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Current Opinion in Toxicology

Air pollution causing oxidative stress

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Abstract

Air pollution remains a major factor for adverse health effects and premature death worldwide. Particulate matter with aerodynamic diameter ≤2.5 μm ($PM_{2.5}$), mainly originating from combustion processes, is considered most toxic. The respiratory and cardiovascular system are particularly affected. Despite all research efforts, the causative relations of air pollutants and exposure-associated health effects are not yet fully established. Recent studies using different methodologies have consistently shown peroxides and reactive oxygen species (ROS) to be crucial mediators of particle toxicity. This review is an excerpt of results from experimental studies and methodological developments of the past 2 years that enhanced our understanding of oxidative molecules in particles, their transmission to the target organ, and the molecular pathways generating ROS in physiological and pathological processes. Further multidisciplinary research towards predicting toxicology from particle-related ROS transmitted to the target organ is required.

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Current Opinion in Toxicology 2020, **20-21**:1–8

This review comes from a themed issue on **Oxidative Toxicology**

Edited by **Yvonne Will** and **Moorthy Bhagavatula**

This review comes from a themed issue on **Oxidative Toxicology**

Available online 26 February 2020

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.cotox.2020.02.006

Keywords

Toxicity, Reactive oxygen species, Particulate matter, Oxidative stress, Oxidative potential, Antioxidant defense.

Air pollution adversely affects human health and is considered the world's largest environmental health threat [1–3]. Accelerated urban development and rapid population growth contribute significantly to poor air quality. Because of this, developing countries are generally most affected [4]. However, the association between air pollution and mortality is still evident in countries, where pollution levels are well below target standards [5]. Traditional classification of air pollution based primarily on particle size and mass concentrations will require complementation concerning the chemical composition and nanostructures of air pollutants [6,7]. Animal inhalation studies have demonstrated adverse effects related to reactive oxygen species (ROS) activity to vary depending on the composition and emission sources of the particles [8]. These have changed over the last few decades, with anthropogenic, combustion-derived air pollutants being the main concern for public health today [9]. Thus, clarification of the causative relation of air pollutants and adverse effects, as well as deciphering the associated molecular mechanisms has become a pressing research topic [10–12].

Air pollution is a heterogeneous, complex mixture of gaseous and particulate components, differing based on the emission source and varying with time and atmospheric conditions. The critical constituents with regard to health are particulate matter with aerodynamic diameter ≤10 μm (PM_{10}) and the gaseous pollutants nitrogen dioxide, sulfur dioxide, ozone, volatile organic compounds and carbon monoxide. Epidemiological studies generally show the fine particle fraction, $PM_{2.5}$, to have a greater impact on health than the coarse PM_{10} fraction [5]. $PM_{2.5}$ mainly derives from combustion processes and consists of carbonaceous particles with associated adsorbed organic molecules like nitrates, sulfates, and polycyclic aromatic hydrocarbons, as well as reactive metals such as iron, copper, nickel, zinc, and vanadium [13]. Physicochemical properties of the particles (i.e. size, structure, chemical composition, reactivity, and solubility) determine their impact on health and the mechanisms by which PM induces adverse effects [8]. Furthermore, primary particles (i.e. those emitted directly from a source) and secondary particles formed by gas-to-particle conversion in the atmosphere may exhibit different toxicity.

Epidemiological and experimental studies have consistently shown the association between $PM_{2.5}$ exposure and a wide range of health effects, mainly on the cardiovascular and respiratory system. Increased mortality from ischemic heart disease, heart failure, thrombotic stroke, and lung diseases, like respiratory infections, asthma, chronic obstructive pulmonary disease (COPD), lung cancer, as well as impaired lung development in children, have been reported [14–16]. $PM_{2.5}$ has also been shown to exert endocrine activity promoting the development of metabolic diseases such as obesity and diabetes, which themselves are well established cardiovascular risk factors [17–20].

