 *Biological Effects of ROS and OP*

Analysing and comparing oxidative stress reactions in lung cells upon PM exposure is challenging. Several technical solutions exist to expose the cells to PM from various sources and online as well as offline particle deposition methods are used in the literature, which makes it difficult to compare results. Online deposition of PM onto cells cultured at the air-liquid interface (mimicking the conditions in the lung) will be essential to assess ROS and OP biological effects due to the short-lived nature of many ROS components. In addition, different lung cell models are currently used, ranging from lung cell monocultures to various co-cultures. It is also largely unexplored how biological effects translate to organs beyond the lung and to systemic effects. Studies often differ in the biological parameters and assays used, making it difficult to compare results.[19] Another challenge is the relatively low exposure to lung cells using air-liquid exposure systems. Cells can only be kept in such exposure systems for several hours, and therefore the experimental window for PM exposure is limited, which poses a challenge to assess long term exposure effects in a lab experiment.

To identify the most toxic PM sources in the polluted atmosphere (outdoors and indoors) and to elucidate the biological mechanisms leading to cellular damage and ultimately to disease and to elucidate the specific role of OP and ROS in these processes, a continued close collaboration between atmospheric sciences and cell biology, toxicology and epidemiology will be essential.

Acknowledgements

MK acknowledges the SNSF Grant No. 200021_192192/1 and ERC grant no. 279405. BRR acknowledges the H2020 EU project ULtrafine particles from TRansportation- Health Assessment of Source (UTRHAS, Grant # 955390) and the Adolphe Merkle Foundation. K.R.D. acknowledges the SNSF Ambizione grant PZPGP2_201992. JJS acknowledge support from the Federal Office for the Environment (FOEN), Grant UTF 684.02.22/BAFU-087.2-64739/1/1.

Received: August 25, 2024

[1] GBD 2019 Stroke Collaborators, *Lancet Neurol.* **2021**, *20*, 795, https://doi.org/10.1016/ S1474-4422(21)00252-0.
[2] WHO, World Health Organisation, Ambient air pollution: A global assessment of exposure and burden of disease, **2016**, ISBN: 9789241511353.
[3] M. Kalberer, D. Paulsen, M. Sax, M. Steinbacher, J. Dommen, R. Fisseha, A. S. H Prevot, V. Frankevich, R. Zenobi, U. Baltensperger, *Science* **2004**, *303*, 1659, https://doi.org/10.1126/science.1092185.
[4] WHO, Global air quality guidelines. Particulate matter (PM2.5 and PM10), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide. Geneva: World Health Organization; **2021**. ISBN: 9789240034228.
[5] P. Gehr, S. Schürch, Y. Berthiaume, V. Im Hof, M. Geiser, *J. Aerosol Med.* **1990**, *3*, 27, https://doi.org/10.1089/jam.1990.3.
[6] F. J. Kelly, *Occup. Environ. Med.* **2003**, *60*, 612, https://doi.org/10.1136/oem.60.8.612.
[7] G. Pizzino, N. Irrera, M. Cucinotta, G. Pallio, F. Mannino, V. Arcoraci, F. Squadrito, D. Altavilla, A. Bitto, *Oxid. Med. Cell Longev.* **2017**, 2017:8416763, https://doi.org/10.1155/2017/8416763.
[8] K. Donaldson, L. Tran, L. A. Jimenez, R. Duffin, D. E. Newby, N. Mills, W. MacNee, V. Stone, *Part. Fibre Toxicol.* **2005**, *2*, 10, https://doi.org/10.1186/1743-8977-2-10.
[9] S. Steiner, C. Bisig, A. Petri-Fink, B. Rothen-Rutishauser, *Arch. Toxicol.* **2016**, *90*, 1541, https://doi.org/10.1007/s00204-016-1736-5.
[10] J. Øvrevik, M. Refsnes, M. Lag, B. C. Brinchmann, P. E. Schwarze, J. A. Holme, *Basic Clin. Pharmacol. Toxicol.* **2017**, *121*, 55, https://doi.org/10.1111/bcpt.12746.
[11] J. T. Bates, T. Fang, V. Verma, L. Zeng, R. J. Weber, P. E. Tolbert, J. Y. Abrams, S. E. Sarnat, M. Klein, J. A. Mulholland, A. G. Russell, *Environ. Sci. Technol.* **2019**, *53*, 4003, https://doi.org/10.1021/acs.est.8b03430.

