HEI
Health Effects Institute

NUMBER 215
DECEMBER 2023

Walter A. Rosenblith New Investigator Award

COMMENTARY BY THE
HEI REVIEW COMMITTEE

Chemical and Cellular Formation of Reactive Oxygen Species from Secondary Organic Aerosols in Epithelial Lining Fluid

Shiraiwa et al.

healtheffects.org

Chemical and Cellular Formation of Reactive Oxygen Species from Secondary Organic Aerosols in Epithelial Lining Fluid

Manabu Shiraiwa, Ting Fang, Jinlai Wei, Pascale S.J. Lakey, Brian C.H. Hwang, Kasey C. Edwards, Sukriti Kapur, Jessica E. Monterrosa Mena, Yu-Kai Huang, Michelle A. Digman, Scott A. Weichenthal, Sergey Nizkorodov, Michael T. Kleinman

---

with a Commentary by the HEI Review Committee

Research Report 215

Health Effects Institute

Boston, Massachusetts

Trusted Science · Cleaner Air · Better Health

CONTENTS

ABOUT HEI

The Health Effects Institute is a nonprofit corporation chartered in 1980 as an independent research organization to provide high-quality, impartial, and relevant science on the effects of air pollution on health. To accomplish its mission, the Institute

- Identifies the highest-priority areas for health effects research
- Competitively funds and oversees research projects
- Provides intensive independent review of HEI-supported studies and related research
- Integrates HEI's research results with those of other institutions into broader evaluations
- Communicates the results of HEI's research and analyses to public and private decision-makers.

HEI typically receives balanced funding from the U.S. Environmental Protection Agency and the worldwide motor vehicle industry. Frequently, other public and private organizations in the United States and around the world also support major projects or research programs. HEI has funded more than 380 research projects in North America, Europe, Asia, and Latin America, the results of which have informed decisions regarding carbon monoxide, air toxics, nitrogen oxides, diesel exhaust, ozone, particulate matter, and other pollutants. These results have appeared in more than 260 comprehensive reports published by HEI, as well as in more than 2,500 articles in the peer-reviewed literature.

HEI's independent Board of Directors consists of leaders in science and policy who are committed to fostering the public–private partnership that is central to the organization. The Research Committee solicits input from HEI sponsors and other stakeholders and works with scientific staff to develop a Five-Year Strategic Plan, select research projects for funding, and oversee their conduct. The Review Committee, which has no role in selecting or overseeing studies, works with staff to evaluate and interpret the results of funded studies and related research.

All project results and accompanying comments by the Review Committee are widely disseminated through HEI's website *(www.healtheffects.org*), reports, newsletters and other publications, annual conferences, and presentations to legislative bodies and public agencies.

CONTRIBUTORS

RESEARCH COMMITTEE

David A. Savitz, Chair *Professor of Epidemiology, School of Public Health, and Professor of Obstetrics and Gynecology and Pediatrics, Alpert Medical School, Brown University*

David C. Dorman *Professor, Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University*

Christina H. Fuller *Associate Professor, School of Environmental, Civil, Agricultural and Mechanical Engineering, University of Georgia College of Engineering*

Marianne Hatzopoulou *Professor, Civil and Mineral Engineering, University of Toronto, Canada, Research Chair in Transport Decarbonization and Air Quality*

Amy H. Herring *Sara & Charles Ayres Distinguished Professor of Statistical Science, Global Health, Biostatistics, and Bioinformatics, Duke University*

Heather A. Holmes *Associate Professor, Department of Chemical Engineering, University of Utah*

Neil Pearce *Professor of Epidemiology and Biostatistics, London School of Hygiene and Tropical Medicine, United Kingdom*

Evangelia (Evi) Samoli *Professor of Epidemiology and Medical Statistics, Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Greece*

Neeta Thakur *Associate Professor of Medicine, University of California–San Francisco*

Gregory Wellenius *Professor, Department of Environmental Health, Boston University School of Public Health and Director, BUSPH Center for Climate and Health*

REVIEW COMMITTEE

Melissa J. Perry, Chair *Dean, College of Public Health, George Mason University*

Sara D. Adar *Associate Professor and Associate Chair, Department of Epidemiology, University of Michigan School of Public Health*

Kiros T. Berhane *Cynthia and Robert Citron-Roslyn and Leslie Goldstein Professor and Chair, Department of Biostatistics, Mailman School of Public Health, Columbia University*

Ulrike Gehring *Associate Professor, Institute for Risk Assessment Sciences, Utrecht University*

Michael Jerrett *Professor, Department of Environmental Health Sciences, Fielding School of Public Health, University of California–Los Angeles*

Frank Kelly *Henry Battcock Chair in Community Health and Policy and Director of the Environmental Research Group, Imperial College London School of Public Health, United Kingdom*

Jana B. Milford *Professor Emerita, Department of Mechanical Engineering and Environmental Engineering Program, University of Colorado–Boulder*

Jennifer L. Peel *Professor of Epidemiology, Department of Environmental and Radiological Health Sciences, Colorado State University, and the Colorado School of Public Health*

Eric J. Tchetgen Tchetgen *University Professor and Professor of Biostatistics and Epidemiology, Perelman School of Medicine, and Professor of Statistics and Data Science, The Wharton School, University of Pennsylvania*

John Volckens *Professor, Department of Mechanical Engineering, Walter Scott Jr. College of Engineering, Colorado State University*

HEI PROJECT STAFF

Annemoon M. van Erp *Staff Scientist (Study Oversight)*

Eva Tanner *Staff Scientist (Report Review)*

Kristin C. Eckles *Senior Editorial Manager*

Hope Green *Editorial Project Manager*

Carol Moyer *Consulting Editor*

Research Report 215, *Chemical and Cellular Formation of Reactive Oxygen Species from Secondary Organic Aerosols in Epithelial Lining Fluid,* Shiraiwa et al.

INTRODUCTION

Atmospheric aerosols are a mixture of organic (e.g., carbon-containing) and inorganic microscopic particles and liquid droplets, known as particulate matter (PM*), that are suspended in the air. Commonly used as a proxy for overall air quality levels, PM can be emitted directly from combustion sources (such as smokestacks, vehicle exhaust, and wildfires) but is also formed by chemical reactions in the atmosphere (World Health Organization [WHO] 2022). Even at relatively low levels of exposure, PM is associated with adverse health effects, namely respiratory and cardiovascular disease and mortality, and is recognized as a leading risk factor of morbidity and mortality worldwide (Global Burden of Disease [GBD] 2020; International Agency for Research and Cancer [IARC] 2016; United States Environmental Protection Agency [U.S. EPA 2019]). Laboratory and human studies show that PM exposure leads to poor health outcomes through various biological mechanisms, including oxidative stress and inflammation, both of which are induced by reactive oxygen species (ROS) (Li et al. 2022).