In environmental health research, ROS are generally considered important. Experimental studies have identified ROS as crucial mediators of particle toxicity, with a particular association to respiratory and cardiovascular disease [21–25]. PM itself contains ROS as well as redox-active components that can lead to ROS generation upon interaction with specimens of biological origin. The capacity of inhaled PM to elicit cellular damage via oxidative reactions is termed oxidative potential (OP) and can be measured using cell-free assays (Table 1A, Figure 1). These have the advantage of being fast, inexpensive, easy to perform and suitable for automation [26]. The most common tests mimic the depletion of endogenous antioxidants like ascorbic acid (AA, OP^{AA}) and glutathione (GSH, OP^{GSH}), or of a surrogate for cellular reductants, such as dithiothreitol (DTT, OP^{DTT}). The values obtained are proportional to the concentration of redox-active species in the analyzed PM sample. Among the above mentioned tests, OP^{DTT} is currently the preferred method to evaluate the OP of $PM_{2.5}$, because of its sensitivity to tracers of combustion derived transition metals and aromatic organic compounds, which largely accumulate in the fine PM fraction [15,27–30]. The OP^{AA} and OP^{GSH} assays mainly capture metals from nontraffic exhaust emissions collected in the coarse PM_{10} fraction. In addition, the horseradish peroxidase/2′7′-dichlorofluorescin (HRP/DCFH) assay, primarily used to evaluate oxidative stress in cells, was adapted to quantify oxidative components of PM [31–34]. The most widely used cell-free assay measures peroxides as indicator of free-radical formation, which is central for oxidative stress. Thereby, the total aerosol peroxide in its three forms (H_2O_2, ROOH and ROOR) is quantified using iodometry-assisted liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS) [30,35]. In addition, there have been substantial efforts to quantify the radical content of PM by electron spin resonance (ESR) spectroscopy [36], and to assess the radical generation upon interaction of particles and aqueous media, like the respiratory tract lining fluid (RTLF) [23,37,38]. The latter is a more complex cell-free assay [38,39]. The liquid lung-lining layer is the first structure a pollutant interacts with upon deposition in the respiratory tract. It consists of the lipid- and protein-containing surfactant film at the air-liquid interface and the underlying aqueous phase containing lipids, proteins and a variety of antioxidants. The lung-lining layer constitutes the first detoxifying environment deposited particles encounter. Thus, its interaction with deposited particles is decisive for the development of adverse effects from particle-associated ROS. In RTLF assays, mixtures of varying composition of antioxidant molecules mimicking the first phase of particle–lung interaction are used to unravel their implication on the oxidative response. This approach allows to study the role of the liquid lung-lining layer as a protective barrier *in vitro* and to discriminate highly reactive compounds (e.g. copper and quinones) responsible for changes in the oxidative response [38,39]. Measurements of the OP of particles also serve as a more refined exposure metric of PM toxicity than mass or number concentrations alone [40,41]. Elucidating the mechanisms underlying particle toxicity remains difficult. Previously, the OP solely described the particles' ROS content. The main challenge is to discriminate cellular damage by the particles' intrinsic OP and that from particle-induced ROS formation [15,42–46]. Further, the term ROS lacks precision, as it covers a variety of reactive species with considerable variability in terms of reactivity, longevity and biological effects.

ROS production is inherent in all aerobic species, primarily as a byproduct of mitochondrial electron transport. At physiological levels, ROS are essential for the regulation of critical signaling pathways involved in cell growth, proliferation, differentiation, and survival [47]. They further contribute to the regulation of blood pressure, cognitive function, immunity and antioxidant defense [48,49]. Excess ROS, mainly resulting from imbalanced antioxidant defense and detoxification, can lead to harmful (i.e. pathological) oxidative stress. The latter has been associated with the development of atherosclerosis, stroke, hypertension, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, COPD, asthma, diabetes, cancer, and even neurodegenerative disorders such as Parkinson and Alzheimer disease [15,17,20,21]. Meanwhile, the simplistic paradigm that low levels of ROS mediate physiological processes and high levels induce toxicity has been gradually replaced by a more refined understanding of redox mechanisms (Figure 2). Redox-active molecules including ROS have been shown to act as both site-specific mediators of cell signaling and central regulators of inflammatory responses [31]. Thus, oxidative stress affecting cellular signaling is likely to cause mitochondrial dysfunction and activation of transcription factors. Of particular importance are the nuclear factor erythroid-derived 2-related factor 2 (Nrf2), regulating antioxidant defense, and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a master regulator of immune response. Activation of these transcription factors may result in the expression of several hundred genes involved in inflammation, endothelial dysfunction, and cell death [50,51].