[12] F. P. H., Wragg, S. J. Fuller, R. Freshwater, D. C. Green, F. J. Kelly, M. Kalberer, *Atmos. Meas. Tech*. **2016**, *9*, 4891, https://doi.org/10.5194/amt-9-4891-2016.
[13] J. Zhou, E. A. Bruns, P. Zotter, G. Stefenelli, A. S. H. Prévôt, U. Baltensperger, I. El-Haddad, J. Dommen, *Atmos. Meas. Tech.* **2018**, *11*, 65, https://doi.org/10.5194/amt-11-65-2018.
[14] R. K. Y. Cheung, L. Qi, M. I. Manousakas, J. V. Puthussery, Y. Zheng, T. K. Koenig, T. Cui, T. Wang, Y. Ge, G. Wei, Y. Kuang, M. Sheng, Z. Cheng, A. Li, Z. Li, W. Ran, W. Xu, R. Zhang, Y. Han, Q. Wang, Z. Wang, Y. Sun, J. Cao, J. G. Slowik, K. R. Dällenbach, V. Verma, M. Gysel-Beer, X. Qiu, Q. Chen, J. Shang, I. El-Haddad, A. S. H. Prévôt, R. L. Modini, *Science of The Total Environment* **2024**, *928*, 172345, https://doi.org/10.1016/j.scitotenv.2024.172345.
[15] S. Goekce, N. Concha-Lauzano, J. J. Sauvain, M. Hemmendinger, A. Portela, E. Sergent, P. Andujar, J. C. Pairon, P. Wild, G. Suarez, *Medicine in Novel Technology and Devices* **2022**, *14*, 100120, https://doi.org/10.1016/j.medntd.2022.100120.
[16] B. Utinger, S. J. Campbell, N. Bukowiecki, A. Barth, B. Gfeller, R. Freshwater, H. R. Rüegg, M. Kalberer, *Atmos. Meas. Tech.* **2023**, *16*, 2641, https://doi.org/10.5194/amt-16-2641-2023.
[17] S. J. Campbell, B. Utinger, A. Barth, Z. Leni, Z.H. Zhang, J. Resch, K. Li, S.S. Steimer, C. Banach, B. Gfeller, F. P. H. Wragg, J. Westwood, K. Wolfer, N. Bukowiecki, M. Ihalainen, P. Yli-Piril, M. Somero, M. Kortelainen, J. Louhisalmi, M. Sklorz, H. Czech, S. de Bucchianico, T. Streibel, M. Deleval, C. Ruger, N. Baumlin, M. Salathe, Z. Fang, M. Pardo, S. D'Aronco, C. Giorio, Z. Shi, R. M. Harrison, D. C. Green, F. J. Kelly, Y. Rudich, S. E. Paulson, O. Sippula, R. Zimmermann, M. Geiser, M. Kalberer, revised manuscript submitted, **2024**.
[18] P. E. Schwarze, A. I. Totlandsdal, M. Låg, M. Refsnes, J. A. Holme, J. Øvrevik, *BioMed Research International*, **2013**, *2013*, 685142, https://doi.org/10.1155/2013/685142.
[19] P. Rossner, T. Cervena, M. Vojtisek-Lom, *Toxicology*, **2021**, *462*, 152953, https://doi.org/10.1016/j.tox.2021.152953.
[20] C. Bisig, S. Steiner, J. Czerwinski, P. Comte, A. Mayer, A. Petri-Fink, B. Rothen-Rutishauser, *CHIMIA*, **2015**, *69*, 68, https://doi.org/10.2533/chimia.2015.68.
[21] L. Müller, M. Riediker, P. Wick, M. Mohr, P. Gehr, B. Rothen-Rutishauser, *J. R. Soc. Interface*. **2010**, *7*, S27, https://doi.org/10.1098/rsif.2009.0161.focus.