ROS are highly reactive oxygen-containing chemicals, such as hydrogen peroxide and hydroxyl radicals, and are part of normal biological function. For example, ROS are byproducts of aerobic metabolism and play a role in such processes as cell differentiation, apoptosis, and immunity (Pizzino et al. 2017). However, the overproduction and accumulation of ROS due to environmental and other insults can damage essential cell components and lead to the onset and progression of disease (Pizzino et al. 2017). In the context of inhaled pollutants and their downstream health effects, understanding ROS formation in the respiratory tract is essential but poorly characterized. Research focused on the epithelial lining fluid (ELF) — a protective liquid layer above the mucosa that contains antioxidants, surfactants, and immune cells — is needed because it is a critical interface between lung tissue and the outside environment. Ambient inhalable aerosols contain components such as transition metals that can catalyze ROS formation in the ELF (Gurgueira et al. 2002; Lakey et al. 2016). In response to aerosol exposure, immune cells called macrophages can also release ROS in the ELF as part of their *kill and capture* campaign primarily intended for microbial invasion. The relative importance of ROS formation by chemical reactions compared with macrophage ROS generation and release is unknown. Better characterization of these ROS formation pathways might lead to the development of clinical or public health interventions to reduce air pollution health effects.

To begin to examine the effects of air pollution exposure on the formation of ROS in lungs, Dr. Manabu Shiraiwa of the University of California, Irvine, submitted an application to HEI titled "Formation of Reactive Oxygen Species by Organic Aerosols and Transition Metals in Epithelial Lining Fluid" in response to HEI's Request for Applications 17-3 Walter A. Rosenblith New Investigator Award. This award was established to provide support for an outstanding new investigator to conduct research on air pollution and health and is unrestricted with respect to the specific topic of air pollution health research. Dr. Shiraiwa proposed to investigate the kinetics and chemical mechanisms of ROS formation in synthetic ELF by different types of air pollutants and to quantify the relative importance of ROS formed by chemical reactions in the ELF compared with ROS released as an immune response to the pollution. HEI funded the study because Dr. Shiraiwa was an established atmospheric chemist who has expertise in ROS kinetic modeling and was proposing interdisciplinary chemistry and toxicology work to elucidate the role of chemically versus biologically produced ROS in air pollution health effects, using state-of-the-art methods to capture formation of a variety of ROS species in a controlled environment.

This Commentary provides the HEI Review Committee's independent evaluation of the study. It is intended to aid the sponsors of HEI and the public by highlighting both the strengths and limitations of the study and by placing the Investigators' Report into scientific and regulatory perspective.

SCIENTIFIC AND REGULATORY BACKGROUND

Aerosol air pollution is associated with a myriad of health effects, including respiratory, cardiovascular, and neurological diseases, cancer, and poor birth outcomes (GBD

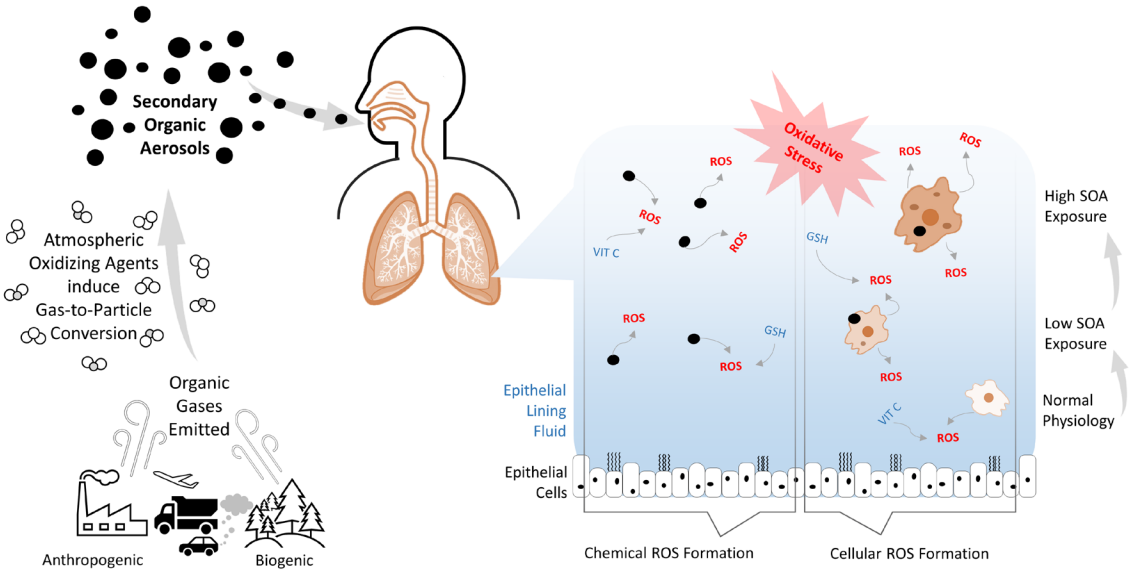
Dr. Manabu Shiraiwa's 3-year study, "Formation of Reactive Oxygen Species by Organic Aerosols and Transition Metals in Epithelial Lining Fluid," began in November 2018. Total expenditures were $450,000. The draft Investigators' Report from Shiraiwa and colleagues was received for review in July 2022. A revised report, received in December 2022, was accepted for publication in February 2023. During the review process, the HEI Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in both the Investigators' Report and the Review Committee's Commentary.

* A list of abbreviations and other terms appears at the end of this volume.

2020; IARC 2016; U.S. EPA 2019). Particle size determines deposition in the respiratory tract and influences subsequent health effects. For example, coarse particles (PM between 2.5 and 10 μm in average aerodynamic diameter) mostly deposit in the upper respiratory tract, whereas fine particles (PM <2.5 μm in aerodynamic diameter, or $PM_{2.5}$) can deposit deep in the lower respiratory tract; they can directly enter the bloodstream, or compounds adsorbed onto the particles can enter the lung and vascular tissues (Li et al. 2022). A substantial body of evidence has led the U.S. EPA to conclude that the link between exposure to $PM_{2.5}$ and mortality is causal (U.S. EPA 2019). Although air pollution levels have decreased over the past few decades in high-income countries, associated health effects are still observed at levels at and below current air quality standards (Brauer et al. 2019, 2022; Brunekreef et al. 2021; Chen and Hoek 2020; Dominici et al. 2019, 2022). Accordingly, the WHO revised its air quality guidelines (WHO 2021), and some governmental agencies, such as the U.S. EPA, have proposed further lowering the regulatory standards for PM (U.S. EPA 2023). They continue to review the scientific evidence to evaluate the need for even lower standards.

Despite their public health importance, the complex time-varying chemistry of atmospheric aerosols is not fully understood. Primary aerosols originate directly from their anthropogenic (e.g., fuel and biomass combustion) or natural sources (e.g., sea salt and mineral dust). The carbonaceous fraction of PM includes such components as organic carbon and black carbon, whereas the inorganic fraction includes such compounds as sulfates and nitrates. Secondary organic aerosols (SOAs) form by gas-to-particle conversion in the atmosphere when organic gases react with oxidizing agents such as ozone (see **Commentary Figure 1**). SOA precursors can be biogenic or anthropogenic in origin. Biogenic precursors, including isoprene and pinene, are primarily emitted from plants and are abundant in the ambient atmosphere (Shrivastava et al. 2017). Anthropogenic precursors such as naphthalene are emitted from fuel burning and coal tar processing (Jia and Batterman 2010). SOAs are the major fraction of organic aerosols in the atmosphere and are thus an important aspect of air pollution health effects (Shrivastava et al. 2017).