The large surface of our respiratory tract is the first structure the inhaled pollutants interact with and cause oxidative damage to. Pulmonary oxidative stress by air pollution depends on the composition of inhaled pollutants, PM deposition efficiency, regional distribution in the respiratory tract, clearance mechanisms at the site of deposition, possible dislocation into or beyond pulmonary cells (systemic and secondary organ distribution), as well as on the susceptibility of the host. All those aspects have to be considered to select appropriate assays and models, when studying health effects

Table 1 Examples of **(A)** studies with cell-free assays elucidating the association of the oxidative potential (OP) with the chemical composition of ambient particulate matter (PM) and **(B, C)** in-vitro and in-vivo studies measuring oxidative stress from exposure to air pollution.

Particles	Targets	Assay	Main findings	Ref.
A) Cell-free assays				
Urban $PM_{2.5}$ (Urbana, Illinois, USA) and quinones	2-OHTA generated by OH	DTT	Additive effects of individual quinone species. Antagonistic and synergistic interactions depending on combination of quinones.	[27]
Urban $PM_{2.5}$ (Atlanta, USA)	OH, H_2O_2	DTT	High efficiency of the ambient PM to convert H_2O_2 to OH.	[28]
Secondary organic aerosols (SOA) generated from naphthalene or phenanthrene	OP of SOA	DTT	OP of SOA depends on primary precursor identity. Oxygenated derivatives show high OP. No significant contributions from peroxides.	[30]
Dust from different emission sources, varying in chemical composition	OP	AA, DTT, DCFH	Different sensitivities of assays to PM components. High sensitivity of: (1) DTT assay to road dust, influenced by water-soluble organic carbon (WSOC); (2) AA assay to metal enriched dust (brake wear); and (3) DCFH assay to crustal components like titanium or aluminum.	[31]
Urban and rural PM_{10} & $PM_{2.5}$ filter samples (Milan, I; San Vittore, CH)	ROS	Online & offline DCFH	Linear relationship between DCFH and increasing PM concentrations.	[32]
SOA generated from α-pinene in smog chamber	OOH content of SOA during ozonolysis	AMS, iodometry	Model simulations provide evidence that photolysis products are highly oxygenated and not more volatile than their precursors, independently of chamber conditions.	[34]
Urban and rural $PM_{2.5}$ filter samples (Po Valley, I)	OP^{AA} in six artificial RTLF	AA	Assay depends on composition of synthetic RTLF used. RTLF containing GSH strongly promotes AA oxidation. Among all surrogates, AA solution generates highest OP^{AA}.	[39]
Water-soluble organic carbon (WSOC) of α-pinene	Organic peroxide discrimination in complex chemical matrix	Iodometry-assisted LC-ESI-MS	Multifunctional organic peroxides decompose to smaller peroxides detectable with iodometry-assisted LC-ESI-MS. Labile organic peroxides can be lost during sample collection and/or after extraction.	[35]
PM_{10} filter samples (Chamonix, F)	OP	DTT, AA, GSH, ESR	Higher OPv values during winter in DTT, GSH and AA assays. Strong correlation between OP^{DTTv} and OP^{AAv}. The ESRv did not show seasonal variation. Combination of different assays essential to capture the wide range of OP determinants.	[25]
B) In-vitro studies (cell cultures)				
Non-volatile (nv) PM from aircraft engine at climb-out and ground-idle conditions, conventional Jet A-1 or alternative (HEFA) fuel.	Cytotoxicity, oxidative stress and pro-inflammatory response in human bronchial epithelial cell line BEAS-2B	Colorimetric LDH test, qPCR (HMOX-1), BioPlex assay (IL-6, IL-8, MCP-1)	Toxicity dependent on engine operating conditions and fuel type. Limited correlation between deposited particle dose (number and mass per surface area) and cell damage. Influence of particle size and nanostructures on cell damage.	[6]
$PM_{2.5}$ samples (Beijing, PRC)	Cellular mechanisms of cardiovascular toxicity in human endothelial cell line EA.hy926 and human monocytic leukemia cell line THP-1	Cell viability (MTS), Western Blotting.	Induction of ROS generation and decrease of cell viability. Phosphorylation of JNK, ERK, p38 MAPK, AKT, and activation of NF-κB. Significant time- and dose-dependent increase in expressions of ICAM-1 and VCAM-1.	[50]
Diesel exhaust particles (NIST® Standard Reference Material SRM®2975)	Intra- and extracellular ROS content in human umbilical vein endothelial cells (HUVEC)	DCFH	No extracellular ROS induction in cell-free assay. Intracellular, dose-dependent ROS generation. Substantial changes in cellular antioxidant/oxidant parameters.	[51]