[22] X. Cao, S. Padoan, S. Binder, S. Bauer, J. Orasche, C.M. Rus, A. Mudan, A. Huber, E. Kuhn, S. Oeder, J. Lintelmann, T. Adam, S. Di Bucchianico, R. Zimmermann, *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **2022**, *874*, 503446, https://doi.org/10.1016/j.mrgentox.2022.503446.
[23] S. S. Steimer, A. Delvaux, S. J. Campbell, P. J. Gallimore, P. Grice, D. J. Howe, D. Pitton, M. Claeys, T. Hoffmann, M. Kalberer, *Atmos. Chem. Phys*. **2018**, *18*, 10973, https://doi.org/10.5194/acp-18-10973-2018.
[24] K. Li, J. Resch, M. Kalberer, *Environ. Sci. Technol*. **2024**, *58*, 3322, https://doi.org/10.1021/acs.est.3c07048.
[25] S. J. Campbell, K. Wolfer, P. J. Gallimore, C. Giorio, D. Häussinger, M. A. Boillat, M. Kalberer, *Environ. Sci. Technol*. **2022**, *56*, 12945, https://doi.org/10.1021/acs.est.2c04101.
[26] Z.-H. Zhang, E. Hartner, B. Utinger, B. J. Gfeller, A. Paul, M. Sklorz, H. Czech, B. Yang, X. Su, G. Jakobi, J. Orasche, Jürgen Schnelle-Kreis, S. Jeong, T. Gröger, M: Pardo, T. Hohaus, T. Adam, A. Kiendler-Scharr, Y. Rudich, R. Zimmermann, M. Kalberer, *Atmos. Chem. Phys*. **2022**, *22*, 1793, https://doi.org/10.5194/acp-22-1793-2022.
[27] S. J. Campbell, B. Utinger, A. Barth, S. E. Paulson, M. Kalberer, *Environ. Sci. Technol*. **2023**, *57*, 13546, https://doi.org/10.1021/acs.est.3c01975.
[28] B. Utinger, A. Barth, M. Kalberer, **2024**, submitted.
[29] J. Zhou, M. Elser, R. J. Huang, M. Krapf, R. Fröhlich, D. Bhattu, G. Stefenelli, P. Zotter, E.A. Bruns, S. M. Pieber, H. Ni, Q. Wang, Y. Wang, Y. Zhou, C. Chen, M. Xiao, J. G. Slowik, S. Brown, L. E. Cassagnes, K. R. Daellenbach, T. Nussbaumer, M. Geiser, A. S. H. Prévôt, I. El-Haddad, J. Cao, U. Baltensperger, J. Dommen, *Atmos. Chem. Phys*. **2019**, *23*, 14703, https://doi.org/10.5194/acp-19-14703-2019.
[30] S. J. Campbell, A. Barth, G. I. Chen, A. H. Tremper, M. Priestman, D. Ek, S. Gu, F. J. Kelly, M. Kalberer, D. C. Green, **2024**, submitted.
[31] K. R. Daellenbach, G. Uzu, J. Jiang, L. E. Cassagne, Z. Leni, A. Vlachou, G. Stefenelli, F. Canonaco, S. Weber, A. Segers, J. J. P. Kuenen, M. Schaap, O. Favez, A. Albinet, S. Aksoyoglu, J. Dommen, U. Baltensperger, M. Geiser5, I. El Haddad, J. L. Jaffrezo, A. S. H. Prévôt, *Nature* **2020**, *587*, 414, https://doi.org/10.1038/s41586-020-2902-8.
[32] D. Bhattu, S. N. Tripathi, H. S. Bhowmik, V. Moschos, C. P. Lee, M. Rauber, G. Salazar, G. Abbaszade, T. Cui, J. G. Slowik, P. Vats, S. Mishra, V. Lalchandani, R. Satish, P. Rai, R. Casotto, A. Tobler, V. Kumar, Y. Hao, L. Qi, P. Khare , M. I. Manousakas, Q. Wang, Y. Han , J. Tian, S. Darfeuil, M. C. Minguillon, C. Hueglin , S. Conil, N. Rastogi, A. K. Srivastava, D. Ganguly, S. Bjelic, F. Canonaco, J. Schnelle-Kreis, P. A. Dominutti, J. L. Jaffrezo, S. Szidat, Y. Chen, J. Cao, U. Baltensperger , G. Uzu, K.R. Daellenbach, I. El Haddad, A. S. H. Prévôt, *Nat. Commun*. **2024**, *15*, 3517, https://doi.org/10.1038/s41467-024-47785-5.