Research has demonstrated that redox active air pollutants, including SOAs, contribute to ROS formation in the lung ELF, where they undergo redox cycling and interact with host antioxidants and immune cells, including macrophages (Pöschl and Shiraiwa 2015). In response to foreign substances, certain types of macrophages classified as M1 macrophages activate to a proinflammatory state and release ROS to kill microbes and signal the disturbance to neighboring cells. Macrophage ROS generation is beneficial when compartmentalized to specific cells and tissues in need, and in healthy tissues ROS are kept in balance by scavenging antioxidants. However, excess ROS can lead to oxidative stress, tissue damage, and overwhelming or chronic inflammation (Canton et al. 2021). Thus, ROS are formed in lung ELF both chemically and cellularly, but the relative importance of each formation pathway is poorly characterized, partly because it is difficult to measure various species of ROS and determine their origin. To shed light on this question, the current study aimed to combine kinetic modeling of ROS formation with experimental quantification



Commentary Figure 1. Anthropogenic and biogenic volatile organic gases are emitted into the atmosphere and react with oxidizing agents such as ozone in a gas-to-particle conversion to form SOAs. SOAs (and other aerosols) are inhaled into the respiratory tract and can form ROS in the ELF through chemical reactions. ROS are also formed cellularly by macrophages under normal physiological conditions. However, aerosols exposure can increase cellular ROS formation. Antioxidants like vitamin C and glutathione (GSH) scavenge and neutralize ROS. When antioxidant systems are overwhelmed, ROS can accumulate and induce oxidative stress, which leads to cell damage and death.

of ROS produced by chemical reactions of SOA in synthetic lung ELF and by macrophages exposed to SOA in vitro.

SUMMARY OF APPROACH AND METHODS

STUDY AIMS AND APPROACH

To begin to characterize the formation of ROS in the ELF of lungs following inhalation of SOAs, Dr. Shiraiwa and colleagues aimed to accomplish the following:

- Identify the mechanisms and kinetics of ROS formation by aqueous chemical reactions of SOAs. Specific tasks were to
  - ◊ Quantify ROS formation by laboratory-generated SOAs
  - ◊ Quantify ROS formation by ambient PM samples
- Quantify the relative importance of ROS formed by chemical reactions compared with the cellular release of ROS by macrophages in synthetic ELF. Specific tasks were to
  - ◊ Use kinetic modeling to estimate ROS concentrations formed by chemical reactions within different lung compartments
  - ◊ Quantify ROS released by macrophage cells when exposed to SOA

Shiraiwa and colleagues first generated SOAs in a controlled laboratory environment by inducing oxidation of selected biogenic and anthropogenic organic compounds in a reaction chamber. They also collected PM from a small number of ambient air samples from the Los Angeles, California, region that were used to represent real-world PM from urban, traffic, and wildfire locations. They conducted various in vitro experiments to quantify the chemical formation of several different types of ROS in water and in synthetic lung ELF from SOAs generated by laboratory precursors or ambient air PM samples. The synthetic lung ELF contained the antioxidants ascorbate (vitamin C), citric acid, glutathione, and uric acid. They also evaluated laboratory-generated and ambient PM for their oxidative potential, a measure of particle capacity to take electrons from (or oxidize) other molecules and generate ROS. In their experiments, the investigators evaluated the effects of different experimental conditions on ROS formation, including the addition of nitrogen oxides (NO_x), the addition of ferrous iron (Fe^{2+}), and changes in pH levels ranging from 1 to 7.4. Both NO_x, a traffic-related air pollutant, and Fe^{2+}, a transition metal of natural and anthropogenic origin, are recognized as important atmospheric oxidizing agents (Pöschl and Shiraiwa 2015). They used a relatively new and sensitive method called continuous wave electron paramagnetic resonance (EPR) spectroscopy with a spin trapping technique to capture the formation of various ROS species.

In addition, Shiraiwa and colleagues used computer modeling to determine the ROS chemical reaction kinetics and developed a combined model of the human respiratory tract and reaction kinetics to estimate ROS burden in different compartments of the respiratory system after inhalation exposure. Finally, in collaboration with a leading toxicologist in the field, Dr. Michael Kleinman, Shiraiwa compared the chemical formation of ROS by SOAs with the ROS released by macrophage cells when exposed to SOAs.

METHODS AND STUDY DESIGN

Aerosol Generation and Sampling

Laboratory-Generated Particles SOA particles were generated by the oxidation of biogenic SOA precursors, including isoprene, α-pinene, β-pinene, α-terpineol, and d-limonene, and such anthropogenic SOA precursors as naphthalene and toluene. Not all precursors were used for all experiments. The investigators used two methods of oxidation (dark ozonolysis and hydroxyl photooxidation). In experiments using dark ozonolysis oxidation, ozone was first injected into an oxidation flow reactor using pure oxygen and an ozone generator. In hydroxyl photooxidation, hydroxyl radicals were generated by ultraviolet photolysis of water molecules. The SOA precursors that formed from ozone or hydroxyl radicals were then separately injected into a potential aerosol mass (PAM) reactor, and SOA particles were collected on to 47-mm polytetrafluoroethylene (PTFE) filters.

Ambient Air Particle Sampling The investigators sampled ambient PM from sites representative of urban, highway, and wildfire environments in the Los Angeles region. First, they used a high-volume sampler with microquartz filters to collect ambient PM samples during periods without nearby wildfires. Samples were separated into PM size fractions <1 micron (PM_1) and 1–10 microns ($PM_{1\text{-}10}$). The single urban site was atop a University of California, Irvine, campus building (20 meters high) and the two highway sites were located within 20 meters of interstates I-5 and I-710, the latter of which has a higher fraction of heavy-duty vehicles. Each sample was collected for 4–12 hours daily over 5–6 days during January and February 2020. Portions of the sampling collection filters were used to measure environmentally persistent free radicals for all sites and to measure ROS and oxidative potential within six months of sampling.

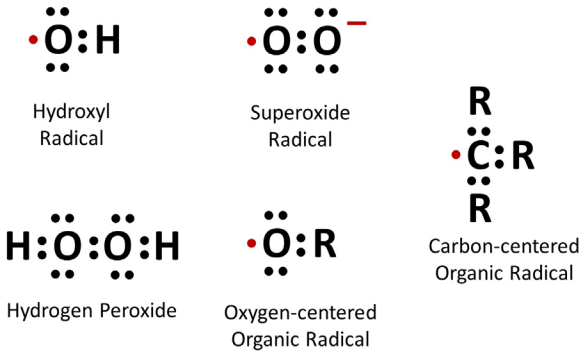
During two major wildfire events that occurred about 20 kilometers away from the Los Angeles sites in October and November 2020, the investigators sampled size-segregated ambient PM using a Micro-Orifice Uniform Deposition Impactor (MOUDI Model 100NR) with Teflon filters. Eight sets of continuous 3-day samples were collected at the rooftop urban site during each fire event. MOUDI samples that were previously collected from the same urban and highway locations earlier that year (January and February 2020) were used as baseline nonfire samples. To normalize the measurements,

hourly mass concentrations of PM_1 and PM_{10} were collected at the urban site using the Purple Air real-time air quality data located within 0.5 kilometers of the sampling site. Purple Air data were not available for the highway sites.