(*continued on next page*)

Table 1 (*continued*)

Particles	Targets	Assay	Main findings	Ref.
C) In-vivo studies (human)				
Metal-rich $PM_{2.5}$ (welding in boilermaker union), personal background $PM_{2.5}$ measurements	mtDNA methylation in blood and heart rate variability in normal male subjects	Bisulfite pyrosequencing	Negative association of mtDNA methylation with $PM_{2.5}$ exposure. mtDNA methylation modified the negative relationship between $PM_{2.5}$ exposure and heart rate variability.	[52]
$PM_{2.5}$ filter samples (Taipei, PRC)	8-oxodG, N7-MeG, 1-OHP and creatinine in urine, GPX-1 and SOD in blood from normal subjects	Questionnaire, LC-MS/MS	No association of PM exposure with urinary 1-OHP. Significant relation between markers for DNA damage (8-oxodG, 1-OHP) and lipid peroxidation (N7-MeG). No association of SOD and GPX-1 in blood with 8-oxodG or N7-MeG in urine.	[54]
PM_{10} and $PM_{2.5}$ filter samples (London, UK)	OP in artificial RTLF	Daily number of nonaccidental, cardiovascular or respiratory deaths from registry, AA and GSH	Positive associations of OP^{AA} and OP^{GSH} with all-cause and cardiovascular mortality in adults (15–64 years) and of OP PM_{10} with respiratory mortality in children (0–14 years). Negative associations of OP^{AA} and OP^{GSH} with cardiovascular and respiratory mortality in adults >65 years.	[43]
Ambient PM_{10}, $PM_{2.5}$, $PM_{10\text{-}2.5}$ and NO_2 (NL)	OP^{DTT}, OP^{ESR}, diabetes prevalence	Cross sectional analysis of national health survey for diabetes diagnosis and annual concentration of multiple pollutants.	Correlation of all pollutants except $PM_{2.5}$ with diabetes prevalence. Most consistent associations between NO_2 and OP^{DTT}. Particle composition may be more important than size.	[44]
$PM_{2.5}$ filter samples (Atlanta, GA, USA)	OP	Poisson log-linear regression of emergency admissions for cardiovascular and respiratory events; DTT	Positive association of OP^{DTT} with respiratory admissions and ischemic heart disease.	[15]
City-level $PM_{2.5}$ (ON, CDN)	OP	Random-effect meta-analysis evaluating the association of prenatal $PM_{2.5}$ exposure and risk of preterm birth (retrospective cohort study); AA, GSH	Positive association of $PM_{2.5}$ exposure (high OP^{GSH}) during first trimester of pregnancy with preterm birth. No association between city differences of OP^{AA} and preterm birth.	[45]

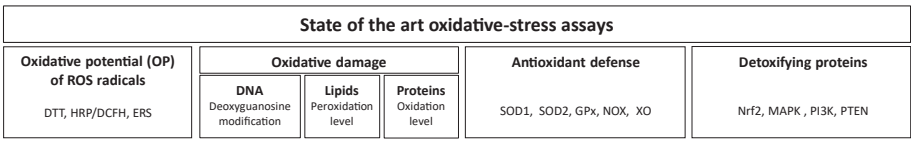
Abbreviations: 1-OHP: 1-hydroxypyrene; 15-F2t-IsoP: 15-F_{2t}-isoprostane; 2-OHTA: 2-hydroxyterephthalic acid; 8-oxodG: 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxodGuo: 8-oxo-7,8-dihydcro-2′-deoxyguanosine; 8-oxoGua: 8-oxoguanine; 8-oxoGuo: 8-oxo-7,8-dihydroguanosine; AA: ascorbic acid; •OH: Hydroxide; AKT: protein kinase B; AMS: aerosol mass spectrometry; Cox-4: Cyclo-oxygenase 4; Cox-5A: cyclo-oxygenase 5a; DCFH: dichloro-dihydro-fluorescein diacetate; DTT: dithiothreitol; EA.hy926: human endothelial cell line; ERK: extracellular signal-regulated kinase; ESR: electron spin resonance; GPX-1: glutathione peroxidase-1; GSH: glutathione; H_2O_2: hydrogen peroxide; HEFA: hydroprocessed esters and fatty acid base/fuel; HMOX-1: heme oxygenase 1; HUVEC: human umbilical vein endothelial cells; ICAM-1: intercellular adhesion molecule-1; IL: interleukin; JNK: c-Jun N-terminal kinase; LC-ESI-MS: liquid chromatography electrospray ionization tandem mass spectrometry; LDH: lactate dehydrogenase; MAPK: p38 mitogen-activated protein kinase; MCP-1: monocyte chemotactic protein-1; mtDNA: mitochondrial DNA; MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NF-κb: nuclear factor kappa B; Nrf1: nuclear respiratory factor 1; Nrf2: nuclear respiratory factor 2; nvPM: nonvolatile PM; OOH: hydroperoxyl radical; OP: oxidative potential; PM: particulate matter; qPCR: quantitative polymerase chain reaction; ROS: reactive oxygen species; RTLF: respiratory tract lining fluid; SOA: secondary organic aerosol; SOD: copper-zinc superoxide dismutase; THP-1: human monocytic leukemia cell line; VCAM-1: vascular adhesion molecule-1; WSOC: water-soluble organic carbon.