[33] J. J. Sauvain, M. J. Rossi, M. Riediker, *Aerosol Sci. Technol*. **2013**, *47*, 218, https://doi.org/10.1080/02786826.2012.742951.
[34] P. A. Dominutti, L. J. S. Borlaza, J.J . Sauvain, V. D. Ngoc Thuy, S. Houdier, G. Suarez, J. L. Jaffrezo, S. Tobin, C. Trébuchon, S. Socquet, E. Moussu, G. Mary, G. Uzu, *Environ. Sci.: Atmos*. **2023**, *3*, 1497, https://doi.org/10.1039/D3EA00007A.
[35] S. J. Campbell, K. Wolfer, B. Utinger, J. Westwood, Z.-H. Zhang, N. Bukowiecki, S. S. Steimer, T. V. Vu, J. Xu, N. Straw, S. Thomson, A. Elzein, Y. Sun, D. Liu, L. Li, P. Fu, A. C. Lewis, R. M. Harrison, W. J. Bloss, M. Loh, M. R. Miller, Z. Shi, M. Kalberer, *Atmos. Chem. Phys*. **2021**, *21*, 5549, https://doi.org/10.5194/acp-21-5549-2021.
[36] J. J. Sauvain, S. Deslarzes, F. Storti, M. Riediker, *Ann. Occup. Hyg*. **2015**, *59*, 882, https://doi.org/10.1093/annhyg/mev024.
[37] J. J. Sauvain, G. Suarez, N. B. Hopf, K. Batsungnoen, N. Charriere, F. Andre, R. Levilly, P. Wild, *Int. J. Hyg. Environ. Health* **2021**, *235*, 113775, https://doi.org/10.1016/j.ijheh.2021.113775.
[38] L. Müller, P. Comte, J. Czerwinksi, M. Kasper, A.C.R. Mayer, P. Gehr, H. Burtscher, J.P. Morin, A. Konstandopoulos, B. Rothen-Rutishauser, *Environ. Sci. Technol*. **2010**, *44*, 2632, https://doi.org/10.1021/es903146g.
[39] S. Steiner, J. Czerwinski, P. Comte, L. Müller, N. Heeb, A. Mayer, A. Fink, B. Rothen-Rutishauser, *Atmos. Environ*. **2013**, *81*, 117, https://doi.org/10.1016/j.atmosenv.2013.08.029.
[40] C. Bisig, S. Steiner, P. Comte, J. Czerwinski, A. Mayer, A. Petri-Fink, B. Rothen-Rutishauser, *Emiss. Control Sci. Technol*. **2015**, *1*, 237, https://doi.org/10.1007/s40825-015-0019-6.
[41] C. Bisig, M. Roth, L. Müller, P. Comte, N. Heeb, A. Mayer, J: Czerwinski, A. Petri-Fink, B. Rothen-Rutishauser, *Environ. Res.* **2016**, *151*, 789, https://doi.org/10.1016/j.envres.2016.09.010.
[42] S. Steiner, J. Czerwinski, P. Comte, O. Popovicheva, E. Kireeva, L. Müller, N. Heeb, A. Mayer, A. Fink, B. Rothen-Rutishauser, *Atmos. Environ*. **2013**, *81*, 380, https://doi.org/10.1016/j.atmosenv.2013.08.059.
[43] Z. Leni, L.E. Cassagnes, K. R. Daellenbach, I. El Haddad, A. Vlachou, G. Uzu G, A. S. H. Prévôt, J.-L. Jaffrezo, N. Baumlin, M. Salathe, U. Baltensperger, J. Dommen, M. Geiser, *PLoS ONE* **2020**, *15*, 11, https://doi.org/10.1371/journal.pone.0233425.