ROS Detection and Quantification

The investigators used several techniques to quantify ROS formation in water, in synthetic lung ELF, and from macrophage cells that were exposed to SOAs generated by laboratory-based biogenic volatile organic compounds and to ambient air PM samples. Radical forms of ROS were detected using a relatively new and sensitive method called continuous wave electron paramagnetic resonance (EPR) spectroscopy with a spin trapping technique that enables detection of some shorter-lived radicals; these included the hydroxyl radical, superoxide radical, and carbon-centered and oxygen-centered organic radicals (**Commentary Figure 2**). To quantify the absolute and relative abundance of the radicals, EPR spectra were fitted and simulated using software packages to process the spin counting methods. Hydrogen peroxide was measured using a fluorometric hydrogen peroxide assay and spectrofluorophotometry; organic hydroperoxides within the SOA particles were measured with an iodometric-spectrophotometric technique. The assays included negative controls and were calibrated with standards. Oxidative potential was quantified with positive controls using the dithiothreitol (DTT) assay and a Liquid Waveguide Capillary Cell coupled to the ultraviolet-visible spectrophotometer and the multiwavelength light detector. Total DTT was calculated using linear regression of time and absorbance.

To quantify the relative importance of chemical versus cellular ROS formation, the investigators used a chemiluminescence assay combined with EPR spectroscopy to detect superoxide formed in the absence and presence of macrophages. The investigators used RAW 264.7 macrophage cells, a cultured mouse cell line, because it had previously been used to study oxidative stress. Experiments were performed in triplicate with positive and negative controls. Note that there was a 15-minute delay in superoxide measurement due to sample preparation; this delay likely missed a large portion of early ROS formation. To account for that, kinetic modeling was applied. Superoxide formation time profiles and total formation were calculated using the mean of triplicates. For imaging, all data points for each sample were averaged from multiple cells ($N > 10$). Unpaired t tests were used to test whether SOA-exposed and control groups of cells differed in superoxide concentrations.



Commentary Figure 2. ROS Chemical Formulas quantified. Red dots represent unpaired electrons.

Kinetic Modeling

Shiraiwa and colleagues developed a kinetic model to simulate the simultaneous formation of hydroxyl radicals and superoxide by aqueous chemical reactions of SOA. This kinetic model combined experimental findings with known information about several individual chemical reactions into a more comprehensive model of SOA and ROS chemistry. Rate-constant model inputs were based on the relative abundance of certain functional groups (published values obtained from experiments by other researchers) or on a Monte Carlo genetic algorithm. Kinetic modeling was also used to simulate ROS formation by aqueous chemical reactions of isoprene-generated SOA with Fe^{2+} in synthetic lung ELF that contained antioxidants.

To estimate the concentration and rate of ROS formation in different regions of the respiratory tract, the investigators built on their previously developed human respiratory tract model and a kinetic multilayer model for surface and bulk chemistry in the ELF (KM-SUB-ELF) (Lakey et al. 2016). The human respiratory tract model estimated particle deposition in the entire tract and within the extrathoracic, bronchial, and alveolar regions with inhalation parameters for a person doing light work while breathing through their nose. The KM-SUB-ELF model incorporated over 50 relevant chemical reactions, included rate constants from literature values when available, and accounted for ROS formation by α- and β-pinene-, isoprene-, and limonene-generated SOAs; by quinones from naphthalene; and by transition metals copper and iron. The models were applied to previously collected urban and roadside, size-segregated PM samples from Atlanta, Georgia (Fang et al. 2017). They modeled the formation of hydrogen peroxide and hydroxyl and superoxide radicals. Lastly, in an attempt to integrate results of the modeling with experimental data, the investigators compared the KM-SUB-ELF modeled ROS formation with oxidative potential as measured by the DTT and ascorbic acid assays for different respiratory tract regions and for the Atlanta PM samples taken in winter versus summer.

SUMMARY OF KEY FINDINGS

ROS FORMATION BY LABORATORY-GENERATED SOAS

Shiraiwa and colleagues evaluated which ROS molecules formed under various conditions to understand various contributing factors better and to obtain detailed data for input into kinetic modeling. They found that the formation of various ROS depended on the method of aqueous oxidation

and the SOA precursor and that reaction kinetics varied (see **Commentary Table 1**). Ozonolysis of α-terpineol exclusively formed hydroxyl radical adducts, and ozonolysis of d-limonene formed 80% superoxide radical adducts. Ozonolysis of isoprene and β-pinene yields were almost evenly split between the hydroxyl and superoxide radical adducts. ROS yields for the hydroxyl photooxidation of all tested biogenic compounds were mostly superoxide radical adducts and excluded the formation of any hydroxyl radical adducts. Carbon- and oxygen-centered organic radical adduct formation was relatively low. In experiments of hydrogen peroxide formation, isoprene-generated SOA formed higher levels than the other SOA precursors, and oxidation by ozonolysis generally formed higher levels than by hydroxyl photooxidation. Superoxide and hydrogen peroxide formation were highly correlated ($R^2 > 0.9$). Kinetic modeling showed that ROS formation varied by time. Hydroxyl radical adduct formation by ozonolysis increased rapidly and reached a steady state after 2 hours. However, superoxide formation by both oxidation methods reached a maximum concentration within 30–40 minutes and then decreased slowly thereafter after exposure to most biogenic precursors.

Commentary Table 1. Summary of Chemical ROS Formation by Oxidation Method, SOA Precursor, and Changes in the Experimental Conditions

		ROS Yield				
	SOA Precursor	**Hydroxyl Radical**	**Superoxide**	**C-Centered**	**O-Centered**	**Hydrogen Peroxide**
Oxidation Method						
Ozonolysis in water	α-terpineol	+++	0	0	0	++
	d-limonene	+	++	+	+	++
	isoprene	++	++	0	0	+++
	β-pinene	++	++	0	0	++
Hydroxyl photooxidation in water	α-terpineol	0	++	+	+	+
	d-limonene	0	++	+	+	+
	isoprene	0	++	+	+	+
	β-pinene	0	++	+	+	+
Variation in Experimental Conditions						
Higher NO_x in water	α-pinene	↓	↓	↓	↓	NA
	naphthalene	↓	↓	0	0	NA
Lower pH in water	α-terpineol	↑	↑	↓	↑	↑
	isoprene	↓	↑	↓	↓	↑
	α-pinene	↑	↑	=	=	↑
	β-pinene	↑	↑	=	=	↑
	toluene	0	↑	↑	0	↑
	naphthalene	0	↑	↑	0	↑
Higher Fe^{2+} in water	α-terpineol	↑	↑	0	0	NA
	isoprene	↑	↑	↑	0	NA
	toluene	0	↑	↑	↑	NA
Higher Fe^{2+} in synthetic lung ELF	α-terpineol	0	0	↑	↑	NA
	isoprene	0	0	↑	↑	NA
	toluene	0	↑	↑	↑	NA

0, negative result; +, low yield; ++, moderate yield; +++, high yield; ↑, increased yield; ↓, decreased yield; =, no change; NA, not tested.