OP^{xx} with xx specifying the type of cell free assay used. OP per μg PM normalized to the corresponding air volume is annotated v for volume (μg m^{-3}) to represent human exposure.

from particle-induced ROS. Despite the benefits of cell-free assays, experimental cell-culture and animal studies are indispensable to investigate adverse effects of air pollution (Table 1B). The caveat is that the experimental setups used differ considerably, which hampers comparability and evaluation of the significance of the results obtained. The respective experimental variables include: (1) the source and composition of particles; (2) the route of particle application (e.g. suspended in liquids or as aerosols, out of a continuous air stream); (3) the number and duration of exposures as well as the time point of analysis to discriminate between acute and chronic exposure; and (4) the cell or animal models used and their susceptibility (e.g. pre-existing diseases, sex and age differences) [42,52,53].

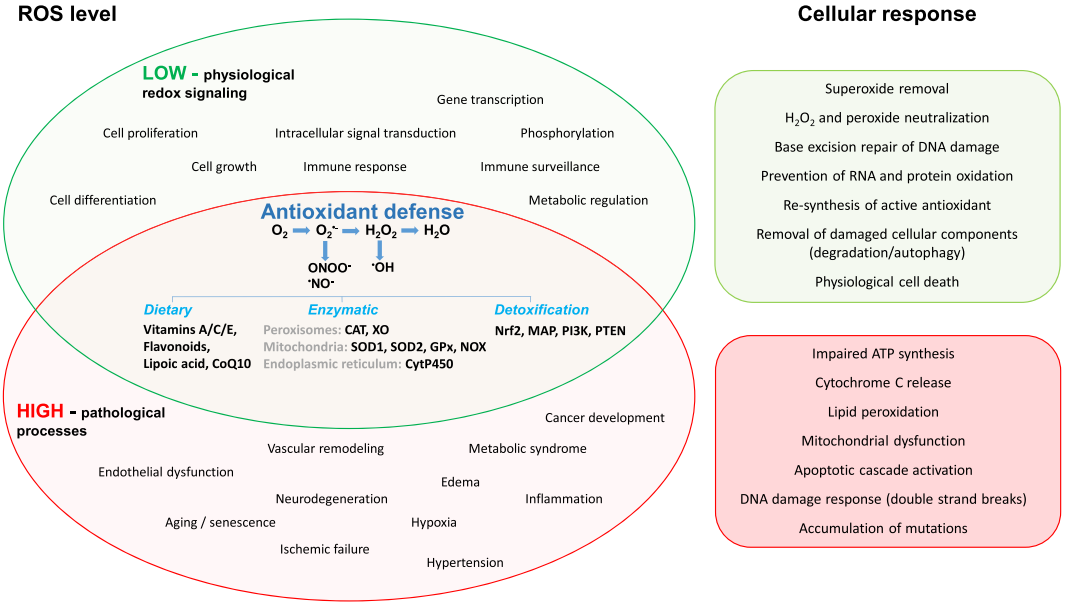
Experimental studies to evaluate air pollution-induced oxidative stress in humans are difficult to perform for ethical and population heterogeneity reasons. There