Different experimental conditions, including the presence of NO_x or Fe^{2+} and changes in pH levels, altered ROS formation in water and synthetic ELF (Commentary Table 1). The introduction of high concentrations of NO_x (700 ppb) to SOA generated by α-pinene or naphthalene decreased hydroxyl radical formation by a factor of 10 and 1.5, respectively, and decreased superoxide formation by a factor of 2 and 3, respectively. NO_x also reacted with the SOA precursors to form many nitrogen-containing compounds, although the individual specifics of which could not be resolved with the current laboratory methods. At neutral pH (7.4), α-terpineol-generated SOAs primarily formed carbon-centered radical adducts; at lower pH (<3.5), the overall ROS radical formation decreased despite small increases in the hydroxyl radical, superoxide, and oxygen-centered radical adducts. Similarly, at lower pH levels the overall ROS formation by isoprene-generated SOAs decreased, despite small increases in superoxide. In contrast, at lower pH levels α-pinene-, β-pinene-, toluene-, and naphthalene-generated SOAs had higher overall ROS radical formation, which was dominated by superoxide adducts. Hydrogen peroxide formation was also increased at lower pH levels for all SOAs.

The introduction of Fe^{2+} to SOA generated by photooxidation of isoprene, α-terpineol, and toluene increased ROS formation in both water and synthetic lung ELF. However, there were some differences in the radical species that would be attributable to the presence of ascorbate in the synthetic lung ELF. Isoprene- and α-terpineol-generated SOA dramatically increased hydroxyl radical adduct formation in water and carbon-centered radical adduct formation in synthetic lung ELF. Radical formation peaked at a reaction time of 20 and 60 minutes for water and synthetic lung ELF, respectively. DTT activity was also higher with the introduction of Fe^{2+} to SOAs, demonstrating a link between aqueous experimental measures of oxidative potential and ROS formation. Overall, chemical ROS formation from SOA was highly complex and heavily dependent on the precursor, oxidation method, and experimental conditions.

ROS FORMATION BY AMBIENT PM SAMPLES

The investigators found that the quantity and composition of ROS formed by ambient PM varied by sampling site in the Los Angeles region, with marked differences between wildfire and nonwildfire samples. ROS formed per air volume were highest in highway and lowest in wildfire samples, and the ROS formed per mass were higher in urban compared with wildfire samples. The total radicals associated with different PM size fractions showed a bimodal distribution for wildfire samples (radical concentrations peaked at 0.056–0.18 and 10–18 μm PM size fractions) and urban samples (radical concentration peaked at 0.56–1 and 10–18 μm PM size fractions), whereas highway samples exhibited a total radical peak in the 0.56–1 μm PM size range. On average, PM from urban and highway sampling sites formed primarily hydroxyl radicals (>84%) with the remaining proportion being carbon-centered radicals for both PM_1 and $PM_{1\text{-}10}$ extracts. Wildfire samples formed mostly carbon-centered radicals (~50%). PM_1 extracts formed 28% hydroxyl, 13% superoxide, and 5% oxygen-centered radicals, whereas $PM_{1\text{-}10}$ extracts formed 49% hydroxyl and 2% superoxide radicals. Carbon-centered radical formation was correlated with oxygen-centered radical formation ($R^2 = 0.53$), whereas hydroxyl radical formation was correlated with superoxide formation ($R^2 = 0.50$), suggesting distinct chemical formation pathways.

Oxidative potential was highest in highway samples. Oxidative potential measurements at different PM size fractions were similar for the highway and urban sites and peaked at the 0.56–1 μm PM size fraction. After normalization, oxidative potential was higher per volume in $PM_{1\text{-}10}$ compared with PM_1 extracts and was correlated with total ROS formation ($R^2 = 0.61$). In contrast, for wildfire samples the oxidative potential measurements were similar across the different PM sizes, but after normalization the measurements were higher per volume and per mass in the PM_1 extracts and did not correlate with total ROS formation ($R^2 < 0.01$).

ROS FORMATION IN THE ELF

Modeling results indicated that ROS formation in the human respiratory tract depended on the size composition of PM exposure and on ELF volume and particle deposition in the different respiratory tract regions. ROS formation was highest in the extrathoracic region and lowest in the alveolar region. The investigators noted that assuming a uniform particle distribution in the respiratory tract would underestimate ROS formation in the extrathoracic cavity and overestimate it in the bronchial and alveolar regions. The estimated formation of hydrogen peroxide was highest, and hydroxyl radical was lowest in ELF. ROS formation was higher for the roadside PM compared with urban ambient PM samples from Atlanta. In terms of specific chemical exposure, the modeled ROS formation was highest for copper, followed by iron; it was an order of magnitude lower for SOAs and lowest for quinones. Copper exposure was estimated to form mostly hydrogen peroxide and superoxide, whereas SOAs primarily formed hydroxyl radicals. The investigators noted that assuming a uniform particle distribution in the respiratory tract would misrepresent ROS formation by iron in the extrathoracic versus alveolar regions. The modeled formation of hydrogen peroxide and superoxide showed moderate to high correlations with the measured oxidative potential, demonstrating good agreement between the two methods, whereas the modeled formation of the hydroxyl radical had low correlation with measured oxidative potential.

CHEMICAL VERSUS CELLULAR ROS FORMATION IN THE SYNTHETIC ELF

Shiraiwa and colleagues found that quinones and isoprene-generated SOA activated macrophage cells to release higher concentrations of superoxide than what was formed through chemical reactions in the synthetic lung ELF. Superoxide formation depended on the concentration of SOA precursor and duration of the exposure. At low concentrations, cellular formation of superoxide was about 10 times higher

than chemical formation, particularly at the beginning of the experiments. The threshold dose for macrophage activation was smaller for quinones than for isoprene. The investigators reported that the decreased superoxide formation by macrophages over longer exposure times was due to the activation of antioxidant processes, oxidative stress, and cell death. The chemical superoxide production modeled by the investigators was similar to the experimental results, providing reassurance that the kinetic modeling approach accurately captured the processes.

HEI REVIEW COMMITTEE'S EVALUATION

This study investigated the potential kinetics and chemical mechanisms of ROS formation in the ELF of the respiratory tract following inhalation of different aerosols. It also quantified the relative importance of ROS formed by chemical reactions in the ELF compared with ROS released by macrophages as an immune response to aerosol exposure. Dr. Shiraiwa and colleagues demonstrated that quantity and composition of ROS formed by aqueous reactions highly dependent on such factors as the specific aerosol mixture, oxidation mechanisms, and other environmental conditions. They also found that for certain exposure conditions, the ROS released by macrophages dwarfed the ROS formation by aqueous chemical reactions.

In its independent evaluation of the report, the Review Committee noted that the investigators made a valuable contribution to the study of the health effects of air pollution mediated through ROS, bridging the fields of chemistry and toxicology. They thought that the work comparing chemical versus cellular ROS formation was novel, and they appreciated the inclusion of ambient aerosol samples from field measurements in addition to the lab-generated aerosols. The results showing higher ROS yields from macrophages compared with yields from aqueous chemical reactions were considered particularly important.

EVALUATION OF THE METHODOLOGICAL APPROACH

The Committee appreciated several aspects of the methodological approach, including the use of both ambient aerosol samples and laboratory-generated SOA, testing the influence of various experimental factors, such as copollutants and pH, and combining both experimental and modeling efforts. They also noted that the use of probes specific to different ROS was superior to other nonspecific methods. However, they thought that it was difficult to translate the results of the experiments to real-world conditions in the human lung after inhalation of ambient pollutants and that the full implications of the results were therefore difficult to determine. Thus, the Committee thought that this work should be considered a valuable initial foray, with considerably more research needed.

It was unclear how well some aspects of the laboratory methods relate to more complex biological systems. The current results advance our mechanistic understanding of substrate influence on radical yields. However, given that results are obtained in a synthetic ELF, the question remains open as to whether they are representative of in vivo outcomes. Although the macrophage cell line used in this study is frequently used in other in vitro toxicity assays and is a useful starting point, it is derived from a leukemia virus-induced mouse tumor. As such, it is not part of immune defense in the respiratory system and does not mimic macrophage behavior in lung tissues well. An immortalized alveolar macrophage cell line that is more responsive to soluble and particulate stimuli would have been more representative of an immune response to inhaled aerosols. It will be important for future work to repeat the experiments using lung-derived macrophage cell lines or primary cells. It would also be useful to examine ROS formation by other key immune system players, such as neutrophils, whose influx to the lung is associated with many respiratory conditions, and to consider the use of co-cultures in which two or more types of lung cell coexist and interact with each other.

Regarding the aerosol generation, the Committee questioned the concentrations of the laboratory-generated SOAs because the experiments used higher SOA precursor concentrations than would be observed in the ambient atmosphere, thereby possibly affecting the interpretation of the results. The investigators acknowledge this difference and note similarities in SOA yield and average oxidation state compared with SOAs formed under more realistic conditions but note that important differences would be expected in the molecular composition of the SOA formed. Future work could shed light on this issue by generating SOA with different equipment. Furthermore, the investigators evaluated only a few ambient air samples that were taken at various times from three locations in Los Angeles. Thus, it is unclear how representative the ROS formation results are from SOA generated by the ambient air samples. The report could have been improved by further discussion of how representative these samples were to aerosol mixtures in other locations in the region, the broader United States, or around the world.

The Committee appreciated the investigators' efforts to evaluate ROS formation using both species-specific probes and oxidative potential by the DTT assay but noted that a more comprehensive evaluation would enhance future work to better align with the overarching goal to elucidate aerosol lung toxicity mediated through ROS. The investigators acknowledged that only a few ROS were quantified within a chemical reaction time range limited by feasibility. Thus, important ROS formation could have been missed. Other important nonradical forms of ROS, such as singlet oxygen, might also play an important role in aerosol-induced health effects. In addition, there is no consensus on which assays are most suitable to measure ROS, because none of the assays currently available show good correlations of redox potential with PM toxicity (Pietrogrande et al. 2022). Although the DTT assay is simple and commonly used, it provides limited insight in this context. Other assays such as dichloro-

dihydro-fluorescein diacetate are currently preferred for studying ROS formation in cells.

Lastly, the Committee thought that the kinetic modeling, particularly with ROS formation in different compartments of the respiratory tract, was well done and demonstrated that the approach was possible. However, as with any modeling efforts, the results hinge on the model inputs, which can change under various realistic conditions. Thus, the results should be interpreted cautiously. Further work in this area might help in efforts to improve our understanding of ROS formation under various conditions and other pathways to mitigate respiratory toxicity. As noted in the report, Dr. Shiraiwa and colleagues later applied their kinetic model to estimate ROS as a metric of exposure to metal components in PM in epidemiological studies and found associations with respiratory and cardiovascular diseases. The Committee did not review the methods and results of these epidemiological collaborations because it was outside the scope of the current report, but they thought that it was a novel and useful approach to exposure assessment.

DISCUSSION OF THE FINDINGS AND INTERPRETATION

A key strength of this study is that it quantified the relative importance of ROS formation by chemical reactions in the ELF compared with the ROS released by activated macrophages. The investigators found that under certain experimental conditions, the ROS macrophage release far outweighed the ROS formed by chemical reactions. This result implies that aerosol-induced respiratory health effects mediated by ROS might not depend on the aerosol composition and associated chemical reactions alone. Instead, future work will also need to consider macrophage and other immune cell activation after aerosol exposure.

In this study, only laboratory-generated SOAs were used to evaluate chemical versus macrophage ROS yields. Future work is warranted to evaluate chemical versus biological yields from real-world aerosol samples. It would also be helpful in future work to use robust negative-control scenarios, for example, by varying the experimental timeframes and by using nonchemical stimuli, such as roughness of the culture surface, and to test a variety of relevant macrophage cell lines and other immune cells, such as neutrophils, as multiple types of cells can be recruited to the respiratory system as part of the immune response.

The investigators reported that ambient PM samples from the two highway traffic locations had higher ROS yields compared with samples from an urban site before and during nearby wildfire events. These results are intriguing and are consistent with a study showing that nontailpipe roadway PM might be more toxic than PM from other sources (Shirmohammadi et al. 2016). The potential for higher toxicity in nontailpipe PM is thought to be due to the synergistic effects of redox-active metals and organics on ROS formation. This theory is also consistent with results in this study showing that laboratory-generated SOAs act synergistically with Fe^{2+} to elicit substantially higher ROS formation. However, it is important to note that only a few ambient samples were collected from just three locations in this study. Thus, the results comparing roadside, urban, and wildfire samples remain preliminary and must be verified in future studies with much larger sets of samples. Wildfire smoke has high compositional variability (Jaffe et al. 2020), and the limited sampling cannot be generalized to other wildfires.

CONCLUSIONS

This study aimed to identify the kinetics and chemical mechanisms of ROS formation in the ELF of the respiratory tract after aerosol exposure and to quantify the relative importance of ROS formed by aqueous chemical reactions in the ELF compared with ROS released by macrophages as an immune response to aerosol exposure. The investigators tested ROS formation under several different experimental scenarios and used both laboratory-generated and ambient air-sampled SOAs. Overall, the Review Committee commended Dr. Shiraiwa and colleagues for the novelty and thoughtfulness in the study approach. The study demonstrated that the quantity and composition of ROS formed by aqueous reactions is highly dependent on such factors as the specific aerosol mixture, oxidation mechanisms, and other environmental conditions. Kinetic modeling suggested that ROS formation in the respiratory tract is highest in the extrathoracic cavity and lowest in the alveolar region. The study also found that under certain experimental conditions, ROS released by macrophages outweighs ROS formed by chemical reactions in ELF. This work is an important first step in understanding the relative importance of chemical versus biological ROS formation in the lung. Further work is recommended by extending the use of PM samples from various ambient sources and including an evaluation of responses in other types of immune cells.

ACKNOWLEDGMENTS

The HEI Review Committee is grateful to Annemoon van Erp for oversight of the study; to Eva Tanner for assistance with review of the report and in preparing its Commentary; to Carol Moyer for editing of this Report and its Commentary; and to Hope Green and Kristin Eckles for their roles in preparing this Research Report for publication.

REFERENCES

Brauer M, Brook JR, Christidis T, Chu Y, Crouse DL, Erickson A. 2019. Mortality–Air Pollution Associations in Low-Exposure Environments (MAPLE): Phase 1. Research Report 202. Boston, MA:Health Effects Institute.

Brauer M, Brook JR, Christidis T, Chu Y, Crouse DL, Erickson A, et al. 2022. Mortality–Air Pollution Associations in Low

Exposure Environments (MAPLE): Phase 2. Research Report 212. Boston, MA:Health Effects Institute.

Brunekreef B, Strak M, Chen J, Andersen ZJ, Atkinson RW, Bauwelinck M. 2021. Mortality and Morbidity Effects of Long-Term Exposure to Low-Level $PM_{2.5}$, BC, NO_2, and O_3: An Analysis of European Cohorts in the ELAPSE Project. Research Report 208. Boston, MA:Health Effects Institute.