Figure 1



Methods and biomarkers employed to assess oxidative stress. Current methods quantify the OP of air pollution either directly or by means of surrogate markers. **Abbreviations**: CAT: catalase, CytP450: cytochrome P450; DNA: deoxyribonucleic acid; DTT: dithiothreitol; ERS: electron spin resonance; GPx: glutathione peroxidase; HPRT/DCFH: horseradish peroxidase/2′7′-dichlorodihydrofluorescein; MAPK: mitogen-activated protein kinase; NADPH: nicotinamide adenine dinucleotide phosphate; NOX: nitric oxidase; Nrf2: nuclear factor erythroid 2-related factor 2; PI3K: phosphoinositide 3-kinase; PTEN: phosphatase and tensin homolog protein; SOD1: superoxide dismutase 1; SOD2: superoxide dismutase 2; XO: xanthine oxidase.

Figure 2



Cellular responses to physiological and pathological levels of reactive oxygen species (ROS). Low, physiological ROS levels are vital for cell and organ function and homeostasis, while high ROS levels are harmful and induce diverse pathological processes. Organisms evolved interactive and synergistic antioxidant defense mechanisms neutralizing free radicals to protect from high ROS levels. Dietary antioxidants, enzymes in various cell compartments and detoxifying gene products are of fundamental importance. **Abbreviations:** CAT: catalase; CoQ10: coenzyme Q10; CytP450: cytochrome P450; GPx: glutathione peroxidase; MAPK: mitogen-activated protein kinase; NADPH: nicotinamide adenine dinucleotide phosphate; NOX: nitric oxidase; Nrf2: nuclear factor erythroid 2-related factor 2; PI3K: phosphoinositide 3-kinase; PTEN: phosphatase and tensin homolog protein; SOD1: superoxide dismutase 1; SOD2: superoxide dismutase 2; XO: xanthine oxidase.

have been efforts to develop and establish non- or minimally invasive methods applicable in humans (Table 1C). A study on mechanisms linking $PM_{2.5}$ exposure to the development of atherosclerosis, respiratory disease and cancer, has demonstrated a statistically significant relationship between both biomarkers for urinary oxidative stress and $PM_{2.5}$ exposure level with DNA methylation [54]. Thus, measurements of methylated DNA in urine samples appear suitable to estimate DNA damage in humans [52,53]. In addition, some studies employed blood samples to investigate the association of mitochondrial DNA methylation [53] and antioxidant enzyme concentration [52,54] with $PM_{2.5}$ exposure levels. Nevertheless, these new and promising methods in human urine or blood samples require further validation regarding reliability and accurate assessment of oxidative stress.

The growing literature unequivocally indicates a correlation between oxidative stress from exposure to air pollution and adverse health outcomes, even at levels below current air quality standards. During the past few years, methodological refinements of assays and in-depth understanding of their readouts have enhanced our comprehension of particle-ROS induced health

effects. The cell-free assays measuring the OP of particles have the potential to fill the gap between atmospheric chemistry and biology with regard to the allocation of particle characteristics to specific adverse effects. The different sensitivities and readouts of the OP-assays bear not only difficulties regarding comparability and interpretation of results. When used in combination, they allow a finer distinction of harmful particle types and characteristics. Experiments using RTLF are an important pillar towards biological models mimicking the inner lung surface, the primary target of inhaled particles upon deposition. Further, the biochemistry- and molecular biology-assisted untangling of the complex redox mechanisms allow a better understanding of direct adverse effects from PM and of oxidative stress-associated development and progression of disease. Because of this, clarification of the dual activity of redox-active molecules, including ROS, in cell signaling and as master regulator of inflammatory processes has been essential for progress in research of air pollution and health effects. Finally, advances in the development of minimal-invasive methods to assess oxidative stress-related cellular damage in humans have opened the possibility for otherwise not feasible studies. Ongoing efforts to establish the causal link between particle properties and specific health impacts are needed. Thereby, cooperation of all disciplines (i.e. multidisciplinary research) is indispensable. In-depth knowledge on mechanisms of particle-ROS induced health effects and the relevance of individual emission sources are required to better regulate ROS emission and increase the effectiveness of efforts towards reducing the risk of air-pollution related health effects.

Acknowledgements

This work was supported by the Swiss National Science Foundation (SNSF), Switzerland, grant CR32I3_166325.

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