Canton M, Sánchez-Rodríguez R, Spera I, Venegas FC, Favia M, Viola A, et al. 2021. Reactive oxygen species in macrophages: Sources and targets. Front Immunol 12:734229; doi:10.3389/fimmu.2021.734229.

Chen J, Hoek G. 2020. Long-term exposure to PM and all-cause and cause-specific mortality: A systematic review and meta-analysis. Environ Int 143:105974; doi:10.1016/j.envint.2020.105974.

Dominici F, Schwartz J, Qian D, Braun D, Choirat C, Zanobetti A. 2019. Assessing Adverse Health Effects of Long-Term Exposure to Low Levels of Ambient Air Pollution: Phase 1. Research Report 200. Boston, MA:Health Effects Institute.

Dominici F, Zanobetti A, Schwartz J, Braun D, Sabath B, Wu X. 2022. Assessing Adverse Health Effects of Long-Term Exposure to Low Levels of Ambient Air Pollution: Implementation of Causal Inference Methods. Research Report 211. Boston, MA:Health Effects Institute.

Fang T, Guo H, Zeng L, Verma V, Nenes A, Weber RJ. 2017. Highly acidic ambient particles, soluble metals, and oxidative potential: A link between sulfate and aerosol toxicity. Environ Sci Technol 51:2611–2620; doi:10.1021/acs.est.6b06151.

Global Burden of Disease (GBD) 2019 Risk Factors Collaborators. 2020. Global burden of 87 risk factors in 204 countries and territories, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet 396:1223–1249; doi:10.1016/S0140-6736(20)30752-2.

Gurgueira SA, Lawrence J, Coull B, Murthy GG, González-Flecha B. 2002. Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. Environ Health Perspect 110:749–55.

International Agency for Research and Cancer (IARC). 2016. Outdoor air pollution. In: Monograph on the evaluation of carcinogenic risks to humans. Vol. 109. Lyon, France:IARC.

Jaffe DA, O'Neill SM, Larkin NK, Holder AL, Peterson DL, Halofsky JE, et al. 2020. Wildfire and prescribed burning impacts on air quality in the United States. J Air Waste Manag Assoc 70:583–615; doi:10.1080/10962247.2020.1749731.

Jia C, Batterman S. 2010. A critical review of naphthalene sources and exposures relevant to indoor and outdoor air. Int J Environ Res Public Health 7:2903–2939; doi:10.3390/ijerph7072903.

Lakey PSJ, Berkemeier T, Tong H, Arangio AM, Lucas K, Pöschl U, et al. 2016. Chemical exposure-response relationship between air pollutants and reactive oxygen species in the human respiratory tract. Sci Rep 6:32916; doi:10.1038/srep32916.

Li T, Yu Y, Sun Z, Duan J. 2022. A comprehensive understanding of ambient particulate matter and its components on the adverse health effects based from epidemiological and laboratory evidence. Part Fibre Toxicol 19:67; doi:10.1186/s12989-022-00507-5.

Pietrogrande MC, Demaria G, Colombi C, Cuccia E, Dal Santo U. 2022. Seasonal and Spatial variations of PM_{10} and $PM_{2.5}$ Oxidative Potential in Five Urban and Rural Sites across Lombardia Region, Italy. IJERPH 19:7778; doi:10.3390/ijerph19137778.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. 2017. Oxidative stress: Harms and benefits for human health. Oxid Med Cell Longev 2017:1–13; doi:10.1155/2017/8416763.

Pöschl U, Shiraiwa M. 2015. Multiphase chemistry at the atmosphere–biosphere interface influencing climate and public health in the Anthropocene. Chem Rev 115:4440–4475; doi:10.1021/cr500487s.

Shirmohammadi F, Hasheminassab S, Wang D, Schauer JJ, Shafer MM, Delfino RJ, et al. 2016. The relative importance of tailpipe and non-tailpipe emissions on the oxidative potential of ambient particles in Los Angeles, CA. Faraday Discuss 189:361–380; doi:10.1039/C5FD00166H.

Shrivastava M, Cappa CD, Fan J, Goldstein AH, Guenther AB, Jimenez JL, et al. 2017. Recent advances in understanding secondary organic aerosol: Implications for global climate forcing. Rev Geophys 55:509–559; doi:10.1002/2016RG000540.

United States Environmental Protection Agency (U.S. EPA) 2019. Integrated Science Assessment for Particulate Matter. Washington, DC:U.S. EPA.

United States Environmental Protection Agency (U.S. EPA). 2023. Public Hearing for the Reconsideration of the National Ambient Air Quality Standards for Particulate Matter. Federal Register 88:6215–6217. Washington, DC:U.S. EPA.

World Health Organization (WHO). 2021. WHO Global Air Quality Guidelines. Geneva, Switzerland:WHO. Available: *https://apps.who.int/iris/handle/10665/345329*.

World Health Organization (WHO). 2022. Ambient (Outdoor) Air Pollution. Available: *https://www.who.int/news-room/fact-sheets/detail/ambient-(outdoor)-air-quality-and-health* [accessed 11 May 2023].

ABBREVIATIONS AND OTHER ITEMS

$Asc^{\cdot-}$	ascorbate radicals
BMPO	5-*tert*-butoxycarbonyl-5-methyl-1-pyrroline-N-oxide
BMPO-OH	·OH
BMPO-OOH	$\cdot O_2^-/HO_2\cdot$
BMPO-R	R·
BMPO-OR	RO·
CMH	1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine
DTT	dithiothreitol
ELF	epithelial lining fluid
ELVOC	extremely low volatility organic compound
EPFR	environmentally persistent free radical
EPR	electron paramagnetic resonance
Fe^{2+}	ferrous iron
FLIM	fluorescence lifetime imaging microscopy
GBD	Global Burden of Disease
H_2O_2	hydrogen peroxide
$HO_2\cdot$	hydroperoxyl radical
HOM	highly oxygenated organic molecules
HULIS	humic-like substances
HX	hypoxanthine
IARC	International Agency for Research and Cancer
KM-SUB-ELF	kinetic multilayer model for surface and bulk chemistry in ELF
MCGA	Monte Carlo genetic algorithm
MOUDI	micro-orifice uniform deposition impactor
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NAD(P)H	reduced nicotinamide adenine dinucleotide [phosphate]
NO_x	nitrogen oxides
O_2	molecular oxygen
$\cdot O_2^-$	superoxide radical
$\cdot O_2^-/HO_2\cdot$	superoxide radical and its conjugated acid HO_2
O_3	ozone
·OH	hydroxy radical
OP	oxidative potential
OP-DTT	oxidative potential measured with DTT assay
PAM	potential aerosol mass
PM	particulate matter
PMA	phorbol 12-myristate 13-acetate
PM_1	PM with aerodynamic diameter ≤1.0 μm (submicron)
PM_{10}	PM with aerodynamic diameter ≤10 μm (coarse)
$PM_{2.5}$	PM with aerodynamic diameter ≤2.5 μm (fine)
PQN	9, 10-phenanthrenequinone
PTFE	polytetrafluoroethylene
R·	carbon-centered organic radicals
RLU	relative light units
RO·	oxygen-centered organic radicals
$RO_2\cdot$	peroxy radicals
ROOH	organic hydroperoxides
ROOR	organic peroxides
ROS	reactive oxygen species
s	second
SOA	secondary organic aerosols
SOA_{O3}	SOA generated from dark ozonolysis
SOA_{OH}	SOA generated from ·OH photooxidation
SOD	superoxide dismutase
SLF	surrogate lung fluid
THG	third harmonic generation
UCI	University of California, Irvine
U.S. EPA	United States Environmental Protection Agency
WHO	World Health Organization
XO	xanthine oxidase

HEI BOARD, COMMITTEES, and STAFF

Board of Directors

Richard A. Meserve, Chair *Senior of Counsel, Covington & Burling LLP; President Emeritus, Carnegie Institution for Science; former Chair, U.S. Nuclear Regulatory Commission*

Jo Ivey Boufford *Clinical Professor of Global Health and Pediatrics, New York University*

Homer A. Boushey *Emeritus Professor of Medicine, University of California–San Francisco*

Jared L. Cohon *President Emeritus and Professor, Civil and Environmental Engineering and Engineering and Public Policy, Carnegie Mellon University*

Stephen Corman *President, Corman Enterprises*

Martha J. Crawford *Operating Partner, Macquarie Asset Management*

Ana V. Diez Roux *Dana and David Dornsife Dean and Distinguished University Professor of Epidemiology, Dornsife School of Public Health, Drexel University; Director, Drexel Urban Health Collaborative*

Michael J. Klag *Dean Emeritus and Second Century Distinguished Professor, Johns Hopkins Bloomberg School of Public Health*

Alan I. Leshner *CEO Emeritus, American Association for the Advancement of Science*

Catherine L. Ross *Regents' Professor Emerita, City and Regional Planning and Civil and Environmental Engineering, Georgia Institute of Technology; Chairman of the Board of Directors of the Auto Club Group, American Automobile Association*

Martha E. Rudolph *Environmental Attorney, Former Director of Environmental Programs, Colorado Department of Public Health and Environment*

Karen C. Seto *Frederick Hixon Professor of Geography and Urbanization Science, Yale School of the Environment*

Research Committee

David A. Savitz, Chair *Professor of Epidemiology, School of Public Health, and Professor of Obstetrics and Gynecology and Pediatrics, Alpert Medical School, Brown University*

David C. Dorman *Professor, Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University*

Christina H. Fuller *Associate Professor, School of Environmental, Civil, Agricultural and Mechanical Engineering, University of Georgia College of Engineering*

Marianne Hatzopoulou *Professor, Civil and Mineral Engineering, University of Toronto, Canada, Research Chair in Transport Decarbonization and Air Quality*

Amy H. Herring *Sara & Charles Ayres Distinguished Professor of Statistical Science, Global Health, Biostatistics, and Bioinformatics, Duke University*

Heather A. Holmes *Associate Professor, Department of Chemical Engineering, University of Utah*

Neil Pearce *Professor of Epidemiology and Biostatistics, London School of Hygiene and Tropical Medicine, United Kingdom*

Evangelia (Evi) Samoli *Professor of Epidemiology and Medical Statistics, Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Greece*

Neeta Thakur *Associate Professor of Medicine, University of California–San Francisco*

Gregory Wellenius *Professor, Department of Environmental Health, Boston University School of Public Health and Director, BUSPH Center for Climate and Health*

Continues next page

HEI BOARD, COMMITTEES, and STAFF

Review Committee

Melissa J. Perry, Chair *Dean, College of Public Health, George Mason University*

Sara D. Adar *Associate Professor and Associate Chair, Department of Epidemiology, University of Michigan School of Public Health*

Kiros T. Berhane *Cynthia and Robert Citrone-Roslyn and Leslie Goldstein Professor and Chair, Department of Biostatistics, Mailman School of Public Health, Columbia University*

Ulrike Gehring *Associate Professor, Institute for Risk Assessment Sciences, Utrecht University*

Michael Jerrett *Professor, Department of Environmental Health Sciences, Fielding School of Public Health, University of California–Los Angeles*

Frank Kelly *Henry Battcock Chair in Community Health and Policy and Director of the Environmental Research Group, Imperial College London School of Public Health, United Kingdom*

Jana B. Milford *Professor Emerita, Department of Mechanical Engineering and Environmental Engineering Program, University of Colorado–Boulder*

Jennifer L. Peel *Professor of Epidemiology, Department of Environmental and Radiological Health Sciences, Colorado State University, and the Colorado School of Public Health*

Eric J. Tchetgen Tchetgen *University Professor and Professor of Biostatistics and Epidemiology, Perelman School of Medicine, and Professor of Statistics and Data Science, The Wharton School, University of Pennsylvania*

John Volckens *Professor, Department of Mechanical Engineering, Walter Scott Jr. College of Engineering, Colorado State University*

Officers and Staff

Elena Craft *President*

Robert M. O'Keefe *Vice President*

Ellen K. Mantus *Director of Science*

Donna J. Vorhees *HEI Energy CEO and Vice President*

Annemoon M. van Erp *Deputy Director of Science*

Thomas J. Champoux *Director of Science Communications*

Jacqueline C. Rutledge *Director of Finance and Administration*

Jason Desmond *Deputy Director of Finance and Administration*

Emily Alden *Corporate Secretary*

Daniel S. Greenbaum *President Emeritus*

Amy Andreini *Science Communications Specialist*

Ayusha Ariana *Research Assistant*

Hanna Boogaard *Consulting Principal Scientist*

Aaron J. Cohen *Consulting Principal Scientist*

Dan Crouse *Senior Scientist*

Philip J. DeMarco *Compliance Manager*

Continues next page

HEI BOARD, COMMITTEES, and STAFF

(Staff, continued)

Cloelle Danforth *Senior Scientist*
Kristin C. Eckles *Senior Editorial Manager*
Elise G. Elliott *Staff Scientist*
Hope Green *Editorial Project Manager*
Lissa McBurney *Senior Science Administrator*
Janet McGovern *Executive Assistant*
Samantha Miller *Research Assistant*
Victor Nthusi *Consulting Research Fellow*
Pallavi Pant *Head of Global Health*
Allison P. Patton *Senior Scientist*
Quoc Pham *Science Administrative Assistant*
Yasmin Romitti *Staff Scientist*
Anna Rosofsky *Senior Scientist and Environmental Justice Program Lead*
Robert Shavers *Operations Manager*
Eva Tanner *Staff Scientist*
Alexis Vaskas *Digital Communications Manager*
Ada Wright *Research Assistant*

NUMBER 215
DECEMBER 2023

COMMENTARY BY THE HEI REVIEW COMMITTEE

Publishing history: This document was posted at *www.healtheffects.org* in December 2023.

Citation for document:

HEI Review Committee. 2023. Commentary. In: Shiraiwa M, Fang T, Wei J, Lakey PSJ, Hwang BCH, Edwards KC, et al. 2023. Chemical and Cellular Formation of Reactive Oxygen Species from Secondary Organic Aerosols in Epithelial Lining Fluid. Research Report 215. Boston, MA:Health Effects Institute.

HEI

Health Effects Institute

75 Federal Street
Suite 1400
Boston, Massachusetts 02110, USA
+1-617-488-2300

healtheffects.org